SECOND EDITION

# Water Pollution Biology

P. D. Abel

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Second Edition

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# Preface to Second Edition

The first edition of this book having been on the whole well received, I have tried to retain as far as possible its general style and character in the second. Nevertheless some sections have needed extensive revision and updating, and I have taken the opportunity to expand or develop some topics, often in response to the many constructive suggestions I have received from readers and reviewers, and from my own students. Inevitably, I have had to delete some material for reasons of space.

The main changes are to revise, update and extend those areas in which there have been significant technical advances, or which were perhaps inadequately covered in the first edition. To keep the book within manageable proportions, I have omitted some material which was included in the first edition; in most cases, this material is by now readily accessible elsewhere and has been appropriately referenced. I have rewritten and/or restructured some sections of the book, to take account of the comments I have received regarding the readers' impression of the most appropriate order in which the material should be presented. Readers of the first edition may detect some greater reliance on secondary sources than was shown in the first. This reflects the increased availability of high-quality monographs and reviews since the first edition. It also, sadly, reflects the experience which I and colleagues at other universities have had over the last few years that students simply no longer have ready access to primary sources, and increasingly do not have the opportunity to develop the skills required to assess and evaluate them. This may be a trend which many deplore, but we have little choice other than, as authors and teachers, to modify our approach to suit the changing circumstances. Nevertheless I still believe that the main purpose of this second edition is the same as the first: 'To impart not only factual information, but also some idea of the areas of uncertainty which must remain; to impart to the reader some sense of how he/she may evaluate for him/herself the quality and reliability of the vast amount of material he/she may encounter; and to concentrate on the elucidation of principles rather than the promulgation of data'. In relation to this aim, I have not been inhibited in preferring sometimes to cite older, original material over more recently-dated sources. The rate of increase in knowledge and understanding, even in science, is often slower than the rate of increase in the number of published works. Also, the

understanding of basic principles and methodology is often more easily achieved through the study of clear and accessible examples, which are sometimes rare in material published under the constraints of space and jargon which are characteristic of many modern journals.

One of the fascinations of studying water pollution is that it demands some familiarity with a wide range of specialisms. In the following pages, I have been foolhardy enough to attempt to touch upon subjects ranging from molecular biology to international law, all of which are relevant to my chosen topic. I cannot write with equal authority and fluency upon all of these subjects, so in many cases I have had to rely heavily upon the assistance and advice of colleagues. I am obstinate enough sometimes to have followed my own path, so must accept responsibility for any errors of fact or interpretation which remain. Many of my colleagues may not even be aware of the extent of their assistance, perhaps because in conversation they have set off a train of thought or drawn my attention to a source of information. Their contributions are no less valued for that, but some have offered me very specific and extensive support or assistance, including Dr A.Abbott, Mr A. Milne, Ms K.Shepherd and Dr D.Reid. I am particularly grateful to Ms L.Kiakides, who has afforded me every form of help, both in the preparation of the book itself and in shouldering many responsibilities which were properly mine in order to allow me to finish it.

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# Water Pollution Problems and Solutions

Water is one of our most important natural resources, and there are many conflicting demands upon it. Skilful management of our water bodies is required if they are to be used for such diverse purposes as domestic and industrial supply, crop irrigation, transport, recreation, sport and commercial fisheries, power generation, land drainage and flood protection, and waste disposal. An important objective of most water management programmes is the preservation of aquatic life, partly as an end in itself and partly because water which sustains a rich and diverse fauna and flora is more likely to be useful to us, and less likely to be a hazard to our health, than one which is not so endowed. To meet this objective, it is necessary to maintain within certain limits factors such as water depth and flow regimes, temperature, turbidity and substratum characteristics, and the many parameters which contribute to the chemical quality of the water. In waters which receive waste discharges, whether by design or by accident, one or more of these variables may come to lie outside the limits which can be tolerated by one or more of the species which live there, and consequently the biological characteristics of the water are altered.

The biologist's role in the monitoring and control of water pollution is to detect and accurately to describe these alterations, to elucidate as precisely as possible the mechanisms by which they are brought about, and to seek to understand the qualitative and quantitative relationships between pollution and its biological consequences. He or she may also need to be aware of the application of biological processes in the control or amelioration of pollution, and of the serious consequences for public health which water pollution threatens. Finally, he or she must be able to offer constructive advice to other specialists—chemists, engineers, administrators and legislators—who share the responsibility for managing our water resources.

All of these topics will be discussed in the following chapters, but first it might be useful to gain some idea of the nature and scale of the problems posed by water pollution.

## 1.1 The Scale of Water Pollution

Water pollution, like other environmental concerns, has been the focus of widespread public interest for about three decades now, and this interest seems to be increasing. While this has many obvious benefits, it sometimes can appear that the public perception of water pollution, as manifested for example by political debate and the activities of pressure groups, does not always accord with the scientific reality. This can lead to ill-advised or cost-ineffective actions, including legislation and regulation, in an attempt to deal with perceived or publicised problems which may, in fact, be less serious than others which are less well publicised or less easy for the educated layman to understand. It is therefore important to gain some idea of the real nature and extent of water pollution.

Britain may perhaps fairly be taken as a representative example of a developed country, and is one of the few countries, even in the developed world, where adequate information is available to undertake a general survey. In fact, national surveys of water quality have taken place at regular intervals since 1958; they currently take place once every five years, the most recent one for which data are available being in 1990 (NRA, 1991). Of approximately 40000km of main river surveyed in England and Wales, 65.3% was classified in 1990 as 'good'; 23.4% as 'fair'; 9.5% as 'poor'; and 1.6% as 'bad'. (The basis of these classifications, and the applications of the survey data, are described and discussed in more detail in Chapter 6.) Thus approximately 35% of the total length of rivers in England and Wales could be said to be significantly influenced by pollution, and about 12% of the total seriously so. Bad as this may seem, it does in fact represent a considerable improvement of the situation as recorded in earlier surveys 30 or so years ago (see Chapter 6), which perhaps gives some confidence that the application of the principles and techniques outlined later in this book is of some use!

The distribution of the more seriously polluted rivers throughout the country is, of course, by no means even; the worst situations tend to be found in and around the larger conurbations and industrial areas. However, the common perception of pollution as being caused primarily by industry is not correct. Although this may once have been true, the most recent survey data clearly indicate that today other sources, particularly agriculture, require much more attention than they sometimes receive.

This is most easily seen by referring to a report on water pollution incidents which occurred in England and Wales in 1991 (NRA, 1992). Approximately 28000 incidents were reported to the NRA in 1991, a number which has increased fairly steadily since records began in 1981, when about 12000 incidents were reported. This increase is probably misleading. An 'incident' may arise through accidental,

negligent or illegal discharge of wastes to water; or through failure, for whatever reason, of waste treatment processes causing them to exceed their discharge limits. The apparent increase in incidents is probably largely accounted for by the increasingly strict regulation and monitoring of known sources of pollution, and by increased public awareness and willingness to report incidents which in earlier times probably went unnoticed. Nevertheless, and allowing for the fact that about 20% of the incidents reported were unsubstantiated or had no significant effect, over 22000 significant incidents did occur, of which 386 were categorised as 'major'. Of the significant incidents recorded, roughly 28% had causes related to the sewage and water treatment industry itself; 23% related to oil discharges; 13% originated from farms; 12% from industrial sources; and 22% from other sources such as road accidents or leaching of contaminated water from waste dumps.

These figures are probably representative of most Western countries in a similar state of economic and industrial development as Britain, but some idea of the scale of water pollution problems in developing countries can be obtained from Brinkhurst's (1993) account of his experiences in China:

> "It is hard to imagine what horrors are associated with the gross levels of water pollution that an aquatic biologist encounters in the Third World. I have visited the People's Republic of China twice attempting to collect aquatic oligochaetes from clean water. The existence of clean-water species in a group of so-called indicator organisms such as the oligochaetes will be mentioned below, but it is sufficient to say that they were nowhere to be found. Reports exist of normal communities of benthos along the Sino-Soviet border from work done during a previous era of cooperation between China and the USSR. Otherwise, the wide rivers, shown on maps of the middle regions of China, usually are reduced to a thin trickle in the middle of a wide valley. The Gobi Desert surges towards Beijing at an accelerating pace. Only the widths of the bridges and the large areas of dry river bed remain as testimony to the former size of these rivers. The water is sometimes jet black, sometimes rainbow-hued with chemicals, or it is steaming with heat. Even springs among the limestone hills of Guilin contain world-wide 'indicator' species, reflecting upstream contamination not alleviated by a spell underground."

Brinkhurst goes on to surmise that similar scenes are to be witnessed in Eastern Europe; certainly, such information as has become available since 1990 (Carter and Turnock, 1993) indicates that the situation there is extremely bad and still deteriorating. Elsewhere in the world, such limited information as exists on a country-by-country basis does little to counter his pessimism (see, for example, UNEP, 1991).

Yet the experience of Britain and some other of the more fortunate countries of the world shows that it is possible to combine economic and industrial development, increased population and the concomitant demands on water resources, with actual improvement of the conditions of the aquatic environment from the dreadful state which we know existed after our own industrial revolution. What is required is the understanding and application of the relevant scientific and technical principles, combined with an appropriate legislative framework and, of course, preferably an informed and sympathetic climate of public opinion. Those scientific principles most likely to fall within the domain of the biologist form the subjects of the succeeding chapters; but perhaps the best introduction to the scope of the problems to be faced, and some of their solutions, is to look at some case studies.

## 1.2 Metalliferous Rivers

In many parts of the world, rivers have become contaminated with heavy metals such as zinc, lead and copper as a result of mining and associated activities. In Britain, three areas of the country are particularly well known because they contain a number of metalliferous rivers: one is in the south-west of England, one is in West Wales and one is in the North Pennine Orefield in northern England. Although these areas were once major centres for the extraction and processing of metal and mineral ores, within the last hundred years or so the mining activities have declined considerably, or ceased altogether. These areas never became major population centres, and were never subject to the intensive pollution pressures of modern urban and industrial society. The effects of the mining activity are still detectable, but these effects have not been compounded and complicated by those of other forms of pollution associated with modern development. For this reason, they offer the opportunity to investigate the impact of an important group of industrial pollutants—heavy metals—on waters which are relatively unaffected by the pressures which have more recently been unleashed on water bodies in more densely-populated areas. They have therefore been extensively studied, and provide a good illustration of the biological effects of pollution, of the methods which may be used in studying these effects, and of the questions which biologists seek to investigate and answer about polluted waters. The following account is largely based upon the review of Green (1984). Mance (1987) also discusses some studies of metalliferous rivers.

Ecological studies on metalliferous rivers in West Wales began about 70 years ago, and have continued, intermittently, ever since. Carpenter (1922, 1924) observed that fewer invertebrate species occurred in these rivers at stations close to the lead mines, and that the differences in abundance of invertebrates appeared to be due to lead in the mine effluents rather than to physical differences in the river bed or any other parameter of water quality. Certain invertebrate groups (Platyhelminthes, Mollusca, Crustacea, Oligochaeta and many Insecta) were always absent from the most heavily polluted sites, but some insect species such

as *Cloeon simile* (Ephemeroptera), *Simulium latipes* (Diptera) and *Velia currens* (Hemiptera) appeared to be tolerant of elevated lead concentrations. Following the closure of a mine and the cessation of pollution, Carpenter (1926) described a process of recovery. The first stage was the establishment of a restricted fauna consisting almost entirely of insect larvae on a substratum bearing a limited covering of algae and bryophytes. Oligochaetes, molluscs, platyhelminthes, crustaceans and many insect species remained absent. Subsequently, the encroachment of chlorophyceous algae was accompanied by an increase in invertebrate species diversity, with oligochaetes, turbellarians, caddis larvae and other insects becoming established. In the final stage of recovery, macrophytes were established in physically-suitable areas and there was a large increase in the numbers of invertebrate taxa present, including molluscs, and the development of fish populations. This process of recovery which occurred following the closure of a mine could also be observed in reverse (Carpenter, 1926) when a mine which had been closed for some years was subsequently reopened. The same stages of succession could also be observed contemporaneously in successive reaches of a polluted river with increasing distance from the source of pollution (Carpenter, 1924). The toxic agent responsible for these changes was at first assumed to be lead, particularly dissolved lead rather than the particulate component of the mine effluents. Carpenter (1925) investigated the toxicity of dissolved lead salts to fish, but at the concentrations of lead found in the river water, lead salts alone were less toxic than the river water. This indicated that the mine effluents contained some additional toxic agent, and zinc was soon identified as being an important factor.

During the decades following Carpenter's early investigations, advances in techniques of taxonomy, ecological sampling and chemical analysis provided more detailed information on the effects of metal pollution in upland rivers. Jones (1940a, b) studied the Rivers Ystwyth and Rheidol, also in Wales, and confirmed the general pattern described by Carpenter. In the Ystwyth it became clear that zinc, rather than lead, was the most abundant toxic agent; by 1958 lead levels had declined markedly but zinc levels remained high (Jones, 1958). This probably occurred because zinc is more soluble than lead, and because the pattern of mining activity in the area tended to result in the fairly efficient removal of lead and silver from the ores, whereas waste material dumped in and around the mines remained rich in zinc. Between 1940 and 1958, there was no substantial increase in the number of species found in the Ystwyth. Molluscs, crustaceans, oligochaetes and leeches were still absent. Among the insects, the fauna remained restricted but *Rhithrogena semicolorata* (Ephemeroptera), *Simulium* spp. (Diptera), and the stoneflies *Leuctra* spp. and *Nemoura* spp. were fairly numerous. Caseless caddis larvae were more numerous than cased caddis larvae.

On the basis of some toxicity tests, the absence of fish, molluscs and crustaceans was ascribed to the presence of toxic metals in the water, but the scarcity of flatworms, and the absence of leeches and oligochaetes was thought to be due to the unfavourable characteristics of the substratum. By 1980, little further improvement had occurred. Brooker and Morris (1980) recorded more species than were found in 1958, but much of the increase was apparently due to the fact that some groups of animals earlier identified to genus, particularly chironomids and simulids, were identified to the species. Paradoxically, the association of particular metal levels with the biological characteristics of the water is becoming more difficult. For example, it is now known that high levels of calcium to some extent protect animals from the toxic effects of heavy metals, whereas low levels can, quite apart from any influence of heavy metals, themselves act as limiting factors.

A great deal more is now known about the influence of purely physical factors on the patterns of distribution of invertebrate species. Therefore it is less easy than it might earlier have appeared to interpret the available biological data solely in terms of the measured heavy metal levels. Nevertheless in the nearby river Rheidol, where metal levels are lower, the invertebrate community is distinctly richer. Whereas Carpenter (1924) recorded 29 species, Laurie and Jones (1938) recorded 103 following a distinct reduction in the metal levels some years earlier; Jones (1949) recorded 130 species, though molluscs and crustaceans were still absent. Brooker and Morris (1980) recorded 134 species.

Elsewhere in Britain and in other parts of the world, metalliferous rivers similar to those in Wales have been widely studied, though rarely over such a long period of time. In south-west England, Brown (1977) studied the River Hayle which is contaminated with zinc, copper and iron. Metalliferous rivers in the North Pennine Orefield have received some attention (e.g. Armitage, 1980). Examples from North America include the studies of Sprague *et al.* (1965) on rivers polluted with copper and zinc; and of Gale *et al.* (1973) on rivers polluted with zinc and lead. Zinc-polluted rivers in Australia have been investigated by Weatherley *et al.* (1967). The river South Esk in Tasmania, which contains cadmium, zinc, copper, iron and manganese, has been investigated by Thorp and Lake (1973), Tyler and Buckney (1973) and Norris *et al.* (1982). There are, of course, many other rivers polluted with heavy metals, but in most of these the effects of heavy metal pollution are compounded or modified by substantial inputs of organic matter and/or other industrial pollutants. Based on his review of investigations relating to rivers polluted solely or predominantly by heavy metals without these other complicating factors, Green (1984) drew attention to the following general points.

In rivers polluted by heavy metals, the invertebrate fauna is affected by the elimination, or numerical reduction, of certain species. If the input of pollution ceases, the invertebrate fauna gradually recovers with the passage of time. A

similar process of recovery can be observed with increasing distance from a source of pollution, as the concentration of pollutants decreases. The nature of the recovery is a gradual increase in the numbers of species and in the numbers of individuals found in the water. Taxa which are universally affected by metal mining and associated activities are Mollusca, Crustacea, Platyhelminthes and Oligochaetes. Some groups, however, appear to behave inconsistently in response to metal pollution. One such group is the larvae of caddis flies (Trichoptera). In the Rheidol and Ystwyth, for example, Jones (1940b) noted the absence of cased caddis larvae from polluted stations, whereas carnivorous, caseless species such as *Rhyacophila dorsalis* and *Polycentropus flavomaculatus* were present. In laboratory experiments, no evidence was found that the concentration of dissolved metal found in the rivers was lethal to cased caddis species, and in fact some species were able to construct their cases, in the laboratory, from particles of solid mine waste. This suggested that metals may exert indirect effects on some species in the field, possibly related to the effects of the metal on the food source of the caddis larvae.

An alternative explanation, of course, may be that sublethal toxicity rather than lethal toxicity plays some part in the elimination of species. Animals may be able to survive, for a short period of time, exposure to levels of metals similar to those found in the rivers, but may be unable to complete their entire life cycles under these conditions. In some cases, however, caddis larvae appear actually to become more abundant than expected in the polluted stretches of metalliferous rivers (Norris *et al.,* 1982; Sprague *et al.,* 1965; Weatherley *et al.,* 1967). Commonly, reduced predation pressure from fish has been suggested as a possible reason for this. There is also some inconsistency in the reported response of another important insect group, the Ephemeroptera (mayflies), to metal pollution. Some invertebrate groups which are commonly reduced or absent from polluted waters appear to be affected by alterations to the physical characteristics of the river bed which are caused by the particulate matter in mine effluents, rather than by the toxic action of the metals themselves. Oligochaetes, platyhelminths and leeches are possible examples.

Another form of stream-bed alteration which may have profound effects is the development of excessive algal growth, as reported in some metalliferous rivers in northern England (Armitage, 1980). One hypothesis to account for this process is that the metals, by removing herbivorous invertebrates, allow the establishment of an unusually luxurious algal mat on the river bed, which in turn interferes with the normal patterns of distribution of animals which are not themselves directly affected by the metal. Algal growth may, of course, be enhanced in rivers with substantial inputs of plant nutrients or organic matter, so this particular phenomenon may not be universal. However, there is evidence (see Chapter 4) that organic matter can afford some protection to aquatic species from the toxic effects of metals, so the outcome in any individual case may be difficult to predict. Indeed, no two rivers

are exactly similar in their physical, chemical andbiological characteristics, and the interactions of living organisms with one another and with their physical environment are so complex that whatever general pattern emerges, it will inevitably be subject to significant variations of detail according to the precise local circumstances.

One major difficulty in studying the effects of pollution in the field and in drawing from them conclusions of general applicability is that it is usually impossible, in any individual case, to compare what is happening in the polluted river with what would have been happening had the river not been polluted. Most commonly, surveys of polluted rivers consist of contemporaneous observations of different sites along a river or within a river system at different distances from the source of pollution. However, it is well known that, even in the complete absence of pollution, the biological characteristics of different points within a river system vary widely according to the physical and chemical conditions prevailing at the different locations. The precise relationships between the biological community and its physical environment are not well understood, and it is therefore extremely difficult to distinguish the consequences of pollution from the response of the community to natural variations in its physical environment. The RIVPACS system (see Chapter 3) represents a recent development towards a solution of this difficulty. Nevertheless, it would be very interesting to have available for study two physically and chemically identical rivers, of which one was polluted (preferably with a single pollutant). In practice, such a pair of rivers probably does not exist, although attempts have been made on a limited scale to approach this situation experimentally. However, a pair of rivers which approaches this ideal as closely as we may reasonably hope is to be found in the North Pennine Orefield in northern England, and may be used as a case study.

### *1.2.1 The Rivers East and West Allen*

The Rivers East and West Allen in Northumberland, England, lie within the North Pennine Orefield. They flow roughly northwards for approximately 18 kilometres before joining to form the River Allen, itself a tributary of the South Tyne (Figure 1.1). The rivers drain adjacent valleys which were heavily exploited for a variety of metal and mineral ores, particularly during the eighteenth and nineteenth centuries. After the early years of the present century, mining activity declined rapidly and virtually ceased in 1946. Sporadic mining for fluorspar took place at the Beaumont mine at the head of the East Allen until 1979, and a very limited amount of mining continued intermittently at Barneycraig on the West Allen until 1981; since that time no activity has been recorded in either valley. Until the end of the nineteenth century, mining in the Allendales was dominated by the production of lead ore. The West Allen mines, in particular, produced substantial quantities of zinc ore, but this seems to have been simply discarded prior to 1899, being

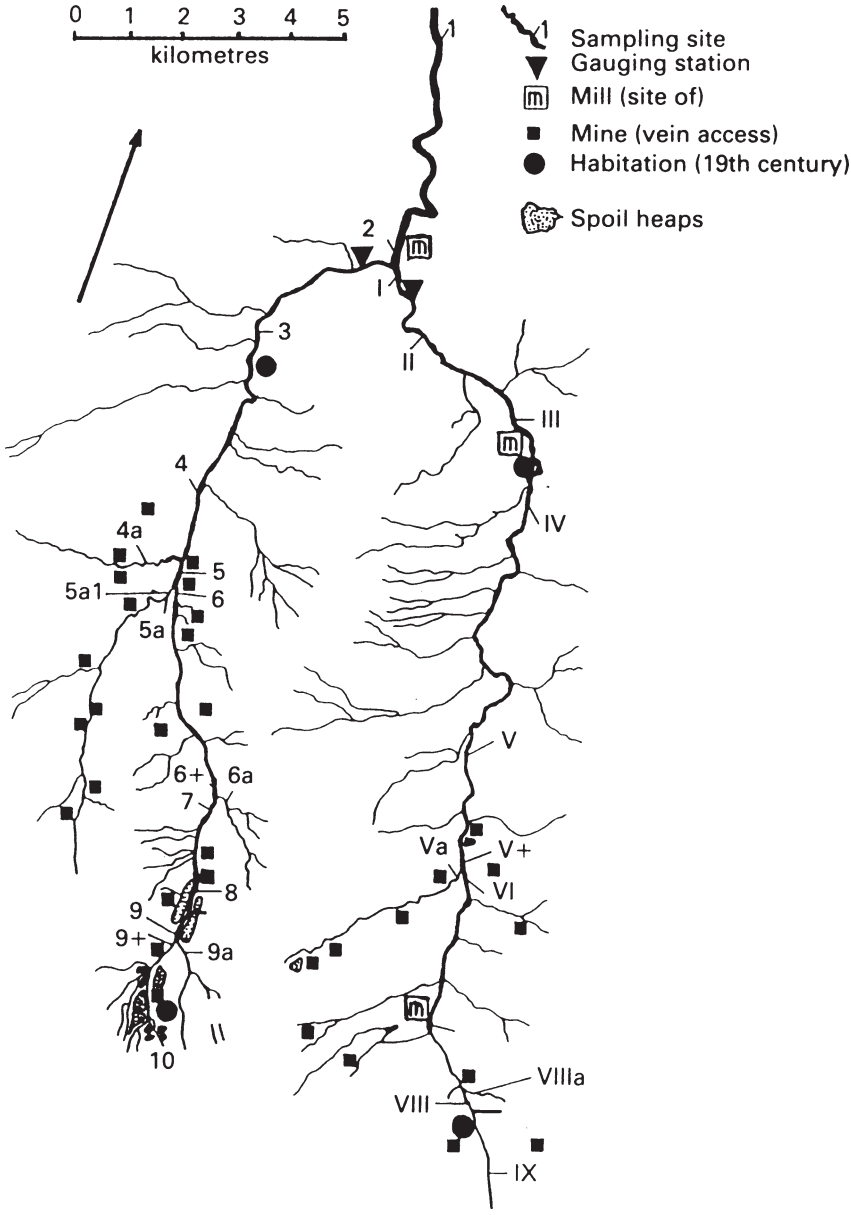



**Figure 1.1** Map of the Rivers East and West Allen, showing sampling stations, derelict mines and workings, and remaining spoil heaps. After Green (1984)

returned as backfill to disused shafts or left in surface spoil heaps. Although the East Allen mines produced far greater quantities of ore than those on the West Allen, the zinc-bearing veins are almost entirely confined to the West Allen. Moreover, in the East Allen valley most of the waste material was removed when the mines closed, while on the West Allen there remain extensive areas of spoil heaps and derelict land associated with mining activity. As a result, the River West Allen today contains considerably higher levels of zinc than the East Allen. A comparative study of these two physically-similar and geographically-adjacent rivers therefore provides a valuable opportunity to study the ecological effects of zinc.

### *1.2.2 Physical and Chemical Survey*

A series of 15 sampling stations was established on the West Allen and its tributaries. A further 12 stations on the East Allen were chosen for

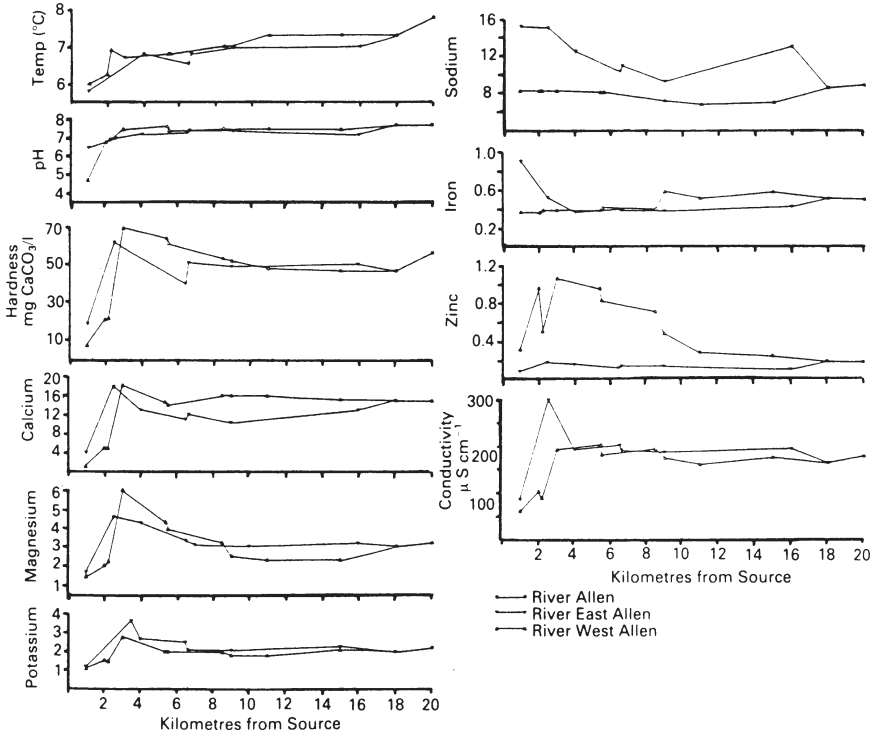



**Figure 1.2** Median values of chemical determinands at mainstream sites on the Rivers East Allen, West Allen and Allen, plotted against distance from source (Green, 1984)

**Table 1.1** Values of chemical variables for one pair of equivalent sites on the East and West Allen, 1979–80. Data from Abel and Green (1981). (Values as mg $l^{-1}$ unless otherwise stated; ND = not detectable)

| | Detection limit | West Allen Site 7 | | East Allen Site VI | |
|---|---|---|---|---|---|
| | | Mean | Range | Mean | Range |
| pH | | 7.7 | 6.7–8.6 | 7.5 | 5.9–8.8 |
| conductivity ($\mu$S $cm^{-1}$) | | 257 | 61–460 | 214 | 62–330 |
| Ca | | 33 | 6–73 | 38 | 5–65 |
| Mg | | 6.39 | 1.35–13.8 | 3.9 | 1.57–13.8 |
| K | | 2.5 | 0.5–4.1 | 2.9 | 0.8–5.2 |
| Na | | 9.53 | 4.5–16 | 12.75 | 5.3–23.4 |
| Mn | | 0.10 | 0.01–2.0 | 0.17 | 0.01–1.0 |
| Fe | | 0.41 | 0.01–1.1 | 0.22 | 0.01–1.0 |
| Pb | 0.1 | | ND–trace | | ND–trace |
| Cu | 0.03 | ND | | ND | |
| Co | 0.06 | ND | | ND | |
| Cd | 0.01 | ND | | ND | |
| Ni | 0.06 | ND | | ND | |
| Zn | 0.001 | 1.31 | 0.45–3.68 | 0.13 | <0.001–0.36 |

comparative study. The East Allen stations were chosen so that each was broadly similar in physical terms (width, depth, distance from source, substratum characteristics, nature of the surrounding terrain) to a site on the West Allen. Thus sites 7, 6a and 6+ on the West Allen could be considered as 'equivalent sites' to VI, Va and V+ on the East Allen (Figure 1.1). Between June 1979 and October 1980, samples for chemical analysis were taken twice monthly from each site. The results are summarised in Figure 1.2. In terms of temperature, pH, conductivity and a wide range of chemical determinands the two rivers are very similar. Zinc levels in the West Allen were consistently up to ten times higher than in the East, placing some stretches of the West Allen among the most heavily zinc-polluted rivers and the East Allen at the upper end of the range found among rivers which may be considered unpolluted. Concentrations of toxic heavy metals other than zinc (copper, cadmium, lead, nickel, chromium) fell consistently below the detection limits of atomic absorption spectrophotometry. Some representative data for one pair of equivalent sites are given in Table 1.1, and levels of dissolved zinc for several pairs of sites are compared in Table 1.2. Compared to the soft, acidic Welsh rivers discussed earlier, the Allens are slightly alkaline and harder, though the zinc levels are broadly comparable.

**Table 1.2** Concentrations of filtrable zinc ($mgl^{-1}$) in river water from equivalent sites on the East and West Allen, 1979–80. Data from Abel and Green (1981). West Allen sites are designated by Arabic numerals, East Allen sites by Roman numerals

| West Allen | | | | East Allen | | | |
|---|---|---|---|---|---|---|---|
| | | Zinc concentration | | | | Zinc concentration | |
| Site | km from source | Mean | Range | Site | km from source | Mean | Range |
| 10 | 1 | 0.24 | 0.04–0.56 | IX | 1 | 0.24 | <0.001–0.83 |
| 8 | 3 | 1.88 | 0.65–4.15 | VII | 4 | 0.19 | 0.1–0.34 |
| 7 | 5.5 | 1.31 | 0.45–3.68 | VI | 6.5 | 0.13 | <0.001–0.36 |
| 6a[a] | 5.5 | 0.09 | <0.001–0.33 | Va[a] | 6.5 | | 0.04–0.49 |
| 6+ | 5.5 | 0.87 | 0.09–1.88 | V+ | 6.5 | | 0.11–0.14 |
| 5 | 9 | 0.51 | 0.12–1.22 | V | 9 | | <0.001–0.23 |
| 4 | 11 | 0.50 | 0.04–1.28 | IV | 13 | | 0.08–0.49 |
| 3 | 15 | 0.34 | 0.08–0.80 | III | 17 | | 0.04–0.31 |

[a]Denotes tributary site.

### *1.2.3 Biological Survey*

Twenty-three sampling stations on the two river systems were surveyed in May, June and October 1980. In addition certain sites were surveyed at monthly intervals during this period. Five replicate Surber samples (see Section 3.3) were taken on each sampling occasion. The results of the survey indicated that the East Allen is, in biological terms, typical of many upland streams and rivers. The fauna is dominated by insects, with molluscs, crustaceans, oligochaetes and platyhelminths also represented. In all 121 taxa were recorded from the Allens during the survey (this number has since increased considerably, partly through improved taxonomic techniques). As expected, the headwaters of the East Allen are relatively poor in species and numbers of individuals, but the fauna develops rapidly and in its lower reaches the East Allen is a moderately good trout fishery. In contrast the West Allen, though initially similar to the East, receives zinc inputs about three kilometres from its source and the invertebrate fauna is markedly affected; some of the survey results are summarised in Figure 1.3. Within the general pattern of reduced species diversity and numbers of individuals at the zinc-polluted sites, the effects of the zinc on particular invertebrate species could clearly be seen. Four species which were common in the East Allen were entirely absent from the mainstream of the West Allen: *Gammarus pulex* (Crustacea), *Ancylus fluviatilis* (Mollusca), *Taeniopteryx nebulosa* (Plecoptera), and *Hydroptila* sp. (Trichoptera). Several other species were absent from many stations on the West Allen, although present

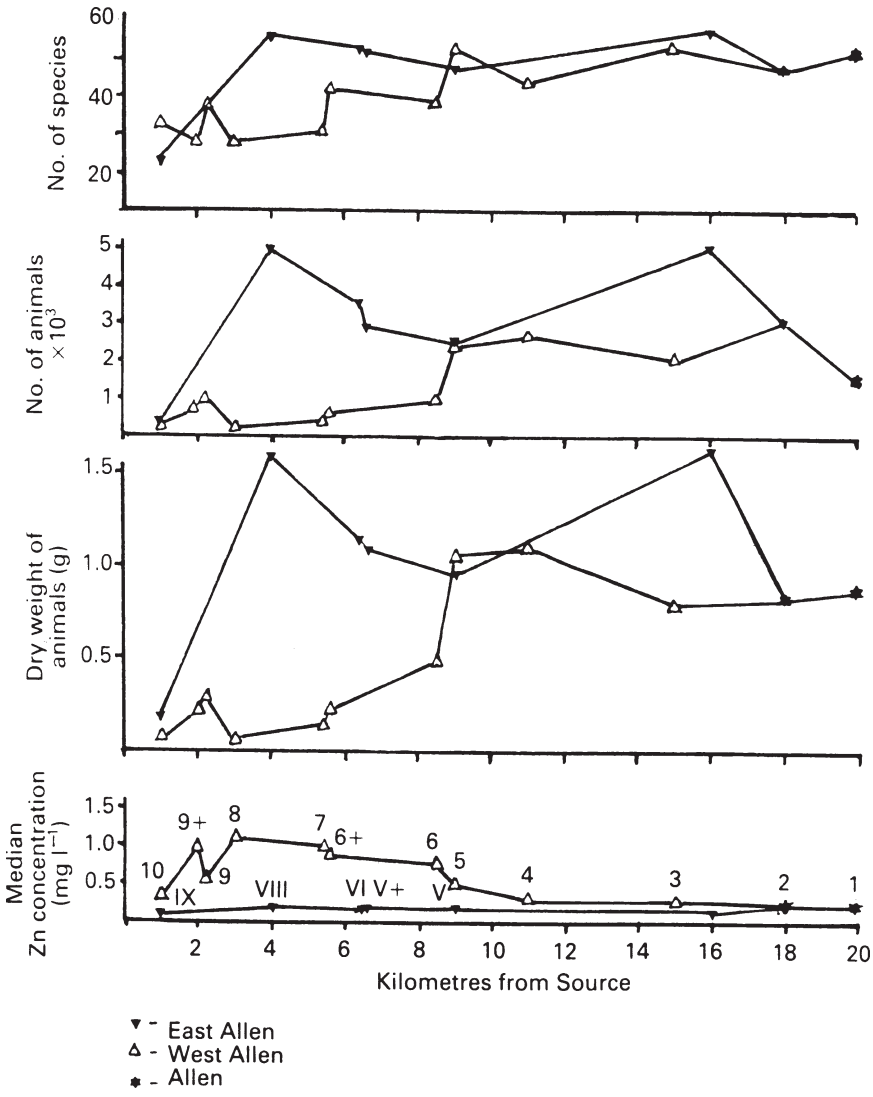



**Figure 1.3** Total number of species, total number of animals and the total dry weight of animals collected from each sampling station during May-October 1980 in the Rivers East Allen, West Allen and Allen (Green, 1984)

in the East (Table 1.3). Species found in both rivers were generally present in greatly reduced numbers in the West Allen; Figure 1.4 shows some data for Plecoptera species, and similar patterns were shown by Ephemeroptera, Trichoptera, Coleoptera and Diptera. Moreover, the survey data indicated that the life cycles of some species were altered in the zinc-polluted stretches. For example, nymphs of the stonefly *Amphinemoura sulcicollis* disappeared

**Table 1.3** Species found along the length of the East Allen, but absent from three or more sites on the mainstream of the West Allen. Data from Green (1984)

| | |
|---|---|
| Plecoptera: | *Leuctra fusca* |
| | *Siphonoperla torrentium* |
| | *Taeniopteryx nebulosa*[a] |
| Ephemeroptera: | *Ecdyonurus venosus* |
| | *Ecdyonurus dispar* |
| | *Baetis muticus* |
| | *Ephemerella ignita* |
| | *Caenis* sp. |
| Trichoptera: | *Hydropsyche instabilis* |
| | *Hydropsyche fulvipes* |
| | *Hydroptila* sp[a] |
| Crustacea: | *Gammarus pulex*[a] |
| Mollusca: | *Ancylus fluviatilis*[a] |

[a]Species absent from all sites on the mainstream West Allen.

from the East Allen in July/August as the mature nymphs emerged from the water as adults. Nymphs of the new generation were abundantly reestablished by October; in the West Allen, however, no new nymphs had appeared by October in the more polluted stretches, perhaps because the peak zinc concentrations tend to occur during the summer and have a particularly marked effect on the early life stages. Possibly the limited populations of this and similarly-affected species in the West Allen are restored later in the year by downstream drift from unpolluted tributaries. The results of some further analyses of the survey data are described in Section 3.4.

### *1.2.4 Zinc Toxicity*

The depletion of the invertebrate fauna in the zinc-polluted stretches may be due to the direct toxic action of the zinc; or may arise as a consequence of, for example, the disappearance of an animal's prey species or some other indirect cause. Green (1984) tested the lethal toxicity of zinc to several invertebrate species under conditions similar to those found in the Allens. One way to measure lethal toxicity is to determine the concentration of the poison which will kill half of a sample of animals within a specific period, such as four days—this value is termed the 96-hour median lethal concentration, or 96 h LC50. Methods of measuring toxicity are discussed in detail in Chapter 4. Table 1.4 shows some results. All of the species tested

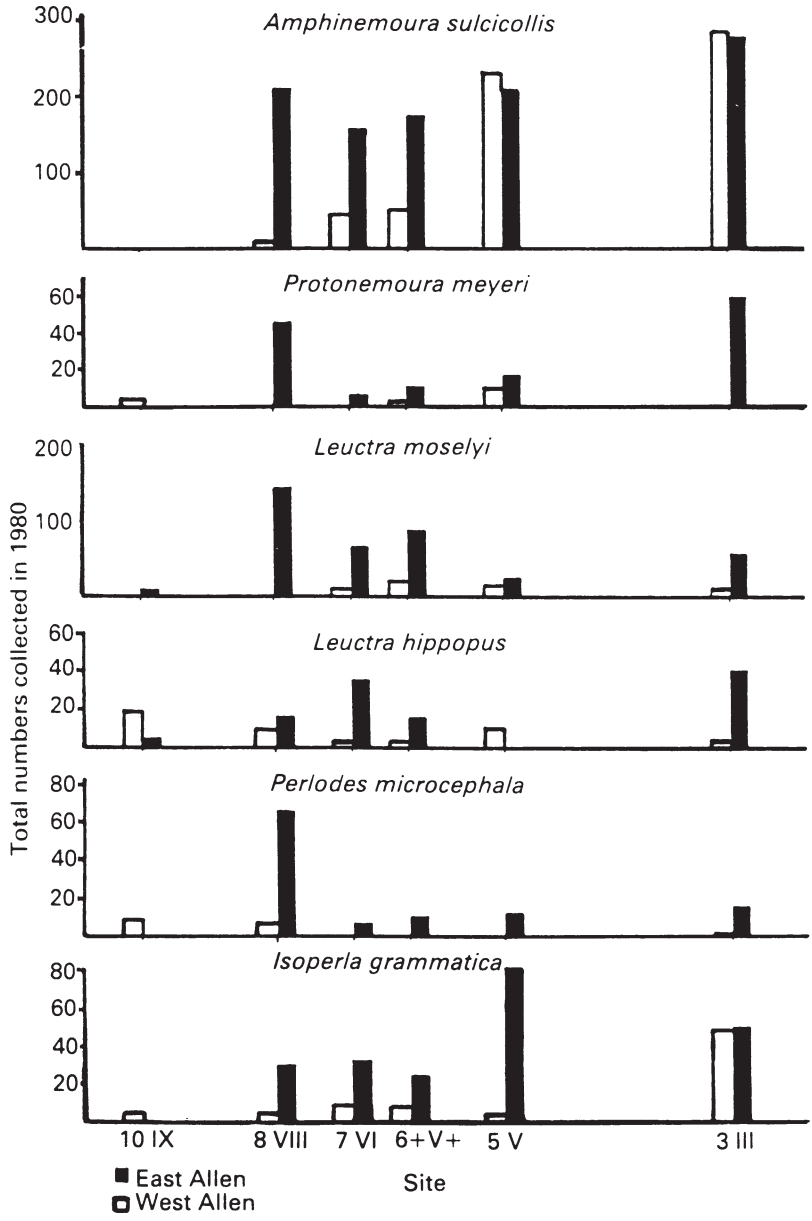



**Figure 1.4** Total numbers of selected species of Plecoptera (stoneflies) from 'equivalent sites' on the Rivers East and West Allen during May-October 1980 (Green, 1984)

**Table 1.4** Toxicity of zinc to some invertebrate species. Data extracted from Green (1984). Where two sets of results are given, the results represent the range of toxicity values recorded in replicate tests carried out at different times

| Species | Result[a] |
|---|---|
| *Gammarus pulex* | 2.0(1.67–2.40) 336 h LC50 0.66 (0.50–0.88) |
| *Baetis rhodani* | 1.3(0.6–2.9) |
| | 31(16.3–58.9) 336 h LC50 16.5 (7.5–36.3) |
| *Rhithrogena semicolorata* | 70(36.8–133) 336 h LC50 52 (34.4–78.5) |
| | 135(71–256) 336 h LC50 68 (45.3–102) |
| *Leuctra moselyi* | 15.7(9.24–26.7) |
| | 55(30.6–99) 336 h LC50 17.5 (4.07–75.3) |
| *Amphinemoura sulcicollis* | 120 h LC50 130 (59.1–286) |
| *Isoperla grammatica* | 90(40.9–198) |
| *Lymnaea peregra* | 2.6(0.8–8.3) |
| *Chloroperla tripunctata* | No mortalities in 144 h at concentrations >360 mg $l^{-1}$ |
| *Deronectes depressus* | No mortalities in 14 h at concentrations >360 mg $l^{-1}$ |
| *Limnephilus* sp. | No mortalities in 288 h at concentrations >360 mg $l^{-1}$ |

[a]96 h LC50 values in mg $l^{-1}$ unless otherwise stated.
Values in parentheses are 95% confidence limits.

were either absent from all or part of the West Allen mainstream, or found only in greatly reduced numbers.

Comparing the zinc levels in the water (Table 1.2) with the values in Table 1.4, three groups of animals can be distinguished. First, those such as *Gammarus pulex* and the snail *Lymnaea peregra,* for which the LC50 values are very close to the zinc concentrations found in the river. It is reasonable to conclude that the river water is rapidly toxic to these species, and that any individuals which found themselves in the polluted stretches would quickly die if they were unable to escape speedily to a less polluted area. Second, there is a group of species which appears to be remarkably resistant to zinc. The stonefly *Chloroperla tripunctata,* a caddis fly of the genus *Limnephilus,* and the beetle *Deronectes depressus* withstood concentrations of zinc greater than 360 mg $l^{-1}$ for up to 12 days without any animals dying at all. This suggests that although the possibility of sublethal toxicity cannot be discounted, it is likely that mechanisms other than the direct effect of zinc on the animals are largely involved. Third, there is an intermediate group for which median lethal zinc concentrations are roughly between 10 and 100 times higher than the zinc levels recorded in the river. For this group, indirect effects may also be involved; however, experience with many poisons and animals, particularly fish, which have been more extensively studied, suggests that sublethal toxicity of zinc is likely to be an important factor in the observed ecological status of these species.

Some species were tested on more than one occasion at different times of the year and the median lethal concentrations varied considerably (Table 1.4). In part, this is probably a reflection of the fact that toxicity often cannot be measured, however it is expressed, with great precision. However, it is possible that some species vary in their susceptibility to zinc at different times of the year, according to the stage of the life cycle which they have reached. The effect seems to be particularly marked in the case of the mayfly *Baetis rhodani*. This feature of the results, along with the various aspects of sublethal toxicity and the nature and extent of the indirect effects of zinc, all need to be further investigated before the effects of the zinc on the receiving water fauna can be fully understood.

### *1.2.5 Some Further Questions*

The investigations of the Allens and other metalliferous rivers show that in each case, the effects of the metal on the receiving water fauna are broadly similar. Generally, it is observed that there is a reduction in the number of species present in the polluted areas, together with a reduction in numbers of individuals of those species which are not eliminated altogether. Certain species appear to be particularly sensitive to heavy metals, others apparently more resistant, and the status of some species with respect to metal pollution appears to vary from one location to another. In other words, there is a perceptible general pattern which is subject to variations in detail. It is important to know to what extent the findings of any particular study are of general application, and to what extent they are restricted to, for example, particular geographic regions, or to waters which have particular physical, chemical or biological characteristics. Therefore it is necessary to consider in more detail the ecotoxicological mechanisms which may underlie the observed effects of the metals. By doing this, it will be possible to illustrate some of the biological questions which may arise out of the investigation of a polluted water.

The role of sublethal toxicity is potentially an important area for further investigation. In the river West Allen, the concentrations of zinc which are lethal to several of the affected species are between 10 and 100 times higher than the zinc levels recorded in the water, that is, within the range where sublethal toxicity may be expected. The measurement of sublethal toxicity gives rise to a number of technical and conceptual difficulties, some of which are discussed in detail in Chapter 4. However, some experiments on sublethal effects of zinc on Limnephilid caddis larvae (which are generally absent from the West Allen) were reported by Abel and Green (1981). These experiments showed that feeding rates of the animals, expressed as the quantity of food consumed per day, were reduced by 30% in animals exposed to the levels of zinc consistently recorded in the river water. The same authors claimed that activity levels, expressed as the percentage of time spent swimming by

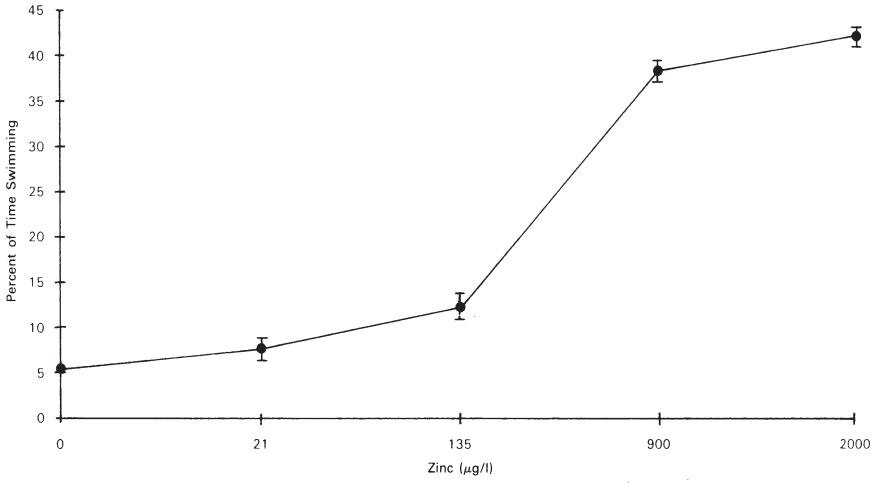



**Figure 1.5** Percentage of time spent actively swimming by *Gammarus pulex* exposed to different concentrations of zinc. Animals were observed via a remotely-controlled video camera to minimise disturbance

animals in an observation chamber, were considerably increased by exposure to the ambient zinc levels of the West Allen. These findings (for the Isopod crustacean *G. pulex*) were confirmed by Austin (1992) using a more sophisticated observation procedure based on analysis of remotely-controlled videotaped observations of the animals under similar conditions (Figure 1.5); and Crane (1995) also found that the feeding rate of *G. pulex* was reduced by 50% at a zinc concentration of 0.5 mg $l^{-1}$.

Whatever the underlying mechanism, a combination of increased activity combined with decreased feeding is potentially of profound ecological significance, leading for example to decreased growth rate and reproductive capacity, and may be associated with the ecological effects of the pollutants on the aquatic fauna. The increased activity levels may represent avoidance reactions on the part of the animals—it has been well documented that fish and invertebrates show measurable avoidance reactions to pollutants in laboratory choice chambers. In the field, hyperactive invertebrates are likely to suffer from increased rates of downstream drift, leading to depopulation of polluted stretches of river. Gilhooley (1988) showed this effect in artificial stream channels at the concentrations of zinc typically found in metalliferous rivers. Similar effects have been recorded in artificially-acidified streams in experiments to assess the ecological impact of acidification of waters (Hall *et al.,* 1980; Ormerod *et al.,* 1987).

Another aspect of sublethal toxicity is the possible accumulation of metals in the animal tissues. Animals may survive exposure to low levels of pollutants without apparent effect, but may continue to accumulate metal from solution, by ingestion

of or contact with particulates, or from their food, until harmful levels of the pollutant in the tissues are reached. These considerations relate not only to heavy metals but also to refractory organic pollutants including certain pesticides. One reason for concern about this phenomenon is that animal tissues containing heavy metals (and other pollutants) may cause harm to consumer organisms—fish consumed by humans are an obvious example—but there are obvious concerns relating to the passage of pollutants up the food chain which may affect all predators. If, however, we consider simply one species without regard to these aspects, we still need to know whether the level of metal in the tissues is harmful to the organism itself. For reasons explained in Chapter 4, this is not always an easy question to answer; however, the presence of the class of inducible metal-binding proteins known as metallothioneins (see Chapter 4) may be used as an indication that the level of metal in the organism under study is abnormally high. Metallothioneins have been tentatively identified as being present in the tissues of some invertebrates living in metalliferous rivers in the North Pennine Orefield (see Figure 4.18).

The effects of other poisons, and of environmental factors which modify toxicity, should also be considered. In many of the rivers referred to above, more than one metal was present in significant quantities. In the zinc-polluted river Nent in the North Pennine Orefield, substantial inputs of organic farm waste were recorded (Armitage, 1980), which may modify the toxicity of the heavy metals present (see Chapter 4). Clearly, some of the differences observed between different rivers may be attributable to such confounding factors. In addition, the toxicity of heavy metals is greatly influenced by levels of calcium, and indeed calcium itself may be a limiting factor; while it may be tempting to ascribe the absence of certain species (particularly molluscs and crustaceans) to the presence of zinc or other heavy metals, they may in fact be limited by levels of calcium lower than 10 mg $l^{-1}$ (Edwards *et al.,* 1978). Even levels of sodium may be limiting to some species (Sutcliffe, 1967, 1983). It is particularly important to take account of such factors in upland, mineral-poor waters where faunistic differences may be too easily and simplistically ascribed to pollution effects whereas in fact they may reflect the effects of natural influences.

The partitioning of the zinc in the river environment is likely to have a great bearing on the correct interpretation of toxicological data. The precise form in which the zinc is present can have a large influence on its toxicity (see Section 4.2). Metals may exist, in the aquatic environment, in dissolved, colloidal or particulate form; in two or more different oxidation states; as simple ions, inorganic complexes or organometal complexes. The biological and toxic properties of these different forms may vary greatly. In laboratory tests, the metal is normally presented to the animals in dissolved form, but it is almost certainly never entirely in the form of simple ions. In the river, the measurements made of 'dissolved' metals are, more accurately, of that fraction of the metals which can pass through a 0.45 μm filter, which is not necessarily the same thing. Another aspect of partitioning is

that many animals burrow into the substratum, and may be exposed to levels of zinc in the substratum which are much higher than those in the overlying water. Green (1984) devised an apparatus for sampling the interstitial water without contamination with overlying water, and in the Allens found zinc levels in the interstitial water up to 25 times higher than those in the water flowing over the substratum. All of these factors complicate the interpretation of toxicological data and their application to the situation in the field.

Finally, the effects of the pollutant on the decomposer organisms of the river may be considered. The productivity of many aquatic ecosystems, particularly rivers, is sustained by the input of allochthonous organic matter (that is, organic material from outside the river) such as dead leaves. Decomposer organisms—bacteria and fungi especially—are thus of great importance in making available to the river fauna the major energy source represented by detritus. Indeed it is likely that most aquatic animals, like animals generally, cannot readily digest plant material unaided by microorganisms. An invertebrate may appear to be eating a dead leaf, but in reality is probably obtaining its nutrition from the bacteria, fungi, protozoa and microinvertebrates which have colonised the leaf and processed it into a form which is more accessible to detritivorous macroinvertebrates. Zinc is known as a fairly potent bactericide and fungicide. Possibly, therefore, one effect of the zinc is to interfere with the processing of detritus by decomposer organisms, thus depriving the invertebrates of a major food source and being indirectly responsible for the depletion of the fauna. Similar arguments have been adduced in relation to the acidification of surface waters (Haines, 1981; Howells, 1990; see Chapter 2). However, Chappell and Goulder (1994) studied the activity of extracellular microbial enzymes in the rivers East and West Allen, and found no evidence of reduced activity in the zinc-polluted river.

These and similar questions will be recurring themes in the following chapters. Even an apparently simple and straightforward case of a river polluted with a single toxic agent can clearly give rise to some questions which are difficult to answer. It is important, however, to understand the mechanisms by which the observed effects of the pollution are brought about, because this understanding may lead us to the means by which the effects can be remedied or at least ameliorated. The next case study shows that with a suitable combination of field and laboratory studies, detailed investigation of the effects of pollutants can provide an empirical basis for tackling practical problems relating to the control of pollution and the management of polluted waters.

## 1.3 Toxicity and the Status of Fisheries

Our second case study illustrates the way in which toxicological data obtained from laboratory investigations can be combined with chemical and biological data from field surveys into a predictive model which suggests specific measures to

protect or improve the status of fisheries in polluted waters. The example is particularly instructive because historically, toxicological research has been dominated by the measurement of lethal toxicity. There is, therefore, a widely-held view that a great deal of toxicological research is misdirected, irrelevant or of limited value in the actual management of polluted waters, since the most intractable problems in practice arise from sublethal, rather than lethal, toxic effects. The following example shows that this view is based on an inadequate understanding of the applications of toxicological data.

The account is based upon the application by Alabaster *et al.* (1972) of the results of a long series of investigations carried out by what was then the Water Pollution Research Laboratory, a Government establishment in Stevenage, UK. These investigations were based upon the work of a substantial number of people carried out over a period of nearly 20 years. The results can be synthesised into an empirical relationship between the presence of certain common toxic pollutants and the ecological status of aquatic communities which can be used for specific management purposes. For example, when several pollutants are present, it allows the identification of those which are responsible for the greatest adverse effects, so that pollution control measures can be selectively directed towards those pollutants whose removal would lead to the greatest improvement. It also allows predictions to be made of the likely effects of additional pollution, or of physical changes in the receiving water environment.

In the more heavily polluted rivers of Britain and of similar industrialised countries, the most abundant toxic pollutants are copper, zinc, phenol, cyanides and ammonia. The toxicity of these poisons to the rainbow trout, *Salmo gairdneri,* was first studied in great detail. The rainbow trout was chosen because it is a widely-available species, is amenable to life in the laboratory, and is a fish of considerable commercial importance. Consequently a great deal is known about many aspects of its biology. Additionally, it is sensitive to most toxic pollutants and reacts more quickly than most species to adverse environmental conditions. Within a few years, the lethal toxicity of the common pollutants to this species was reliably determined. The effects of common environmental variables on pollutant toxicity were also studied. In particular temperature, water hardness, pH and dissolved oxygen concentration were found to have significant effects on the toxicity of many pollutants. Finally, methods were devised for the study of the effects of fluctuating concentrations of poisons, and for determining the toxicity of poisons in different combinations of varying composition. Much of this work is described in greater detail in Chapter 4.

A crucial idea in the development of this approach is that of the unit of toxicity, or toxic unit. A toxic unit was defined as the concentration of a pollutant which would kill half of a sample of rainbow trout in 48 hours (i.e. the 48-hour median lethal concentration or 48 h LC50 as described in Chapter 4). Because trout react

quickly to most poisons at lethal concentrations, the 48 h LC50 is close to the lethal threshold concentration, that is, the concentration which would just kill half the sample of fish during an exposure of indefinite duration. A few simple examples will illustrate how toxic units are used.

Assume that, under a certain set of conditions, it is found that the concentration of zinc which will kill half the trout in 48 hours is 2 mg $l^{-1}$. Under these conditions, one toxic unit of zinc is 2 mg $l^{-1}$. Now, assume that the experiment is repeated under similar conditions, but in water which has a greater degree of hardness. It may be found that the 48 h LC50 for zinc is now 10 mg $l^{-1}$. Under these new conditions, one toxic unit of zinc is 10 mg $l^{-1}$, and 2 mg $l^{-1}$ zinc is now equivalent to only 0.2 toxic units. Now assume that we wish to measure the toxicity of copper to trout. In soft water, one toxic unit of copper may be equal to 0.5 mg $l^{-1}$ and in hard water 2.5 mg $l^{-1}$. Now assume that we wish to know whether, in the soft water, fish would survive in the presence of 1.5 mg $l^{-1}$ zinc together with 0.25 mg $l^{-1}$ copper. In this case, 1.5 mg $l^{-1}$ zinc equals 0.75 toxic units, and 0.25 mg $l^{-1}$ copper equals 0.5 toxic units. The total number of toxic units is therefore 1.25, whereas we know that by definition, 1 toxic unit will kill half the fish in 48 hours. Therefore we would expect that significantly more than half of the fish would die within 48 hours. In the hard water, however, zinc would contribute 0.15 toxic units, and copper 0.1 toxic units, a total of only 0.25 toxic units. We might therefore expect that the majority of fish would survive these concentrations of zinc and copper in the hard water. These expectations assume, of course, that the effect of the poisons in combination is neither more nor less than the sum of their individual effects. This point has been extensively investigated (see Section 4.2.3) and fortunately, with some important exceptions, appears to be generally true for this group of pollutants. It is not true, however, for all combinations of pollutants. The toxicity of mixtures of poisons is discussed more fully in Chapter 4.

The next stage is to use this technique to estimate the expected toxicity of polluted river water to fish, and to compare the results with the observed status of fish populations. It is not difficult to measure the concentrations of zinc, copper, phenol, cyanides and ammonia in samples of river water. The temperature, pH, hardness and dissolved oxygen concentration of the water at the time of sampling must also be determined. Using these data, the measured concentrations of each pollutant, in mg $l^{-1}$, can be converted into toxic units. Summing these 'fractional toxicities' will give a measure, in toxic units, of the total toxicity of the river water. Obviously, the toxicity of the river water will vary with time, depending upon the amount of effluent being discharged, the quantity of water available for dilution, and the prevailing environmental factors. It would not be sensible to try to associate the observed status of the fish population of a stretch of river with the toxicity of the water on any single occasion. It is necessary to take a relatively large number of samples over a reasonably long period of time, ideally a whole

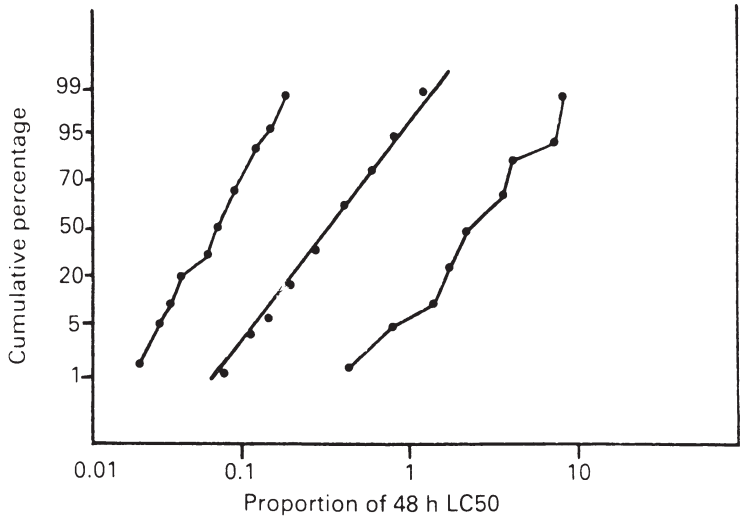



**Figure 1.6** Distributions of toxicity calculated by the method of Alabaster *et al.* (1972). The line on the left is typical of a station which supports fish; the line on the right represents a fishless station; and the central line is reconstructed from the boundary distribution of toxicity between fishless and fish-supporting stations in the River Trent catchment. See text for explanation

year, and to calculate the toxicity of the water on each occasion. A graph of the kind shown in Figure 1.6 then is constructed. Each sampling station investigated generates one line on the graph.

The line on the left of Figure 1.6 represents the distribution of toxicity over time at a sampling station which sustains a fish population. It is constructed by plotting the cumulative frequency with which a given level of toxicity occurs in a number of samples taken from the site under investigations over a period of time. It is convenient to plot the graph using a logarithmic scale for the toxicity values and a probability scale for the y-axis. Thus, in the example shown, on 5% of sampling occasions the toxicity was equal to or less than 0.04 toxic units. On 20% of occasions, it was equal to or less than 0.055 toxic units, on 50% of occasions it was equal to or less than 0.08 toxic units, and so on. The line on the right of Figure 1.6 represents toxicity distribution typical of a fishless station. The increased toxicity of the water is represented by a shift of the line towards the right. Plotting a large number of these lines (one for each of the sampling stations studied) showed that the lines fell in various places on the diagram, depending upon the distributions of toxicity recorded at each station. However, lines corresponding to stations where fish were normally present were grouped to the left of the diagram, and lines corresponding to stations which were normally fishless were grouped to the right; a line can be constructed through the

**Table 1.5** Coordinates of approximate boundary distribution of 48 h LC50 between fishless and fish-supporting waters in the River Trent catchment area, England. See Figure 1.5 and text for explanation. Data from Alabaster *et al.* (1972)

| Per cent probability | 1 | 5 | 10 | 25 | 50 | 75 | 90 | 95 | 99 |
|---|---|---|---|---|---|---|---|---|---|
| Sum of proportions of LC50 | 0.07 | 0.10 | 0.13 | 0.18 | 0.28 | 0.41 | 0.60 | 0.73 | 1.1 |

narrow zone of demarcation between the two groups. From this line it is possible to read off the 'coordinates of boundary distribution between fishless and fish-supporting waters' (Alabaster *et al.,* 1972). The central line in Figure 1.6 has been reconstructed from the coordinates determined by Alabaster *et al.* (1972) for sampling stations in the catchment area of the River Trent. The values of the coordinates are shown in Table 1.5.

These data show that a water will only sustain a fish population if for at least 50% of the time the toxicity of the water is less than 0.28 toxic units; and if for 90% of the time its toxicity is less than 0.6 toxic units, for 95% of the time less than 0.73 toxic units, and so on. Where the toxicity of the water exceeds 1.0 toxic unit for as little as 2 or 3% of the time, fish will generally be absent. The simplest way to determine whether or not a sampling station is likely to support fish is to draw the line of toxicity distribution. If the line, or any substantial portion of the line, falls to the right of the boundary line, the water will be unlikely to support fish. The further to the left the line falls, the smaller is the likely effect of toxic pollution on the fish population. Where the line falls to the left of the boundary but close to it, the fishery may be of only marginal quality.

Although this relationship between toxicity and fishery status is purely empirical, it has considerable potential value in water management, as the following examples show. Assume that a stretch of river is fishless, and that we wish to know the most effective means of re-establishing a fish population. Chemical analysis of the water on a number of occasions reveals that several pollutants are present, and that the dissolved oxygen concentrations are generally low. Many poisons are more toxic at low dissolved oxygen levels. Could the fishery be restored by increasing dissolved oxygen levels, or is the removal of specific toxic substances required? In the toxicity distribution represented by the line on the right of Figure 1.6, the toxicity has been calculated using the dissolved oxygen calculations measured on each sampling occasion. These values can be recalculated, on the assumption that the dissolved oxygen concentration will be increased to any desired level. A new line can therefore be constructed using the hypothetical oxygen values, and this line will be displaced to the left.

If the displacement carries the line beyond the boundary distribution, it is expected that a fishery could be re-established by increasing the levels of dissolved oxygen in the water. This could be achieved relatively easily and cheaply, for example by imposing stricter controls on the discharge of sewage or organic wastes which cause reduced dissolved oxygen levels; by upgrading sewage treatment plants; or by utilising one of the various methods available for aerating rivers such as constructing a weir. (Any of these strategies are also likely to reduce problems associated with ammonia levels, since ammonia is produced in significant quantities by the decay of organic matter.)

If, however, the displacement does not carry the line beyond the boundary distribution, the desired effect is likely to be achieved only by the removal of specific toxic substances, which may be more difficult and expensive. A similar line of reasoning can be applied in situations where, for example, it is proposed to site a new discharge on a river and we wish to predict the likely effect of the new discharge. The new discharge may contain additional toxic pollutants, and/or may alter the temperature, dissolved oxygen or other environmental characteristics of the receiving water. If the effect of the proposed new conditions is significantly to shift the existing distribution of toxicity towards the boundary conditions, or to carry the existing distribution beyond the boundary conditions, the decision may well be made that the proposed new discharge is unacceptable, or should be subjected to more rigorous control.

A further application of this technique is the determination of the relative contributions of each of several pollutants to the overall toxicity of the water. It is frequently the case that several pollutants are present in significant quantities, but chemical analysis alone cannot reveal which of them is exerting the most serious adverse effect on the biota. If the most significant pollutants can be identified, specific control measures may be directed against those, rather than against pollutants whose biological impact may be small, thus affording greater efficiency in the allocation of resources. Alternatively, we may wish to know whether the addition of a new pollutant, or an increase in the expected concentration of an existing pollutant, is likely to have any serious effect. Since the fractional toxicities of each pollutant are initially determined separately, plotting them in the manner shown in Figure 1.7 allows these questions to be answered.

In one example given by Alabaster *et al.* (1972), the toxicity distribution of ammonia alone was plotted, giving a line similar to that shown on the left of Figure 1.7. A second line, showing the toxicity distribution of metals and ammonia combined, displaced the line significantly to the right. This indicates that metals were contributing substantially to the overall toxicity of the water. A third line, showing the effect of including cyanide in the calculation, similarly indicated a smaller, but substantial contribution from cyanide. Phenol, however, contributed little to the overall toxicity, although it was present in the water; its inclusion in the calculation gave only a very slight displacement to the right. Using the same method, the authors were able

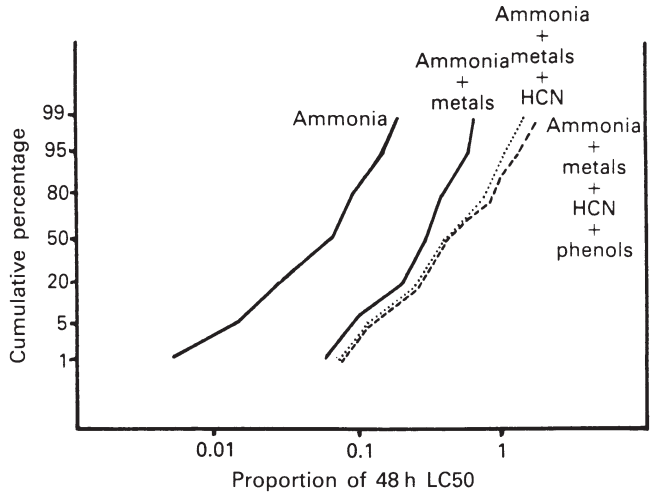



**Figure 1.7** Contribution to toxicity from ammonia, metals, hydrogen cyanide and phenols at a heavy-polluted site in the River Trent catchment (Alabaster *et al.*, 1972)

to show that among the metals present, copper and zinc were identifiable as major pollutants in the sites they studied, whereas nickel, chromium and cadmium contributed little to the overall toxicity of the water. This was in spite of the fact that nickel alone formed 20% of the total metal present in chemical analyses. However, nickel and, in particular, cadmium are known to be very slow-acting poisons (see Chapter 4). The model in use relies upon the 48-h LC50 as the definition of the toxic unit, and this is only satisfactory if the 48-h LC50 is a good approximation of the lethal threshold concentration. Since this is not the case for nickel and cadmium, the calculations were revised using the lethal threshold concentration as the definition of the toxic unit. Under these circumstances, the results indicated that nickel and cadmium (but not, in the cases studied, chromium) were significant pollutants in some instances.

The synthesis of data from field and laboratory studies into the potentially useful model for the management of polluted waters has many advantages. Toxicological data, particularly those derived from the study of lethal toxicity, can rapidly be accumulated and replicated under controlled conditions, but are difficult to apply to real situations in the field because our knowledge of ecotoxicological mechanisms is inadequate. We might expect, for example, that if the level of pollution in the water was equal to the 48-h LC50, that half or more of the fish would die and that sublethal toxicity and mechanisms involving the relationships of fish with other members of the aquatic community might be sufficient to account for the total absence of fish from a particular site. The empirical finding that fish are absent if the toxicity exceeds 1.0 toxic unit for 1% of the time, or exceeds 0.07 toxic units for 99% of the time (Table

1.5) is, however, more directly useful to us. It does not actually matter, in the day-to-day management of the water, why exactly this happens—whether, for example, it arises through sublethal toxicity, avoidance by the fish of specific adverse conditions which they have detected, or because the pollutants are adversely affecting the food organisms of the fish. Knowledge of ecotoxicological mechanisms is ultimately valuable because it allows the interpretation and application of data in the frequent circumstances that decisions have to be made on the basis of incomplete information. In these same circumstances, purely empirical relationships are also useful for precisely the same reasons.

An alternative approach would be to rely entirely upon data from field surveys. In principle, any particular management decision could be formulated with reference to experience of what has happened previously in a similar situation elsewhere. In practice, the database of previous information which would be required is so vast that even if it existed, the task of collating it and analysing it in such a way as to extract from it the specific information required would be impossible. In any case, new situations are constantly arising; and water bodies differ so widely in their physical, chemical and biological characteristics that purely anecdotal evidence is of limited predictive value without some attempt to understand and apply fundamental principles. In practice, the control and amelioration of the problems raised by water pollution are best achieved by a combination of approaches—field based and laboratory based, empirical and fundamental.

# 2

# Sources and Effects of Water Pollutants

There are hundreds, perhaps thousands of pollutants whose effects are of actual or potential concern. Their numbers increase annually, as new compounds and formulations are synthesised. A substantial minority of these find commercial applications and become significant pollutants of water during their manufacture and in subsequent use. It is clearly impossible within the scope of a short book to discuss all of these in detail; instead, the sources and effects of broad categories of pollutants will be discussed in general terms, and where appropriate reference will be made to further sources of information.

## 2.1 The Nature of Effluents

Water pollution is most commonly associated with the discharge of effluents from sewers or sewage treatment plants, drains and factories. Outfalls of this kind are known as 'point-source discharges'. Most cases of accidental, negligent or illegal discharge are also from point sources. The concentration of pollutant in the receiving water is initially high, decreasing as the distance from the point of discharge increases. The effects of the pollution are therefore frequently easy to observe. Some of the more serious forms of pollution arise, however, from 'diffuse' sources, that is the pollutant does not enter the water from a single point. For example, in agricultural areas, surface water runoff and groundwater infiltration into lakes and rivers can introduce plant nutrients (from fertilisers) and pesticides in substantial quantities to water bodies. The effects of pollution from diffuse sources can be serious, but are often less immediately obvious than those from point sources as there is no adjacent unpolluted area with which comparisons may

**Table 2.1** Analysis of sewage effluents after primary and secondary treatment. Figures given are in $mg\,l^{-1}$, and are the range of values found at four different treatment works, as summarised by Bond and Straub (1974). Treatment plants operating under adverse conditions yield higher values than those shown here, but values at the upper end of the ranges shown would in many countries exceed modern effluent standards

| Analysis | Range |
|---|---|
| Total solids | 640–1167 |
| Suspended solids | 15–51 |
| Biological oxygen demand | 2–70 |
| Chemical oxygen demand | 31–155 |
| Organic carbon | 13–20 |
| Anionic detergents | 0.75–1.4 |
| Ammonia | 1.9–22 |
| Nitrate | 0.25–38 |
| Nitrite | 0.2–1.8 |
| Chloride | 69–300 |
| Sulphate | 61–270 |
| Phosphate | 6.2–9.6 |
| Sodium | 144–243 |
| Potassium | 20–26 |

be made. Many pollutants also enter water through fallout from the atmosphere. Historically, control and prevention of water pollution have concentrated on point sources as these are more obvious, easily identifiable and in theory easier to regulate at the point of origin. As awareness has increased of the significance of diffuse sources of pollution, control strategies have been under development but are based more on the application of good practices designed to reduce pollutant impact rather than on regulation of specific sources of input (see, for example, MAFF, 1991).

Most effluents are complex mixtures of a large number of different harmful agents. These include toxic substances of many kinds, extreme levels of suspended solids, and dissolved and particulate putrescible organic matter. In addition, many effluents are hot, of extreme pH value, and normally contain high levels of dissolved salts. Detailed compilations of data on the composition of sewage and industrial effluents of many kinds are given by Bond and Straub (1974), and by Sittig (1975). Some representative values for treated sewage effluent are given in Tables 2.1 and 2.2. Most effluents also vary in their strength and composition, on a seasonal, diurnal or even hourly basis. Most sewage treatment plants report regular diurnal peaks and troughs in their output according to patterns of water use. Sometimes storm-water drains are connected to the sewerage system, so the strength of the sewage effluent will vary with rainfall. Alterations in the

**Table 2.2** Levels of heavy metals recorded in the final effluent of a typical sewage treatment plant operating under good conditions. Data from Bond and Straub (1974)

| Metal | Concentration range ($\mu g\,l^{-1}$) | Typical % removal compared to raw sewage |
|---|---|---|
| Zn | 85–190 | 65 |
| Al | 460–550 | 77.5 |
| Fe | 160–290 | 70.5 |
| Cr | 36–70 | 76 |
| Cu | 31–38 | 60.5 |
| Pb | <20 | |
| Ag | 11–12 | 17.3 |
| Cd | <20 | |
| Ni | <10 | |
| Sr | 280–450 | 17.6 |
| B | 240–260 | 13.3 |

strength and composition of sewage also influence the efficiency of the sewage treatment process, so that dilution of the influent does not necessarily cause an improvement in the quality of the effluent. In industrial plants, variations in the quality of the raw materials, or changes in specification of the finished product, will require changes in the operating conditions of the plant and lead to changes in the composition of the effluent. Many industrial processes are 'batch' rather than continuous processes, so that some effluent discharges will be intermittent rather than continuous.

Nevertheless, it is often possible to generalise about the effects of different kinds of effluent on their receiving waters. Broadly speaking, the effects of sewage effluent, kraft pulp mill effluent, coal mine effluent and so on are consistent wherever they occur. However, a detailed understanding of the effects of individual components of an effluent, and of the consequences of variation in the composition of an effluent, is essential for pollution control. Waste-water treatment is expensive, and in order to devise cost-effective treatment processes it is necessary to identify those components of the effluent which cause the greatest damage to the environment. This is because it is usually impossible to devise an economically-feasible process which will be equally effective against all the components of a complex effluent. Frequently, treatment of an effluent to remove one component will exacerbate the problem of removing another; it is notoriously difficult, for example, to treat satisfactorily effluents which contain both cyanides and phenolic compounds, although many basic industrial processes produce just such an effluent. Therefore in order to devise the optimum pollution control strategy it is necessary to study, in the laboratory and in the field, the effects of effluents and of their individual components.

## 2.2 The Environmental Requirements of Aquatic Organisms

The effects of pollutants in aquatic ecosystems cannot be understood without some knowledge of the ecophysiology and basic biology of the aquatic biota. Those aspects which have most bearing on the interaction between water pollutants and aquatic organisms are briefly discussed here. There are many authoritative and readable texts on freshwater ecology which may be recommended to readers who require a fuller treatment. Among these are Hynes' (1970) classic text and more recent volumes such as Moss (1988), Jeffries and Mills (1990), Maitland (1990), Calow and Petts (1992, 1994) and Allan (1995).

In order to survive, a living organism must spend its life in an environment which meets its needs: a suitable physical habitat which provides space, shelter, and a sufficient supply of food, oxygen and other metabolic requirements; and which is not subject to extremes of temperature or other physical variables which lie outside the range which the organism can tolerate. Obviously, different habitats have very different physical characteristics, and organisms have evolved a fascinating array of adaptations which have enabled them to colonise every part of the Earth. To a greater or lesser extent, every living organism is adapted—in its morphology, physiology and behaviour—to the environment it normally inhabits. Some are remarkably specialised, that is they are adapted to specific places and/or modes of life in which they are very successful, but are excluded from living in most habitats. Others are more generalised in their adaptations, perhaps being nowhere particularly abundant but able to survive reasonably well in a wider range of habitats. Few organisms, however, are universally distributed.

Any of a living organism's many individual requirements may be a *limiting factor* preventing the establishment, survival or reproduction of a species in a particular habitat. Aquatic plants, for example, are commonly limited in their distribution and abundance by the availability of nutrients, such as phosphorus or nitrogen. An abundance of phosphorus is of no use to the plant if it has no nitrogen, and vice versa. Further, the nutrients must be present in a form which the plant can use. Photosynthetic plants, of course, also require light, an important limiting factor in most aquatic habitats. The non-photosynthetic flora (fungi, bacteria) are more likely to be influenced by the levels of dissolved or particulate organic material present. Animals, in turn, are greatly influenced by the quality and quantity of the aquatic flora, because many animals rely upon plants for food, shelter, as a repository for eggs, and so on. Animals are also influenced by the physical environment—current speed, nature of the substratum, temperature—but in addition are generally much more susceptible than plants and bacteria to the prevailing levels of oxygen.

Although some aquatic animals are air-breathers, the majority have to obtain their oxygen from water. Oxygen is not very soluble in water, and water is a rather dense and viscous medium. Moving through water requires a great deal of energy expenditure, and therefore a high oxygen consumption. To obtain from the water

the meagre amount of oxygen dissolved therein requires that the respiratory surfaces be moved through the water, or the water be moved over the respiratory surfaces. Further, as the water temperature increases, the solubility of oxygen in water decreases, while the oxygen requirement of the animal actually increases. Thus the survival of animals in water is crucially dependent upon the extent to which their oxygen demand can be matched to the availability of oxygen from the environment.

Alexander (1970) describes elegantly and in detail the respiratory predicament of fishes. A fish at rest in well-oxygenated water ventilates its gills fairly slowly. Respiratory exchange is efficient, and the fish removes most of the oxygen from the water passing over its gills. If, however, the fish ventilates more quickly, as it must for example if it becomes physically active, the water passing over the gills has less time to equilibrate with the blood, and the efficiency of respiratory exchange drops. Thus, in order to double the rate of oxygen uptake, the fish must more than double the amount of water pumped over the gills. Measurements of the ventilatory mechanisms in fish suggest that a resting fish can remove 80% of the oxygen in the water passing over the gills, but this figure drops to about 30% in an actively swimming fish. Therefore, to increase its oxygen uptake by a factor of five, a fish must pump about 15 times as much water per unit of time. The density and viscosity of water are such that the muscles which pump the water over the gills themselves consume a significant proportion of the oxygen obtained from the water. This proportion increases dramatically as the fish's activity increases, or as the temperature rises, or as the dissolved oxygen level in the water falls. Alexander (1970) calculated that a resting fish in water containing 8 ml oxygen per litre might under typical conditions expend 0.025 ml oxygen to work the respiratory muscles, in order to obtain 6ml oxygen. However, if the oxygen concentration in the water fell to 1 ml per litre, the fish would expend 0.25 ml oxygen in order to obtain only 0.3 ml. At this point, the fish is clearly close to asphyxiation and has no ability to engage in physical activity.

Clearly, oxygen is an important limiting factor to fishes. Equally clearly, however, limiting factors interact with one another. An oxygen concentration which is acceptable at 10°C is limiting, even lethal, at 20°C, because as the temperature rises the metabolic rate of the fish also rises; thus most aquatic animals need more oxygen at higher temperatures, but the solubility of oxygen in water decreases with temperature, as does the efficiency of respiratory exchange. Varley (1967) described very simply the interactions of temperature and oxygen and their influence on the distribution patterns of freshwater fishes in Britain. Similar principles are involved among the invertebrates. Nymphs of stoneflies (Plecoptera), for example, are particularly fastidious in their requirements for oxygen, and are absent from waters whose oxygen concentration drops much below saturation value for appreciable periods of time. Ephemeroptera (mayfly) nymphs are a little more

tolerant, but are generally more sensitive to oxygen depletion than Trichoptera (caddis fly) larvae. The amphipod crustacean *Gammarus pulex* and the isopod *Asellus aquaticus* are remarkably similar in their habits and environmental requirements, but above a critical oxygen concentration *Gammarus* predominates, while below that level it tends to be replaced by *Asellus*. The distribution of individual species is often correlated with dissolved oxygen levels in the water. Indeed, the response of invertebrates to the prevailing levels of dissolved oxygen is so well known that their distribution patterns can be used as indicators of the prevailing environmental conditions (see Chapter 3).

Other correlates with distribution patterns can be found. An excellent example is Maitland's (1980) study of mayfly (Ephemeroptera) species in the British Isles. There are 47 species of Ephemeroptera recorded in the British Isles, a rather smaller number of species than is found in continental Europe. This illustrates an important general biogeographical principle; the British Isles, like other islands situated offshore from the continental land mass, has a relatively poor fauna and flora. Second, there is a distinct pattern of distribution in relation to altitude, each species having its own upper and lower limit of distribution, some being confined to upland areas and others to lowland waters. Altitude, of course, will tend itself to be correlated with other factors, such as temperature, and a variety of physical and chemical characteristics of the water, so in fact it would be expected that several correlations may be found between physico-chemical parameters and the presence or absence of species. At its simplest level, Maitland's (1980) study identified four specific habitat types which would *not* be expected to sustain mayfly nymphs: small water bodies liable to desiccation; water bodies susceptible to saline intrusion; nutrient-poor upland waters in exposed areas; and lowland waters subject to eutrophication or pollution.

The patterns of distribution and abundance of organisms cannot, of course, be explained solely in ecophysiological terms. A habitat may be perfectly adequate, physically and chemically, for a herbivorous animal, but if the habitat is not suitable for the animal's food plants the herbivore will be absent. Where a habitat is marginally acceptable to a species, the organism may nevertheless be absent because another species, with similar requirements, is better adapted to those conditions and competes more successfully for the available resources. Some organisms are absent from particular habitats not because the habitats are unsuitable, but simply because the species concerned have so far failed to overcome a geographical barrier which prevents their spread. In other words, the observed patterns of distribution and abundance of species are the net result of a complex interaction of physical, chemical and biological factors. Patterns of distribution in relation to these factors are, however, discernible and with an adequate database and suitable methods of analysis can be formed into very useful tools for assessing the impact of pollution on a habitat. The type of study represented by Maitland (1980) was developed into

RIVPACS (River Invertebrate Prediction and Classification System), which is now widely used in pollution monitoring. RIVPACS is described more fully in Chapter 3. Even with simple methods of analysis, within a habitat it is frequently possible to observe a zonation pattern in the distribution of species in response to directional changes in the physical environment. The zones may be sharply delineated, as on a steeply-sloping seashore, or they may merge gradually one into another, as is more typically found along the length of a river (Hawkes, 1975; Hynes, 1970). Pollution may directly influence one or more components of the dynamic equilibrium of physical, chemical and biological phenomena which give an ecological community its characteristics. The influence of the pollution will extend even to organisms which are not directly susceptible, as the system accommodates itself to the new conditions. Indeed, it is frequently possible to observe consistent zonation patterns in the biological community which occur in response to sources of pollution. The response to organic pollution in rivers, for example, is particularly well known.

## 2.3 Organic Pollution

The discharge of excessive quantities of organic matter is undoubtedly the oldest, and even today the most widespread form of water pollution. Its significance to human health is discussed in Chapter 5; the present discussion is concerned with its effect on the receiving waters.

The major sources of organic pollution are sewage and domestic wastes; agriculture (especially runoff from inadequately stored animal wastes and silage); various forms of food processing and manufacture; and numerous industries involving the processing of natural materials such as textile and paper manufacture. Most organic waste waters contain a high proportion of suspended matter, and in part their effects on the receiving water are similar to those of other forms of suspended solid (see Section 2.8). However, the most important consequences of organic pollution can be traced to its effect on the dissolved oxygen concentration in the water and sediments. In an unpolluted water, the relatively small amount of dead organic matter is readily assimilated by the fauna and flora. Some is consumed by detritivorous animals and incorporated into their biomass. The remainder is decomposed by bacteria and fungi, which are themselves consumed by organisms at higher trophic levels. The activity of microorganisms results in the breakdown of complex organic molecules to simple, inorganic substances, such as phosphate and nitrate, carbon dioxide and water. During these metabolic processes, oxygen is consumed. However, where the organic load is light, the oxygen removed from the water is readily replaced by photosynthesis and by re-aeration from the atmosphere.

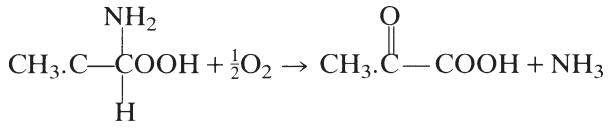
Where the input of organic material exceeds the capacity of the system to assimilate it, a number of changes take place. How far the sequence of

**Table 2.3** Approximate analysis of the organic component of sewage (after Higgins and Burns, 1975)

| | |
|---|---|
| Lipids | 30% |
| Amino acids, starch, glycoproteins | 8% |
| Hemicellulose | 3% |
| Cellulose | 4% |
| Lignin | 6% |
| Protein | 25% |
| Alcohol-soluble fraction | 3% |
| Ash | 21% |

changes proceeds depends upon the severity of the organic load and the physical characteristics of the receiving water. Initially, the enhanced level of organic matter will stimulate increased activity of the aerobic decomposer organisms. When their rate of oxygen consumption exceeds the rate of re-aeration of the water, the dissolved oxygen concentration in the water will fall. This alone may be sufficient, as argued earlier, to eliminate some species, which may or may not be replaced by others with less rigorous demands for oxygen. If the drop in oxygen concentration is very severe, the aerobic decomposers themselves will no longer be able to function, and anaerobic organisms will become predominant.

The biochemical reactions involved in the breakdown of organic matter, and the microorganisms involved, are described in general terms by Dugan (1972) and by Higgins and Burns (1975). The composition of organic waste varies according to its source, and in particular according to the relative abundance of material of plant, animal or microbial origin. Typically, however, the composition of organic waste is approximately as shown in Table 2.3. Most effluents, of course, also contain other materials, in particular toxic matter (see Tables 2.1 and 2.2), derived from various sources. To illustrate the effects of the breakdown of organic matter on the receiving water, proteins may be used as an example. The first stage of decomposition of proteins is usually their breakdown by hydrolysis to their constituent amino acids. A typical amino acid is alanine, and its breakdown under aerobic conditions may be summarised:



Alanine Pyruvic acid Ammonia

Pyruvic acid is an important substance in the metabolism of most living organisms. It is produced in normal metabolism during the glycolytic (anaerobic) phase of the breakdown of carbohydrates and, as in this case, from the breakdown of excess

amino acids. In aerobic organisms, the pyruvate enters the citric acid cycle, the primary means by which compounds are broken down to release energy, carbon dioxide and water. Under aerobic conditions, therefore, proteins will be broken down ultimately to these relatively innocuous compounds, while providing a source of metabolic energy for the organisms responsible for the catabolism. Ammonia is also a common end product of the metabolism of nitrogenous compounds (such as amino acids) and in aquatic organisms is generally excreted as such. Normally, the ammonia diffuses rapidly into the environment, but where the level of organic enrichment is high, it can create difficulties for living organisms as it is very toxic (see Section 2.7.2). Typically, therefore, organic wastes contain high levels of ammonia; the eventual fate of the ammonia is very relevant to the effects of water pollution and is discussed below.

Under anaerobic conditions, the breakdown of amino acids takes place through different metabolic pathways. Some amino acids, such as cysteine, contain sulphur as well as nitrogen, and its breakdown is used as an example in the following sequence of reactions which are catalysed by acid-producing and methanogenic bacteria:

$$4CH_3H_7O_2NS + 8H_2O \rightarrow 4CH_3COOH + 4CO_2 + 4NH_3 + 4H_2S + 8H$$
$$4CH_3COOH + 8H \rightarrow 5CH_4 + 3CO_2 + 2H_2O.$$

In this case, the products of decomposition include (in addition to ammonia, carbon dioxide and water) acetic acid, hydrogen sulphide and methane. These compounds are very toxic to most forms of aquatic life and, in addition, they are aesthetically undesirable by virtue of their unpleasant odours.

The fate of the ammonia largely depends upon the level of oxygen present. Under aerobic conditions, nitrifying bacteria predominate and the ammonia is converted to nitrite (e.g. by bacteria of the genus *Nitrosomonas*) and subsequently to nitrate (e.g. by *Nitrobacter* spp.). Thus the toxic ammonia is oxidised to the less toxic nitrite and to the relatively innocuous nitrate. Since, however, both ammonia and nitrate are important plant nutrients, problems related to eutrophication can arise as a consequence of organic inputs to water (see Section 2.4). Under anaerobic conditions, denitrification of the nitrate typically takes place under the influence of other bacteria such as *Thiobacillus denitrificans* and some *Pseudomonas* species. These cause the reduction of nitrate to elemental nitrogen, which readily displaces the less-soluble oxygen from solution and contributes still further to the deoxygenation of the water. The processes are most easily understood by reference to Figure 2.1 which summarises the nitrogen cycle in the aquatic environment. Note that the gaseous phases of the cycle are usually of limited significance in the aquatic environment.

These chemical changes, combined with the blanketing effect of fine organic particles on the substratum, lead to the deoxygenation of the water

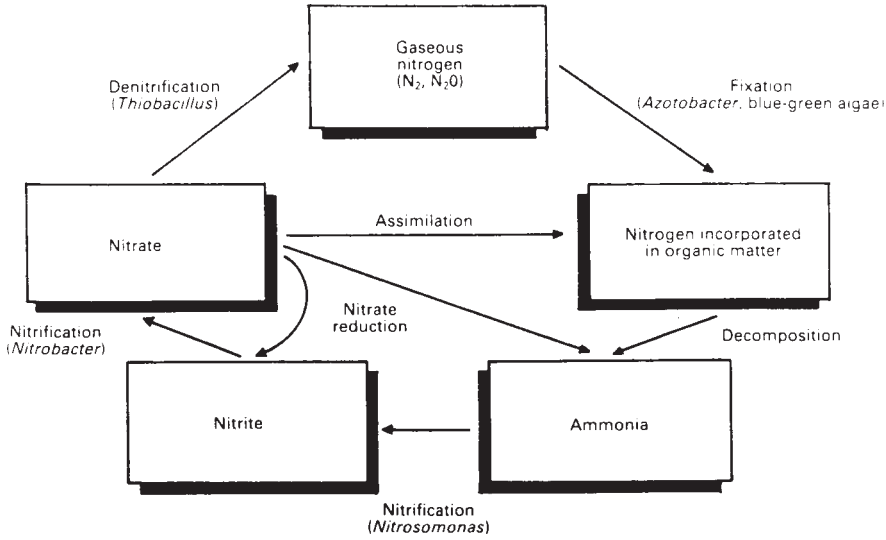



**Figure 2.1** Summary of the nitrogen cycle in fresh-water ecosystems

and substratum, and readily bring about profound changes in the fauna and flora of the receiving water. In severe cases, animal life may be completely eliminated. Hynes (1960) summarised in diagrammatic form the changes which typically occur in a river below a discharge of organic effluent (Figure 2.2).

The strength of an organic effluent is frequently expressed in terms of its biological oxygen demand (BOD). This is defined as the quantity of oxygen utilised, expressed in mg $l^{-1}$, by the effluent during the microbial degradation of its organic content. BOD is typically measured by taking a sample of water or effluent and aerating it until it is saturated with oxygen. The dissolved oxygen concentration in part of the aerated sample is then determined by one of the well-known standard procedures. Another part of the aerated sample is incubated, in a sealed bottle of known volume for a period of (typically) five days, at a controlled temperature which is usually 15°C or 20°C. Usually the incubation is carried out in the dark, to eliminate oxygen production by any photosynthetic organisms which may be present. At the end of the incubation period, the concentration of dissolved oxygen remaining in the sample is determined. The difference between the initial and final dissolved oxygen concentrations is used to determine the BOD of the sample, which is expressed as milligrams of oxygen consumed per litre of sample. The basic assumption of the method is that oxygen is mainly consumed by aerobic microorganisms during the metabolism of organic matter. This is not necessarily true, since many effluents contain chemically-reduced compounds which undergo oxidation by purely chemical reactions.

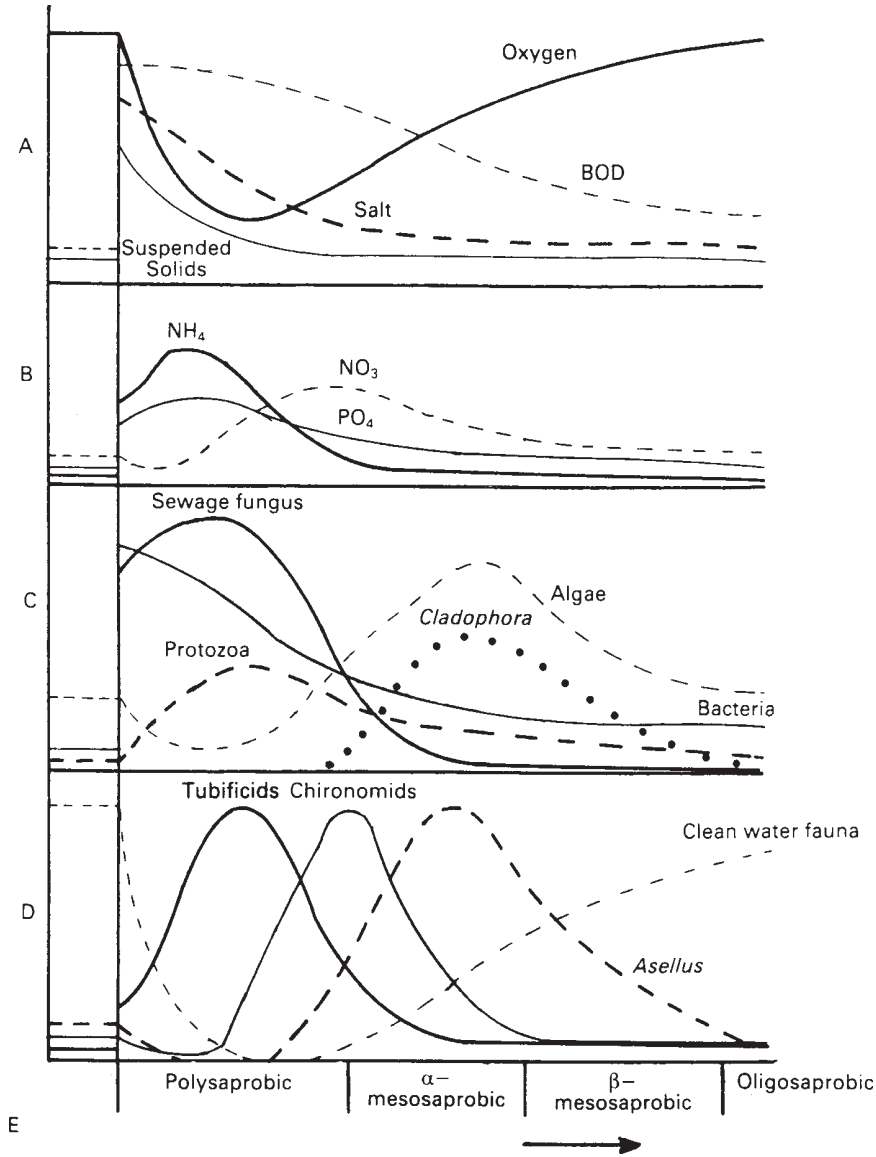



**Figure 2.2** Changes in water quality and abundance of selected organisms typically found in a river below the discharge of an organic effluent, after Hynes (1960). See text for explanation. (E) shows the zones described by Kolkwitz and Marsson (1909). Other descriptive systems have been used (see Hellawell (1986) for a summary) but are not significantly different

For this reason, it is often necessary to carry out other determinations (for example, of chemical oxygen demand, or of permanganate value) in addition to BOD in order to characterise precisely the likely effects of an effluent or correctly interpret the significance of a BOD value. Within these limitations, BOD values are generally useful as indicators of the organic loading of water. They typically range from one or two milligrams per litre in unpolluted water, to 10000 milligrams per litre or more in raw wastes, untreated effluents or severely polluted receiving waters. The wide range of expected values means that the precise details of the method of determination have frequently to be modified according to the circumstances; raw samples, for example, often need to be diluted before the determination is carried out. The incubation period of five days (rather than some shorter period) in practice compensates for the fact that some samples will have, initially, very few microorganisms present, while others will contain a large inoculum. Detailed descriptions of the procedures which may be employed for BOD and other relevant determinations, and for the interpretation of their results, are available in several handbooks, for example APHA (1995). Note that in recent years, the term *biochemical oxygen demand* has become a popular synonym for *biological oxygen demand*. The present author prefers the term *biological* oxygen demand as it has historic precedence and avoids confusion with the *chemical oxygen demand,* which refers to the oxygen demand imposed by the *chemical* oxidation of reduced substances in the water.

Figure 2.2(A) shows the immediate effect of the effluent on the BOD of the receiving water. Correspondingly, the dissolved oxygen (DO) level drops, gradually recovering as the BOD falls with increasing distance downstream. The oxygen sag curve varies in its dimensions according to the strength of the effluent and the physical characteristics of the river. (Knowing the BOD of the effluent and certain physical characteristics of the river, oxygen sag curves can be predicted according to mathematical models. This can be useful in planning, for example by predicting the likely effect of a proposed new outfall on a receiving water.) Figure 2.2(A) also shows the typical changes in the suspended solids and dissolved salt concentrations of a receiving water downstream of an organic effluent. Figure 2.2(B) shows how ammonia, nitrates and phosphates typically behave. Ammonia in the receiving water reaches peak levels at the point of greatest deoxygenation, but declines as aerobic conditions are re-established and reduced nitrogen compounds are oxidised to nitrates.

Figures 2.2(C) and (D) show the responses of the plant and animal communities to these physical and chemical changes. ‘Sewage fungus’ is a characteristic and conspicuous feature of waters which are heavily polluted with organic matter. It is not, in fact, a fungus at all, although its dense, blanketing growths of matted, greyish-brown filaments suggest this idea to many people. It is an aggregation, of varying composition, of bacteria, algae, fungi and protozoa, frequently dominated by the

slime-forming bacterium *Sphaerotilus natans* (Curtis and Curds, 1971). Algae are generally reduced initially (partly because the high levels of suspended solids prevent photosynthesis), but increase rapidly in abundance downstream as light penetration improves and levels of nitrate and phosphate remain high. *Cladophora* is a particularly conspicuous attached filamentous alga, widely associated with mild organic pollution or with the early stages of recovery from more severe pollution, and appears to be especially responsive to elevated phosphate levels (Pitcairn and Hawkes, 1973; Whitton, 1970).

The animal community (Figure 2.2(D)) also shows a clear pattern of response. The 'clean-water fauna' initially declines, and may be entirely eliminated, in the region immediately below the outfall. Tubificid worms, being typically tolerant of low dissolved oxygen levels and silty substrata, usually dominate the fauna in the more seriously affected areas. The larvae of the midge *Chironomus* (Diptera) spp. typically become established further downstream, followed by the isopod crustacean *Asellus,* and the gradual re-establishment of the 'clean-water fauna' as the river returns to its normal physical and chemical status. In any particular situation, the total number of species involved can be very large. The general pattern, subject to many variations in detail, is of a zonation, with increasing distance from the source of pollution, every bit as obvious as that along the length of an unpolluted river, or along a transect on a steeply-sloping seashore. The distribution of many individual species in response to organic pollution has been studied in detail in many different parts of the world. Hellawell (1986) gives a detailed summary of these observations. Several early investigators attempted to provide systematic descriptions of the zonation pattern of plants and animals observed in response to organic pollution with increasing distance from the pollution source, and one such description is shown in Figure 2.2(E). These descriptive systems eventually gave rise to the idea of indicator organisms, and to the development of numerical indices (pollution indices and biotic indices), which are widely used in the biological surveillance of water quality. These topics are further discussed in Chapter 3.

## 2.4 Nutrient Pollution

Plant growth in water may be limited by any of several factors, including light and the physical characteristics of the habitat. In many cases, however, the limiting factor is the availability of inorganic nutrients, particularly phosphate (Moss, 1988). Increased input of nutrients can therefore trigger increased plant growth which, if excessive, leads to changes in the biological characteristics of the receiving water. The discharge of organic matter to water is an important source of plant nutrients, since the aerobic decomposition of organic matter results in the release of phosphate, nitrate and other nutrients. Domestic sewage typically contains high levels of phosphate because detergent washing powder formulations normally contain high levels of phosphate. For example, the level of phosphate typically found in treated

sewage effluent (Table 2.1) may be compared with the levels normally found in unpolluted waters, which range from about 0.001 to 1 mg $l^{-1}$ (Moss, 1988). Food-processing effluents are often high in nitrate and phosphate, and in agricultural areas runoff from land carries nutrients into the water, especially if artificial fertilisers are used. Many agricultural and forestry practices lead to increased soil erosion, carrying plant nutrients from the land to the water. Intensive rearing of livestock contributes significant nutrient loads to surface waters.

Increased plant growth can sometimes be considered beneficial, especially in oligotrophic waters where primary productivity is nutrient-limited. Moderately-increased plant growth can provide increased productivity of herbivorous and detritivorous animals, leading to increased overall productivity. It is not unknown, for example, for fishermen deliberately to 'fertilise' lakes to increase fish yield. The increased spatial heterogeneity of the habitat can also give rise to an increase in species diversity. Excessive plant growth, however, has four main adverse consequences. The blanketing effect of macrophytes and filamentous algae can result in major faunal alterations owing to physical changes in the habitat. Respiration of dense plant growths can produce depressed dissolved oxygen levels, not only at night when photosynthesis ceases but also during the day if the density of plant growth reduces light penetration. Some algal species, under the influence of elevated nutrient levels, 'bloom'—that is they reproduce rapidly and dominate the flora. These algal blooms give rise to several problems, including tainting and discolouration of the water (rendering it unsuitable for potable supply) and the production of toxins which are harmful to fish and invertebrates. Following an extensive outbreak of algal blooms during 1989 in Britain, the National Rivers Authority produced a useful report which summarises the problems associated with algal blooms (NRA, 1990). Finally, the eventual decay of the plant biomass has exactly the same effect as the input of a large quantity of allochthonous organic matter.

## 2.5 Eutrophication

The phenomenon of eutrophication is particularly associated with lakes and slow-flowing waters. It is widely, and erroneously, believed that pollution by plant nutrients and organic matter actually causes eutrophication. It is more accurate to say that pollution accelerates what is probably a natural process. To understand the causes and consequences of eutrophication requires some knowledge of the special characteristics of lakes.

In temperate latitudes, most lakes were formed by glaciation. Moving glaciers gouged out hollows in the earth, and when the ice retreated these hollows became filled with water from the melting ice. Such lakes are not, therefore, geologically ancient phenomena. In modern times, substantial man-made lakes have become common in many parts of the world. A lake

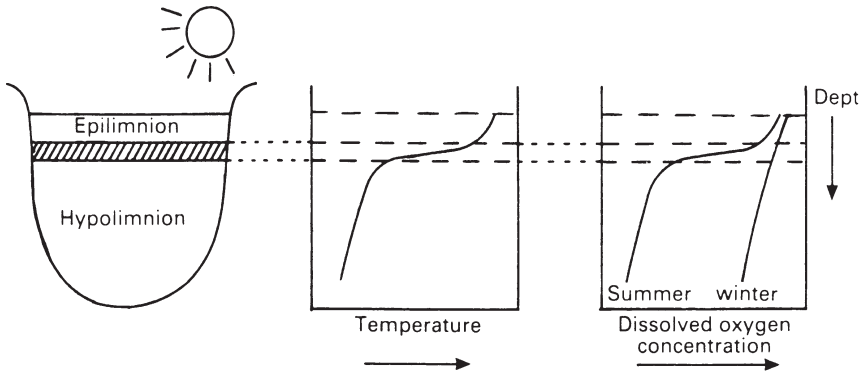



**Figure 2.3** Thermocline formation in a typical lake. The diagram on the left shows the stratification which develops, the thermocline being represented by the shaded area. The centre diagram shows the temperature profile. The diagram on the right shows the deoxygenation of the hypolimnion which occurs in eutrophic lakes in the summer. The vertical mixing which occurs in winter tends to abolish the stratification, redistributing oxygen and plant nutrients through the water column

is a body of water which is very slow-moving. Some lakes have rivers flowing into or out of them. Even those which do not, however, are not static; water moves slowly into or out of the lake via the ground. Because the water moves only very slowly, some physical and chemical processes occur in lakes which do not occur in moving waters. Of particular importance are stratification, and temporal variations in chemical quality of the water.

Stratification occurs because the lake water is heated by the sun at the surface. Because warm water is less dense than cooler water, and water is a poor conductor of heat, during the warmer months of the year an upper layer of warm water, the epilimnion, becomes established and sharply delineated from a lower layer of cooler, denser water, the hypolimnion. Between them is a very narrow zone, the thermocline, within which the water temperature drops very sharply with only a slight increase in water depth (Figure 2.3). Little or no vertical mixing can take place, the lake being effectively divided horizontally into two distinct layers separated by the thermocline. Obviously, stratification cannot occur in very shallow lakes.

Photosynthesis can only occur in shallow water, where light can penetrate. At the lake margins, emergent plants and rooted aquatic macrophytes occur, but as the depth of the water increases, primary production is possible only by phytoplankton in the surface waters, within the epilimnion. During the winter, phytoplankton growth is restricted by low temperatures and low light intensity. In spring and summer increasing temperatures and light intensity stimulate phytoplankton growth, leading to an increase in population density and the depletion of nutrients in the water of the epilimnion. Plant growth

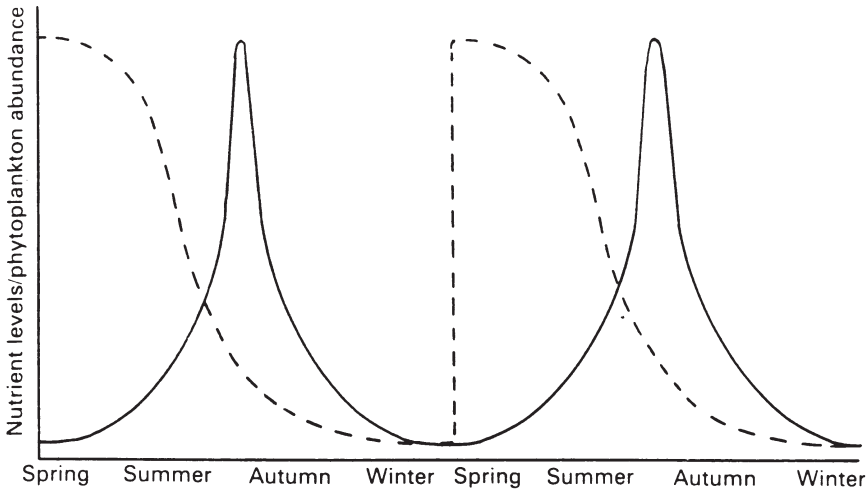



**Figure 2.4** Seasonal cycling of nutrients and phytoplankton in the surface waters of a lake. Nutrient levels (dotted line) decline as phytoplankton growth (solid line) takes place. Stratification leads to nutrient depletion of the surface layers, so the phytoplankton population declines and senescent cells fall to the hypolimnion. Abolition of the thermocline in winter redistributes the plant nutrients allowing the cycle to begin again the following spring

and reproduction slow down, and as the plant cells senesce and die, they sink into the hypolimnion and eventually to the bottom of the lake, where they begin to decompose. The inorganic nutrients which are the products of decomposition remain in the hypolimnion, however, as the stratification prevents vertical mixing of the water and upwards diffusion is slow. As the autumn approaches, reduced temperatures, light intensity and limited nutrients accelerate the decline of the phytoplankton population. In the winter, the epilimnion cools and becomes more dense. Its water sinks, displacing the hypolimnion which is now warmer and lighter than the epilimnion. The lake waters become thoroughly mixed, and nutrients from the hypolimnion are brought to the surface, bringing about conditions suitable for the start of the next annual cycle (Figure 2.4).

Underlying these annual cycles is a progressive change in the physical and chemical characteristics of the lake. At its formation, the lake contains few plant nutrients or dissolved minerals of any kind, and a negligible quantity of organic matter. With the passage of time, dissolved minerals including plant nutrients enter the lake from surface runoff and groundwater infiltration, at a rate which depends largely upon the climate and the geology of the surrounding area. As the nutrient levels rise, a flora and fauna becomes established and develops, contributing an increased content of organic matter in the lake. Organic matter is also gradually accumulated from outside the lake, progressively building up a

layer of sediment on the lake bottom. Airborne dust also falls into the lake, and the lake begins slowly to fill up. The rate at which this happens varies from the barely detectable up to a few millimetres per year. The gradual deposition of material on the floor of the lake basin causes the lake to shrink, new land being formed at its edges (Figure 2.5). This new land is colonised by terrestrial plants, and in some lakes it is possible, by walking away from the lake's edge, to see clearly the various stages of development of the terrestrial flora, a classic example of ecological succession. In areas where these processes have occurred, for various reasons, at different rates in different lakes, it is possible to see contemporaneously all the stages of a lake's development from nutrient-poor, sparsely-populated lakes of low productivity, through various stages of nutrient enrichment, to swamp or marsh and eventually dry land.

The term *eutrophication* is applied to the process whereby the nutrient levels of lakes increase from oligotrophic (nutrient poor) to eutrophic (nutrient rich). It appears to be a natural process, although some authors have argued that it is not inevitable or intrinsically unidirectional (Moss, 1988). Since its basic cause is, however, the accumulation of plant nutrients and organic matter in the lake basin, clearly anthropogenic influences will accelerate it. The transition from oligotrophic to eutrophic is accompanied by qualitative and quantitative changes in the biota. Since plant growth is commonly limited by nutrient levels, a gradual increase in nutrient levels would be expected to lead to successional changes in the plant community and corresponding changes in the animal community. Animals, in particular, are likely also to be affected by deoxygenation of the hypolimnion. In eutrophic lakes, the stratification which leads to nutrient depletion of the epilimnion also causes oxygen depletion of the hypolimnion. Oxygen demand due to aerobic decomposition of detritus is high in the hypolimnion, but the absence of either vertical mixing or photosynthesis in the hypolimnion prevents re-oxygenation of the hypolimnion from the atmosphere (Figure 2.3). The sensitivity of aquatic animals to dissolved oxygen levels, and its consequences for their patterns of distribution and abundance, has already been discussed.

Moss (1988), Mason (1991) and Jeffries and Mills (1990) discuss in detail some well-studied case histories of eutrophication, and a very full account is given by Harper (1992). Attempts have been made to restore eutrophic lakes, with varying degrees of success, or to manage lakes and their catchment areas in such a way as to reduce the effects of anthropogenic activities on eutrophication (Harper, 1992).

## 2.6 Thermal Pollution

The extent of the use of water for cooling is formidable. Howells (1983) estimated that in Britain 28% of rainfall and 50% of river flow were utilised

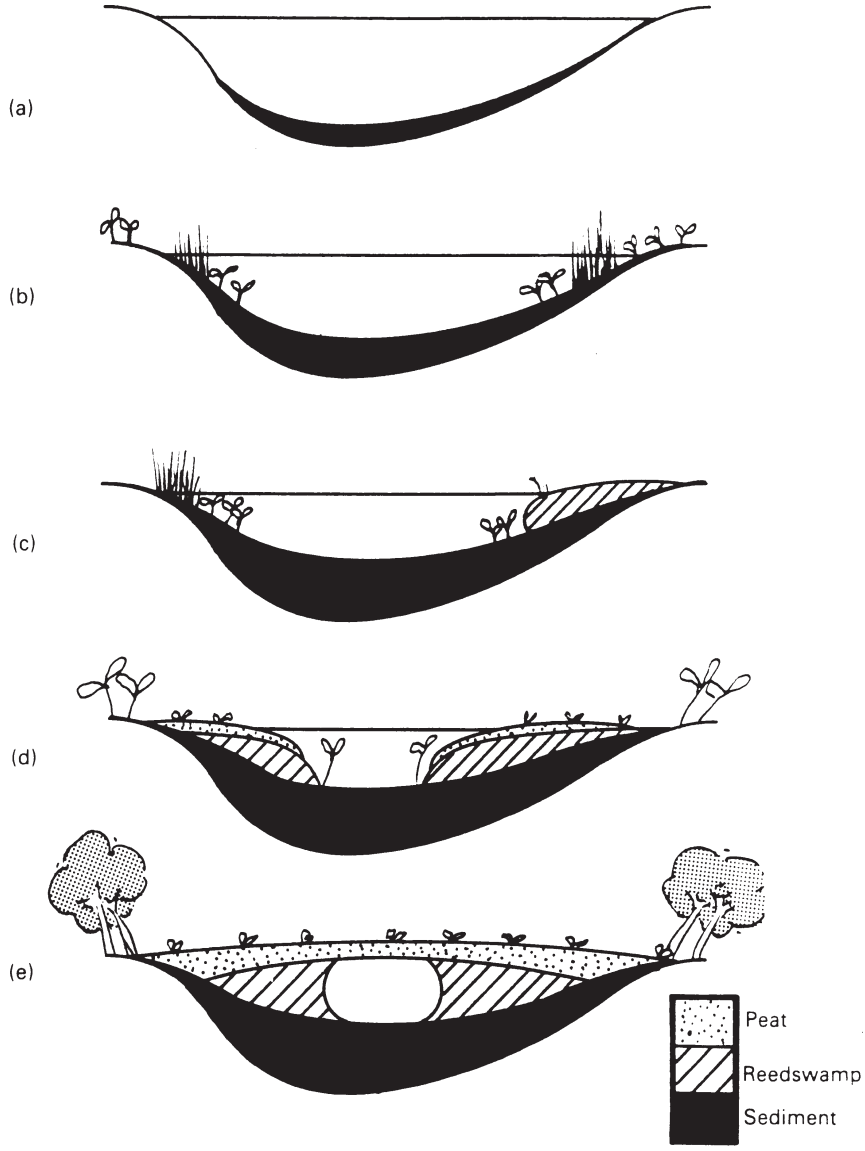



**Figure 2.5** Stages of development of a typical glacially-formed temperate lake from oligotrophic (upper diagrams) to eutrophic (lower diagrams)

for this purpose. More recently, attempts to reduce these percentages have been made in view of increasing demands on water for other purposes, and the availability of improved technology. In fact, Britain has relatively few large inland power stations, and the bulk of the demand falls upon a single river system, the Trent (Lester, 1975) which supplies cooling water for approximately one-third of the

nation's power-generating capacity; most of the remainder is situated on coastal or estuarine sites. In Britain, direct cooling is rare; water abstracted from the river is recirculated within the plant and much of the excess heat is dissipated through cooling towers. Direct cooling would in fact require far more water than is available from the relatively small rivers. In the USA, direct cooling is more common, and about 10% of runoff is used for cooling (Castenholtz and Wickstrom, 1975). Very large rivers are, of course, more common in large continental land masses; further, the climate in much of the USA is such that river temperatures of 30°C or more are not uncommon, whereas in Britain river temperatures above 24°C are extremely rare. To this extent, the problems of dissipating waste heat differ widely from one location to another.

Temperature is of such profound importance in chemical and biological processes that the effect of temperature alterations on aquatic biological communities is potentially large. Hot effluents from industrial processes and power generation can cause temperature increases in the receiving water of 10°C or more. Some effluents, such as water pumped from deep mines or regulating reservoirs, may be significantly colder than the receiving water, although the effects of cold effluents have received relatively little attention. Because the density of water alters with temperature, hot effluents often form a surface plume rather than mixing quickly with the receiving water. This can exacerbate some of the adverse effects, but may sometimes act to minimise the influence of the effluent on the benthic community, and fish can avoid the elevated temperature by remaining in deeper water.

Elevated temperatures can influence aquatic organisms directly, as the organisms respond physiologically or behaviourally to the new conditions; or indirectly, as the changed temperature influences the chemical environment. For example, increased temperature reduces the solubility of oxygen in water. At the same time, it may increase BOD by stimulating more rapid breakdown of organic matter by microorganisms. Temperature affects the toxicity of some poisons (see Chapter 4) either through its effect on the organisms themselves or because the dissociation of ionisable pollutants (such as ammonia or cyanides, see Section 2.7.2) is temperature-dependent. The direct effects of elevated temperature on fishes have been particularly well studied and may be used to indicate the potential impact of thermal pollution on aquatic animals generally. Varley (1967) gives a very readable introduction to the relationships between fish and their thermal environment, and their consequences for fish distribution patterns. Alabaster and Lloyd (1980) provide a detailed review of the literature relating to the temperature requirements of freshwater fishes, and Hellawell (1986) provides a concise summary of the effects of thermal pollution on the aquatic environment.

The maximum temperature which fish can withstand varies from species to species, and also within a species according to the environmental history of the fish. Generally, fish can acclimate to gradually-rising temperatures, so that the lethal temperature depends to some extent on the temperature to which the fish was initially acclimated. Relatively small, sudden changes

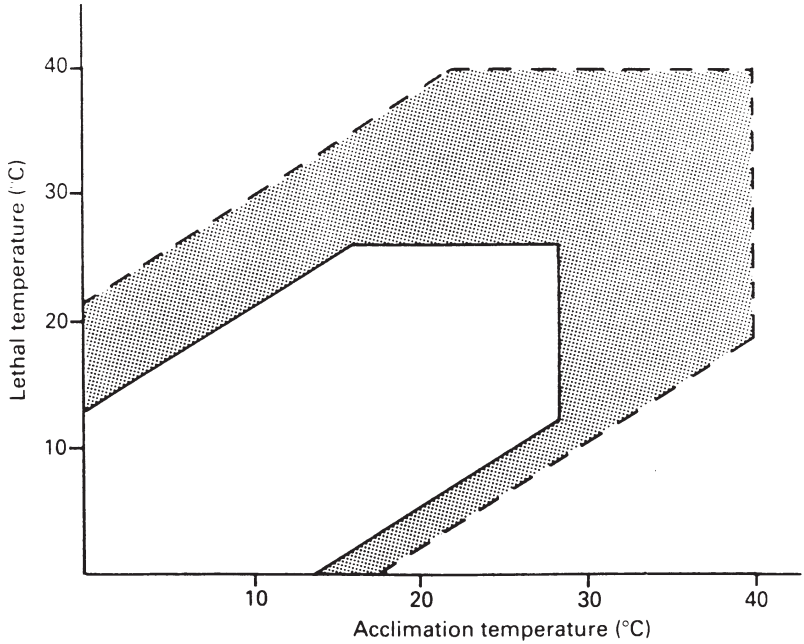



**Figure 2.6** Temperature tolerance diagrams for (dotted line) a eurythermal warm-water-adapted fish and (solid line) a stenothermal cold-water-adapted fish. The upper lethal temperature increases with acclimation temperature until the ultimate upper lethal temperature is reached. The lower lethal temperature is also influenced by acclimation temperature. Only temperature changes within the boundaries are tolerated

of temperature which do not allow the acclimation process to occur can be more harmful than larger, more gradual changes. Acclimation to altered temperature is probably achieved by the induction and synthesis of isoenzymes. Many enzymes are known to exist in several forms, each having the same function but each modified to perform optimally at a particular temperature, or under some other specific condition. This is one way in which poikilothermic animals can continue to function over wide ranges of temperature, although the adjustments involved require periods ranging from several hours to several days. The relationship between acclimation temperature and the upper lethal temperature may be summarised in a temperature tolerance diagram (Figure 2.6). The temperature at which the upper lethal temperature ceases to rise with acclimation temperature is termed the ultimate upper lethal temperature. Values range from about 24°C for Salmonid fishes to 40°C or more for fishes characteristic of warmer environments. In general, the ultimate upper lethal temperature for a particular species is several degrees higher than any temperature likely to be encountered in its normal habitat. Therefore death of fish due to high

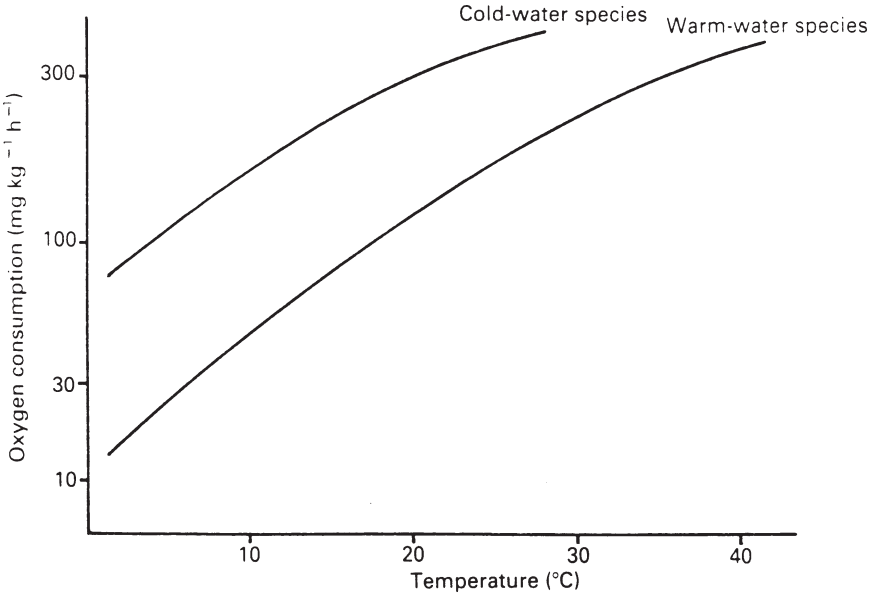



**Figure 2.7** Relation between oxygen consumption and temperature for fish at rest. The upper line represents a cold-water-adapted species, the lower line a warm-water-adapted species

temperature alone is probably rare, and the adverse effects of elevated temperature are due to more subtle mechanisms.

The effects of temperature on the respiratory physiology of fish are particularly important. Even under favourable conditions, aquatic animals face formidable difficulties in balancing their needs for oxygen against the meagre quantity available from water and the high energy cost of obtaining it (see Section 2.2). Increased temperature both reduces the amount of oxygen available and increases the animal's demand for it. In addition, for animals in the wild it is not sufficient to survive passively; survival presupposes the need to engage in physical activity, which imposes still further demands for oxygen. In fish, constraints imposed by the size, structure and efficiency of the gills, and by the limited solubility of oxygen in water, limit the maximum possible oxygen consumption to a value of about 400 mg $kg^{-1}$ $h^{-1}$ in most species.

In Figure 2.7, the effect of temperature on the oxygen consumption of two fish species is shown. Warm-water species require to reach the maximum possible rate of oxygen expenditure only at fairly high temperatures. As the temperature falls, their metabolic rate falls until it is too low to permit physical activity, and the fish become torpid. In the wild, they would not survive because they would be unable to find food, avoid predators, or even maintain their position in a current. Cold-water species, such as Salmonids, however, must be active at low

temperatures. They are adapted to high rates of oxygen consumption at low temperatures. However, their rate of oxygen consumption increases with temperature at about the same rate as that for cold-water species (Figure 2.7). Therefore they reach the maximum possible rate at a much lower temperature. As argued in Section 2.2, when the fish reaches this condition the energy cost of working the respiratory muscles is such that there is no oxygen available for the tissues, and the fish dies. Further, the need to retain a margin of capacity for physical activity is important. At its ultimate upper lethal temperature, the fish has no scope to indulge in physical activity other than respiratory movements. At very low temperatures, it has little scope for muscular activity of any sort, since the efficiency of muscle tissue decreases with temperature. At intermediate temperatures, the difference between basal metabolism (oxygen consumption at rest) and active metabolism (oxygen consumption during physical activity) reaches a distinct maximum (Figure 2.8). In practice, the temperature regime which is favourable for the indefinite survival of the fish includes a much narrower range of temperatures than that which would allow the survival of the fish under laboratory conditions.

The range of temperatures which is suitable for growth, reproduction and development of fishes is also generally rather narrower than that which simply allows survival. Temperature is, in conjunction with photoperiod, an important trigger for the onset of reproductive cycles. Temperature also governs the rate of development of fish eggs. In most species, the average temperature multiplied by the time taken for the eggs to hatch is a constant. In Salmonids, eggs typically require 410 degree-days; for example, 41 days at 10°C or 51 days at 8°C. This relationship only holds good, however, within a certain temperature range—below about 5°C and above about 15°C, the proportion of eggs successfully developing is markedly reduced. In Cyprinid fishes, development of eggs may be much quicker, typically three days at 20°C in carp, with other species showing intermediate values. Thus quite short-term anomalies in the temperature regime, if they occur at a critical period, can exert a serious effect on fish reproduction. Studies of the growth of many fish species, particularly those which are widely cultured, show that temperature has critical effects on growth rate and on food conversion efficiency (i.e. the ratio between the increase in weight of the fish and the quantity of food consumed). (In fish, sexual maturity normally occurs when the animal has reached a critical size rather than a critical age.) Clearly, temperature anomalies can have a major influence on the reproductive success of fish, without necessarily giving rise to any obvious immediate effects on the adult population. In practice, fish have considerable powers of mobility and in laboratory experiments show clear preferences for particular temperatures, ranging from about 13°C for Salmonids to 37°C for carp (Varley, 1967). Thus areas subject to thermal anomalies may be devoid of fish through avoidance reactions, but without any serious long-term effects on the population, provided that the fish do have access to cooler water.

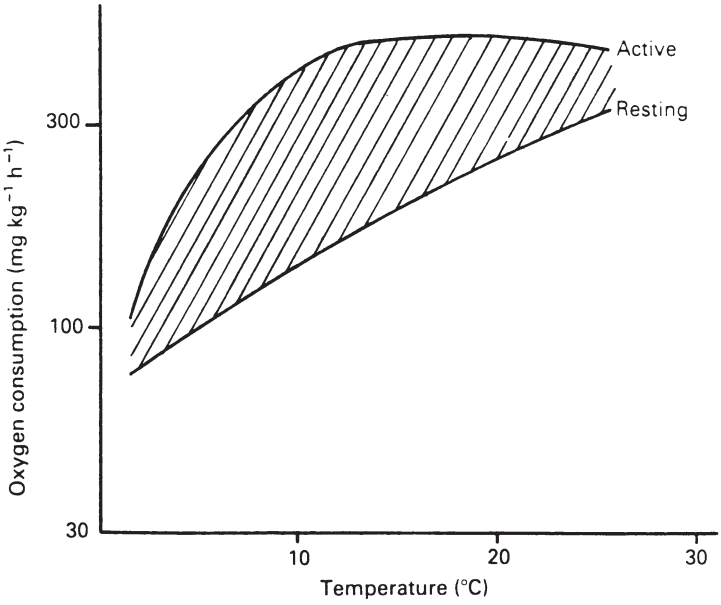



**Figure 2.8** Effect of temperature on (lower line) the resting or standard metabolism of a fish and (upper line) the active metabolism. The shaded area represents the 'scope for activity' of the fish

In view of the prevalence of heated effluents, and of the potential importance of elevated temperatures indicated by laboratory studies, it is perhaps surprising that unequivocal examples of ecological damage by thermal pollution are rare. Reviewers such as Castenholtz and Wickstrom (1975), Howells (1983) and Hellawell (1986) cite no clear instances of any readily-detectable adverse effect of elevated temperature, as such, on the ecology of a receiving water, apart from numerous autecological studies whose significance to the overall ecosystem is unknown. Poff and Matthews (1986) did produce some data which appear to show that in a North American stream receiving power-station effluent, invertebrate communities showed reduced diversity and changes in species dominance, compared to nearby undisturbed streams. This is the type of effect which would be expected, but it seems that river communities are often more resilient to thermal disturbance than theory would predict. However, the review of Alabaster and Lloyd (1980), based largely upon research (including field studies) in Eastern Europe which was previously little-known, suggests that temperature increases of between 2 and 8°C do produce significant alterations in the biota of receiving waters.

The apparent difficulty of detecting the ecological effects of thermal pollution may be due to any of several causes. In Eastern Europe, where such effects are more apparent, the prevailing climate is significantly more extreme than in Western Europe or the USA where most studies have been carried out. In many

circumstances, heated effluents are discharged to rivers which are also polluted with toxic or organic matter, and the effects of elevated temperature may be difficult to distinguish from other pollution effects. Since *low* temperatures can also limit biological processes, in some circumstances artificially-elevated temperatures may actually be beneficial in terms of overall productivity. Also, local conditions undoubtedly influence the impact of heated effluents. In many countries, power generation reaches its maximum during the winter months when river temperatures are low and river discharges high. In others, depending upon climatic and economic factors, demands for electric power (for refrigeration or air-conditioning) in summer, when river discharges are lowest and temperatures naturally highest, may approach or exceed winter levels. The effects of heated effluents on the ecology of receiving waters may therefore be expected to vary from one region to another. Finally, the effects of elevated temperatures may be difficult to predict or detect without detailed knowledge of specific local circumstances. For example, increased temperature will accelerate microbial decomposition of organic matter. In sluggish, poorly-aerated waters this will accentuate the effects of organic pollution; but in turbulent waters which re-aerate rapidly from the atmosphere, the effect of elevated temperature would be to reduce the extent of the zone within which the adverse effects of organic inputs manifested themselves, and in a lightly-polluted water might lead to a beneficial increase in overall productivity. It is therefore unwise to attempt generalisations on the effects of thermal pollution; each case must be considered individually.

## 2.7 Toxic Pollution

There are about four million different chemical substances in existence, a number which increases by about 300000 every year. Of these, about 63000 are in common use (Maugh, 1978). Goodman (1974) estimated that about 10000 chemicals are produced in quantities exceeding 500 kg $yr^{-1}$. A large proportion of these thousands of chemicals are, presumably, only produced and/or used in only a small number of locations. Nevertheless the number of pollutants which can be considered as widespread is still formidably large. A realistic figure is indicated by the 1978 Great Lakes Water Quality Agreement between the US and Canadian governments (reprinted in Nriagu and Simmons, 1983). Appendix 1 of this agreement lists 271 different substances which, on the basis of toxicological and discharge data, are considered hazardous to the North American Great Lakes. Appendix 2 of the same agreement lists a further 106 'potentially hazardous polluting substances'.

The present discussion is confined, of necessity, to a general description of the sources and characteristics of some of the major categories of toxic pollutant. The categories chosen are for convenience, and are not necessarily mutually exclusive;

many pesticides, for example, contain heavy metals. It is not at all easy to give specific descriptions of the effects of a given pollutant, because of the diversity of the chemicals involved, the wide range of environmental conditions which prevail in different receiving waters, the fact that poisons frequently occur in complex mixtures, and the enormous differences in the physiology of the organisms which are exposed to them. The study of pollutant toxicity and toxic effects is described in some detail in Chapters 1 and 4. Specific information on the toxicity and toxic effects of pollutants is best sought in review articles and other data compilations, which are referred to where appropriate. A very comprehensive reference work is that of Hellawell (1986). It is important to note, however, that toxicity data obtained from such compilations can be extremely misleading, for reasons which are explained fully in Chapter 4. It is recommended that wherever possible the original sources are consulted, and the validity of the data assessed in relation to the methodological criteria described in Chapter 4.

### *2.7.1 Heavy Metals*

'Heavy metals' is an imprecise term that is generally taken to include the metallic elements with an atomic weight greater than 40, but excluding the alkaline earth metals, alkali metals, lanthanides and actinides. The most important heavy metals from the point of view of water pollution are zinc, copper, lead, cadmium, mercury, nickel and chromium. Aluminium (see Section 2.9) may be important in acid waters. Some of these metals (e.g. copper, zinc) are essential trace elements to living organisms, but become toxic at higher concentrations. Others, such as lead and cadmium, have no known biological function. Industrial processes, particularly those concerned with the mining and processing of metal ores, the finishing and plating of metals and the manufacture of metal objects, are the main source of metal pollution. In addition, metallic compounds are widely used in other industries: as pigments in paint and dye manufacture; in the manufacture of leather, rubber, textiles and paper; and many others. Quite apart from industrial sources, domestic wastes contain substantial quantities of metals because the water has been in prolonged contact with copper, zinc or lead pipework or tanks. The prevalence of heavy metals in domestic formulations, such as cosmetic or cleansing agents, is frequently overlooked. Some forms of intensive agriculture give rise to severe metal pollution; copper, for example, is widely added to pig feed and is excreted in large quantities by the animals. Mance (1987) gives a detailed review of metal pollution.

Heavy metals may be classed (see Table 2.4) generally as toxic or very toxic to aquatic animals and to many plant species, though large interspecific differences in susceptibility occur even within closely-related groups of

**Table 2.4** Degrees of pollutant toxicity, classified according to the scheme proposed by the Joint IMCO/FAO/UNESCO/WHO Group of Experts on the Scientific Aspects of Marine Pollution (1969)

| Degree of toxicity | Acute toxicity threshold, ($mg\,l^{-1}$) |
|---|---|
| Practically non-toxic | Above 10 000 |
| Slightly toxic | 1000–10 000 |
| Moderately toxic | 100–1000 |
| Toxic | 1–100 |
| Very toxic | Below 1 |

organisms. Relatively little is known about the interaction of heavy metals with the aquatic microbial flora. Algae, macroinvertebrates and fishes have, however, been widely studied. In general, the heavy metals may be listed in approximate order of decreasing toxicity as follows: Hg, Cd, Cu, Zn, Ni, Pb, Cr, Al, Co. However, this sequence is very tentative and the position of each element in the series will vary with the species tested and the conditions of the experiment. Apart from some remarkable interspecific variations in susceptibility to metals, the toxicity of most metals varies enormously with the environmental conditions, mainly because of the effect of environmental conditions on the chemical speciation of the metal (see Section 4.2). Study of the ecological effects of heavy metals as water pollutants is often hampered by the fact that other pollutants are normally additionally present. However, there is an enormous literature on heavy metal toxicity (see, for example, Alabaster and Lloyd, 1980; Hellawell, 1986; Mance, 1987; Whitton and Say, 1975) and some important aspects of the impact of heavy metals as water pollutants have already been discussed in Chapter 1.

Two features of heavy metal toxicity which should not be overlooked are their ability to form organometal complexes and their potential for bioaccumulation. There is some evidence (see Section 4.2) that the presence of organic substances can reduce heavy metal toxicity considerably, at least as measured in conventional toxicity tests. However, a number of organometal compounds are known to be particularly hazardous to aquatic life. Tributyltin, for example, a constituent of antifouling paints, is implicated in severe environmental damage in harbours, boatyards and inland waters, and appears in the 'Black Lists' of substances compiled by international organisations such as the European Union and United Nations Environment Programme. Similarly, the dangers associated with methylated forms of mercury are well known. Many metals, whether organically-complexed or not, are known to accumulate in plant and animal tissues to very high levels, posing a potential toxic hazard to the organisms themselves, or organisms higher in the food chain including humans, which may consume them.

### *2.7.2 Ammonia, Cyanides and Phenols*

Ammonia, cyanides and phenols are considered together because, with copper and zinc, they are the most widespread and serious toxic water pollutants in industrialised countries. Ammonia and its compounds are ubiquitous constituents of industrial effluents because ammonia is a staple raw material in many branches of the chemical industry; it is, therefore, a common end-product of industrial processes as well as an important by-product of others, notably the production of coke and gas from coal, from power generation and from most processes involving the heating or combustion of fuel. It is also a natural product of the metabolism of organic wastes in treatment plants and receiving waters. The toxicity of ammonia to fish is well documented, and although less is known of its effect on invertebrates it appears that levels of ammonia which are tolerable to fish present little danger to most invertebrates (Alabaster and Lloyd, 1980). In aqueous solution, ammonia forms an equilibrium between unionised ammonia, ammonium ion and hydroxide ions:

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-.$$

Unionised ammonia is very toxic to most organisms, but ammonium ion is only moderately toxic. The toxicity of the solution therefore depends on the quantity of unionised ammonia. This in turn depends upon the pH and temperature of the water; as pH and temperature rise, the proportion of unionised ammonia also rises. The effect of pH and temperature on ammonia toxicity is therefore considerable. In order to know whether a given level of total ammonia is likely to be toxic, it is necessary to use the pH and temperature values to calculate the corresponding level of free ammonia. (Some authors, such as Alabaster and Lloyd (1980) and Hellawell (1986), have published tables or nomographs to facilitate the calculation.) As an example, the European Inland Fisheries Advisory Commission (EIFAC) recommends that unionised ammonia concentrations should not exceed 0.025 mg $l^{-1}$. In a water of pH 8.5 at a temperature of 20°C, this corresponds to a *total* ammonia concentration of 0.22 mg $l^{-1}$. In a cooler, acid water, however (pH 6.5, 5°C), a concentration of *total* ammonia of 63.3 mg $l^{-1}$ would be acceptable, whereas at pH 6.5 and 20°C, the maximum acceptable concentration of *total* ammonia would be 20 mg $l^{-1}$.

Cyanide is also a very common constituent of industrial effluents, being produced from processes involving coking and/or combustion such as steelworks, gas production and power generation. Cyanides are also used in the hardening, plating and cleaning of metals. Cyanides dissociate in aqueous solution:

$$KCN + H_2O \rightleftharpoons H^+ + K^+ + HCN \rightleftharpoons CN^- + H^+.$$

The dissociation, and consequently the toxicity of cyanide, is pH-dependent, low pH favouring the formation of undissociated HCN which is highly toxic. Cyanide ions readily form complexes with heavy metal ions. The stability and toxicity of

these complexes varies according to the metal and also with the pH. Thus the toxicity of cyanides and effluents containing cyanides (which commonly contain substantial amounts of heavy metal) is, as with ammonia, greatly influenced by pH, but is less well understood. It is clear, however (see Chapter 1), that the contribution of cyanides to the overall toxicity of complex industrial effluents can be identified, and is often significant.

Phenolic substances include the monohydric phenols (phenol, cresols and xylenols) and the dihydric phenols including catechols and resorcinols. They are found in a wide range of industrial effluents, and are particularly associated with gas and coke production, the refining of petroleum, power generation, many branches of the chemical industry and the production of glass, rubber, textiles and plastics. Alabaster and Lloyd (1980) and Buikema *et al.* (1979) provide detailed reviews of the literature. The latter review includes discussion of phenol derivatives which are used for specialised purposes such as pesticides. Apart from these, phenolic substances rarely occur as pollutants except as components of complex effluents which contain a variety of other polluting substances. In practice, therefore, the major concern is to determine the extent to which phenols contribute to the overall toxicity of an effluent, and an example was discussed in Chapter 1.

### *2.7.3 Pesticides*

Pesticides are a diverse group of poisons of widely-varying chemical affinities, ranging from simple inorganic substances to complex organic molecules. Of the latter, some are natural metabolites, particularly of plants, while others are synthetic derivatives of natural products or completely synthetic substances produced in chemical factories under conditions which do not exist in the natural world. They have in common only that each pesticide is highly toxic to some forms of life and of intermediate or negligible toxicity to others, and that they have been widely introduced into the natural environment. Pesticides are introduced into aquatic systems by various means: incidentally in the course of their manufacture, and through discharge consequent upon their use. Surface water runoff from agricultural land and the side-effects of aerial spraying are especially important, and many serious pollution incidents arise through the accidental or negligent discharge of concentrated pesticide solutions which have been used for agricultural purposes such as sheep-dipping. Additionally, many pesticides are deliberately introduced into water bodies to kill undesirable organisms such as insect or molluscan vectors of human diseases, weeds, fish and algae.

Pesticides are also used in many industrial processes, for example in the manufacture of textiles and in the production and processing of perishable materials such as paper and timber products. They are therefore an important component of many industrial effluents. Because of their enormous diversity and their importance as pollutants, pesticides have attracted an enormous literature. Useful introductions are given, for example, by Khan (1977), Perring and Mellanby (1977), Brown (1978) and Muirhead-Thompson (1987). A valuable summary of pesticides as water

pollutants is given by Hellawell (1986). In many countries, the special significance of pesticides as pollutants (and as widely-used toxic chemicals in the working environment) has led to the development of strict controls on their use. One example is the Control of Pesticides Regulations, operated in Britain by the Ministry of Agriculture.

Insofar as it is possible to generalise about the polluting effects of such a diverse group of substances, the following points are perhaps of greatest significance. First, effective pesticides are more or less selective in their effects, that is they are extremely toxic to some forms of life and relatively harmless to others. Second, their modes of application vary according to the circumstances. In some cases, pesticides are applied in relatively high concentrations for relatively short periods. This pattern of application typically occurs where pesticides are applied to water to kill weeds, disease vectors or other undesirable organisms, or as an incidental effect of aerial spraying of crops. Here, the principal concern may be to determine their short-term toxicity to non-target organisms, and it may be advantageous to devise specific toxicity testing protocols in order to estimate the impact of the pesticides on the receiving-water biota (see Chapter 4). However, in lowland rivers draining agricultural areas, pesticides are more likely to be present at low but fairly consistent levels, and in this case the major areas of toxicological interest will be their potential sublethal effects, their capacity to accumulate in individual organisms and via the food chain, and the development of resistance through acclimation and/or genetic adaptation. Many pesticides are known to be refractory to chemical and biological degradation, and their persistence in the environment has for many years been a cause for concern. Probably the best example of this is the well-known case of DDT, which has been used in such enormous quantities in the last 50 years that no part of the world is now free from measurable contamination, and its manufacture and use in many countries are now banned or severely restricted. One consequence of this, however, is that other pesticides are used as substitutes; many of these are of much more recent discovery than DDT, and may eventually be found to be equally or more dangerous when sufficient information about them has been accumulated.

## 2.8 Suspended Solids

Virtually all effluents contain suspended particulate matter, but especially those associated with mining and quarrying for coal, china clay, stone and other mineral materials. Dredging, engineering works and boat traffic commonly introduce particulate matter into suspension. Storm-water drainage and surface water runoff also contribute substantial loads. Suspended matter may be organic or inert, and some forms are chemically reactive (for example, the ferric hydroxide precipitate associated with acid mine drainage (see Section 2.9)). The present discussion is confined to the physical effects of suspended solid matter. The effects of suspended matter on the receiving water biota are both direct and indirect. Direct effects include physical abrasion of body surfaces, and especially of delicate structures

such as gills. Physical damage of this kind interferes with respiration and renders the animals susceptible to infections. High levels of suspended particulates may interfere with the filter-feeding mechanisms of invertebrates, and possibly with the feeding of fish which locate their food visually. In laboratory experiments high levels of suspended solids can kill fish. The concentrations of suspended solids at which these effects occur vary with the species and the nature of the particulate matter. Herbert and Merkens (1961) found that kaolin and diatomaceous earth in suspension at a concentration of 270 mg $l^{-1}$ caused substantial mortality to trout over a period of 10–15 days, but other investigations have shown large differences between the lethal concentrations of different types of suspended matter (Alabaster and Lloyd, 1980).

Indirect effects are mainly due to increased turbidity and the blanketing effect of the particulates when they eventually settle. Increased turbidity will reduce or prevent photosynthesis, leading to a reduction in primary productivity or the complete elimination of plants. Alternatively, certain forms of silting can, depending on the physical conditions, bring about major changes in the community by promoting the formation of stable weed beds. Salmonid fishes require aerated gravel beds for egg-laying sites, and the silting of gravel beds can eliminate salmonid populations by depriving the fish of suitable nest sites. In many rivers, salmonid population density is governed by the availability of nest sites rather than by biological or chemical factors. Invertebrate distribution patterns are profoundly influenced by the size of the particles composing the substratum (Hynes, 1970). Mayfly nymphs with exposed abdominal gills (e.g. *Ecdyonurus, Rhithrogena, Ephemerella*) may be replaced by species with covered gills or which are adapted for burrowing (e.g. *Caenis, Ephemera*). Insects which crawl upon the substratum may generally be disadvantaged in favour of species whose means of locomotion is better suited to a soft substratum, such as leeches, oligochaetes and some molluscs. Thus the input of even inert fine particulates can readily bring about major community changes. Herbert *et al.* (1961) described the effects of china clay wastes on the ecology of trout streams, and discussed the mechanisms by which alterations occurred. Other field and laboratory studies are reviewed by Alabaster (1972) and Alabaster and Lloyd (1980). The majority of unpolluted British rivers contain fewer than 50 mg suspended solids per litre of water, and about half have under 30 mg $l^{-1}$. Alabaster and Lloyd (1980) tentatively suggest that waters containing more than 80 mg $l^{-1}$ are unlikely to support good fisheries.

## 2.9 Extreme pH and Acidification

Many effluents, especially if untreated, are strongly acidic or alkaline. All natural waters have some buffering capacity, that is the ability to absorb acid or alkaline inputs without undergoing a change in pH. This buffering capacity is usually expressed in terms of the acidity (ability to neutralise alkalis) and alkalinity (ability to neutralise acids) of the water, and is determined by titration in the presence of a suitable indicator. The relationship between the pH, acidity and alkalinity of a

water is not simple. Acid waters (pH<7) can have measurable alkalinity, and alkaline waters (pH > 7) can have measurable acidity. Where the buffering capacity of the water is exceeded by the input of an effluent, the pH of the water will change. Unpolluted natural waters show a pH range from 3.0 to 11.0 or more; those lying between 5.0 and 9.0 generally support a diverse assemblage of species and this range may be considered broadly acceptable (Alabaster and Lloyd, 1980). This does not mean, however, that pH changes within the range 5.0–9.0 are of no consequence. For example, pH is an important determinant of the distribution patterns of aquatic species, as can be seen from the study of invertebrate communities in unpolluted rivers (Haines, 1981; Sutcliffe, 1983; Sutcliffe and Carrick, 1973). In addition, apparently small changes in pH can have major effects on the toxicity of pollutants such as ammonia, so that the effect of a given level of pollutant can vary, depending upon pH, from being scarcely noticeable to being extremely serious.

A very common form of pollution involving extreme pH is acid mine drainage. Coal mines are the most common source of acid mine drainage, but it can occur wherever mineral ores are mined. A series of reactions commencing with the oxidation of the common mineral pyrite ($FeS_2$) is responsible, and certain autotrophic bacteria are closely involved (Lundgren *et al.,* 1972). The reactions may be summarised:

$$2FeS_2 + 2H_2O + 7O_2 \longrightarrow 2FeSO_4 + 2H_2SO_4$$
$$4FeSO_4 + 2H_2SO_4 + O_2 \longrightarrow 2Fe_2(SO_4)_3 + 2H_2O$$
$$Fe_2(SO_4)_3 + FeS_2 \longrightarrow 3FeSO_4 + 2S$$
$$2S + 3O_2 + H_2O \longrightarrow 2H_2SO_4$$
$$Fe_2(SO_4)_3 + 6H_2O \longrightarrow 2Fe(OH)_3 + 3H_2SO_4.$$

These reactions occur chemically, but only very slowly at acid pH values. The sequence of reactions may therefore be self-limiting. However, certain bacteria, especially *Thiobacillus thiooxidans* and *Ferrobacillus ferroxidans* possess enzymes which bring about these reactions rapidly. These bacteria are abundant in acid mine waters, especially since they are tolerant of low pH whereas many species commonly found in soil and water are not. Further, they are autotrophic, that is they require no substantial amount of organic matter for growth, and soon dominate the flora.

The effects of acid mine drainage are threefold. The low pH itself has adverse effects on the receiving water flora and fauna. It also promotes the solubilisation of heavy metals, which exert their own toxic effects. Third, as the drainage water is diluted and the pH rises, ferric hydroxide precipitates and discolours the water, producing the effects of suspended particles. As the hydroxide settles, it forms a gelatinous layer over and within the substratum, causing both direct and indirect

effects on the receiving water community. In general, the result is a marked reduction in species diversity and biomass in the affected areas (Koryak *et al.,* 1972; Letterman and Mitsch, 1978; Scullion and Edwards, 1980). The effect on the receiving water community is influenced by the alkalinity of the water or the presence of nearby alkaline discharges; where the acid is neutralised, the effects are due to the ferric hydroxide alone, rather than to the combined effect of hydroxide and low pH.

A phenomenon which has recently received widespread attention is that water bodies in some parts of the world, especially in North America and Northern Europe, appear to be becoming steadily more acidic. The reason, it is suggested, is that airborne pollutants from industrial areas are transported by prevailing winds to areas remote from their source, and precipitated in 'acid rain'. The subject has caused much controversy both within the scientific community and among the general public, particularly since the problem not only has grave implications for important economic interests, but has already given rise to differences between the governments of some countries. It is not yet clear, for example, whether the main cause is sulphur dioxide emissions from power stations, or motor vehicle exhausts. Some countries have already begun expensive programmes to limit $SO_2$ emissions, while others argue that the extent of acid rain is not controlled by the amount of $SO_2$ emitted but by chemical reactions in the atmosphere and that the reduction of $SO_2$ emissions would therefore not lead to a decrease in acid rain. It is also clear that certain forestry practices lead to acid runoff from the soil into surface waters, so that the source of the problem may lie not in distant industrial areas, but within the affected areas themselves. These controversies do not directly concern us, but it is appropriate to consider the extent to which natural waters are becoming acidified, whether or not this is a natural phenomenon, and what its biological consequences may be.

The consequences of acidification are undoubtedly serious. Haines (1981) and Howells (1990) provide general reviews, and Baker and Schofield (1985) discuss the impact of acidification on North American fish populations. It is generally accepted that as pH decreases, both the diversity of species and the overall productivity of aquatic ecosystems decline. These phenomena can readily be observed through contemporary studies on waters of different pH values. Several mechanisms appear to be involved. First, every species has its own zone of tolerance to pH. The pH range which permits survival may be wider than that which will permit successful reproduction. This is particularly well documented for fish, both in laboratory experiments and through field observations (Baker and Schofield, 1985). Sutcliffe (1983) draws attention to the important influence of pH on the mechanisms of ionic regulation in aquatic species. Naturally-acid waters tend to be found in areas of base-poor geology and tend to be low in nutrients, dissolved

ions and buffering capacity. Therefore they have low primary productivity, present unusual difficulty to animals and plants in maintaining ionic balance, and are particularly susceptible to extraneous inputs of acid. The distribution of invertebrates in relation to pH was investigated in a catchment in northern England (Sutcliffe, 1983; Sutcliffe and Carrick, 1973) and revealed a pattern which is widely repeated elsewhere (Haines, 1981). Phytoplankton, zooplankton and macrophytes behave similarly in relation to pH. Generally, the pattern is of reduced species diversity as pH declines, although in favourable circumstances acid-tolerant species may become locally abundant. Undoubtedly, an apparently small change in pH can bring about major changes in community structure. (It may be appropriate to recall that a reduction of 1 pH unit represents a tenfold increase in hydrogen ion concentration; and that a pH change from, say, 6.9 to 6.6 means that the hydrogen ion concentration has approximately doubled.)

In addition, low pH values in theory could strongly and progressively reduce the rate of decomposition of organic detritus, presumably through the effect of low pH on the fungal and bacterial organisms responsible for this process (Haines, 1981). Many aquatic ecosystems depend on the decomposition of allochthonous detritus (i.e. organic material from outside the system, such as fallen leaves) as the main source of energy for the animals in the system. Primary (photosynthetic) production of organic matter in acid waters is naturally low, being normally limited by the low availability of nutrients; it may also be itself inhibited by the susceptibility of phytoplankton and macrophyte species to low pH. The alternative source of energy for animals, organic detritus, is only available in the presence of microbial decomposers, since animals cannot digest plant material unaided. Therefore the overall productivity of the system will decline. However, Gilmour (1992), reviewing the results of experimental acidification studies, concluded that the effects of acidification on microbial decomposition processes do not in practice become significant until the pH drops below 5, by which time other more direct effects on the fauna and flora will already have taken place. Field studies on acidified lakes in Europe largely point towards a similar conclusion (Howells, 1990). Indeed, such evidence as exists suggests that microbial decomposers and their associated biogeochemical processes are more robust to reduced pH than are higher organisms, and that nutrient cycling may actually be accelerated by low pH.

A third effect of acidification is to increase the threat of heavy metal toxicity. Aluminium, a metal which does not commonly cause serious problems of toxicity to aquatic life, has received particular attention. Burrows (1977) and Odonell *et al.* (1984) have summarised the role of aluminium as a toxic water pollutant. Although it is a very abundant metal, forming up to 7% of the earth's crust, it is highly reactive and readily forms stable compounds of very low solubility. In most natural

waters which have been investigated, the dissolved aluminium concentration lies between 0.05 and 10 mg $l^{-1}$. Within the pH range of approximately 5.5 to 7.0, aluminium is practically non-toxic and is certainly harmless at the concentrations found in most waters. The chemistry of aluminium and its compounds at low pH is poorly understood, but it appears that as pH falls aluminium compounds become more soluble and that the proportion of free aluminium rises. Below pH 4, the toxic effects of free hydrogen ion are so severe that the presence of aluminium is probably of little significance. However, between pH 4 and 5.5, the toxicity of aluminium is high, reaching a maximum at around pH 5. At this pH, the level of aluminium naturally present in water is acutely toxic to fish. High levels of calcium appear to offer some protection, although acid waters with high calcium levels are unusual. Aluminium toxicity is likely, therefore, to be a major contributor to the effects of acidification. Surges of acidity in upland rivers associated with snowmelt are not uncommon, and can be particularly damaging to salmonid fish populations which tend to be breeding at the time when snowmelt is likely to occur, leaving young fry vulnerable (Howells, 1990).

More rarely, aluminium can also cause toxicity in alkaline waters, as the solubility of aluminium compounds also increases with pH above 7.0. Aluminium compounds are widely used in the treatment of potable waters, and discharges from water-treatment works can have adverse consequences (Hunter *et al.,* 1980).

Finally, the question of whether acidification is a recent phenomenon, and whether it is a cyclic or an irreversible change, can only be answered indirectly. Accurate measurement of pH, particularly in weakly-buffered solutions like natural surface waters, has only been possible for about 30 years, and even within this timespan few water bodies have been studied systematically. It is also difficult to distinguish long-term trends from diurnal or seasonal pH variations, so the data available even from recent measurements of surface water pH are not altogether reliable. However, contemporary observations on the distribution of diatoms in relation to water pH show that the diatom community shows distinct qualitative changes according to the pH of the water. Analysis of the diatom community therefore indicates approximately the pH of the water. The characteristic siliceous frustules of diatoms accumulate at the bottom of lakes and decay only very slowly, so analysis of a core of lake sediment allows the changes in the diatom community over long periods of time to be determined. Thus, indirectly, any changes in the pH of the water may be inferred. Flower and Battarbee (1983) used this technique to estimate changes in the pH of two lakes in Scotland over several hundred years (Figure 2.9), and their results are typical of many studies. It appears that the pH of lake water declines slowly for natural reasons; rain is naturally acidic. However, the rate of decline of lake pH

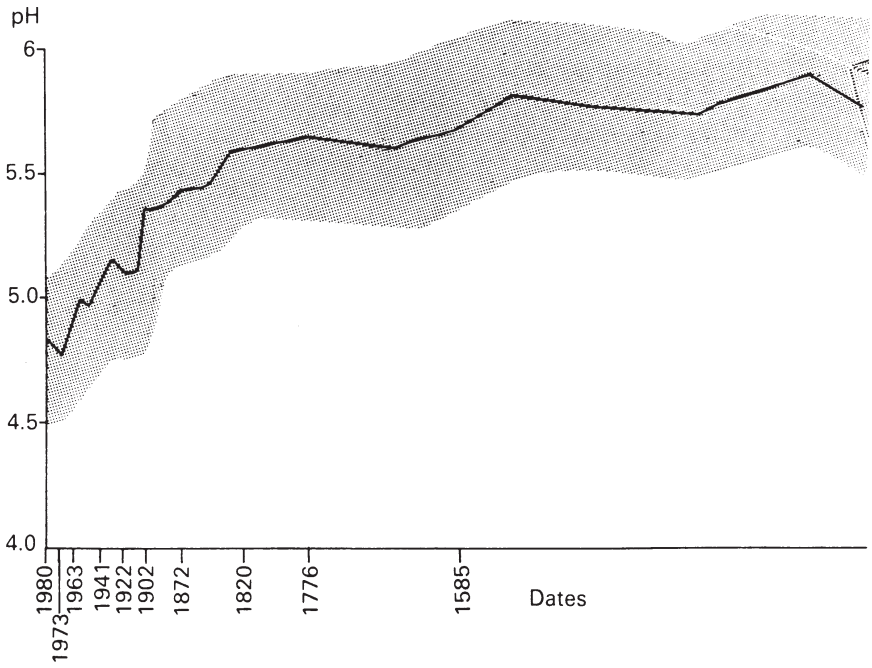



**Figure 2.9** Changes in pH of a lake in Scotland with time, inferred from the composition of the diatom community in lake sediment cores at different depths (Flower and Battarbee, 1983). (Reproduced by permission from *Nature*, **305**, pp. 130–132; copyright © 1983 Macmillan Magazines Ltd)

appears to have accelerated rapidly within the last 150 years or so, corresponding to the period during which Britain became heavily industrialised. Further discussion of many aspects of acidification is given by Howells (1990).

## 2.10 Detergents

Synthetic detergents are an interesting group of pollutants because they were virtually unknown before 1945, yet within a few years became responsible for some spectacular water pollution problems which, unusually, came rapidly to the attention of the general public. The alkylbenzene sulphonate detergents (Figure 2.10) rapidly replaced soap as domestic and industrial cleaning agents because of their cheapness and greater efficiency, and particularly because they did not cause precipitation of calcium salts in areas supplied with hard water. Unfortunately they were not readily broken down by sewage treatment processes, giving rise to problems of toxicity to the receiving-water biota, and of foaming in watercourses and treatment works.

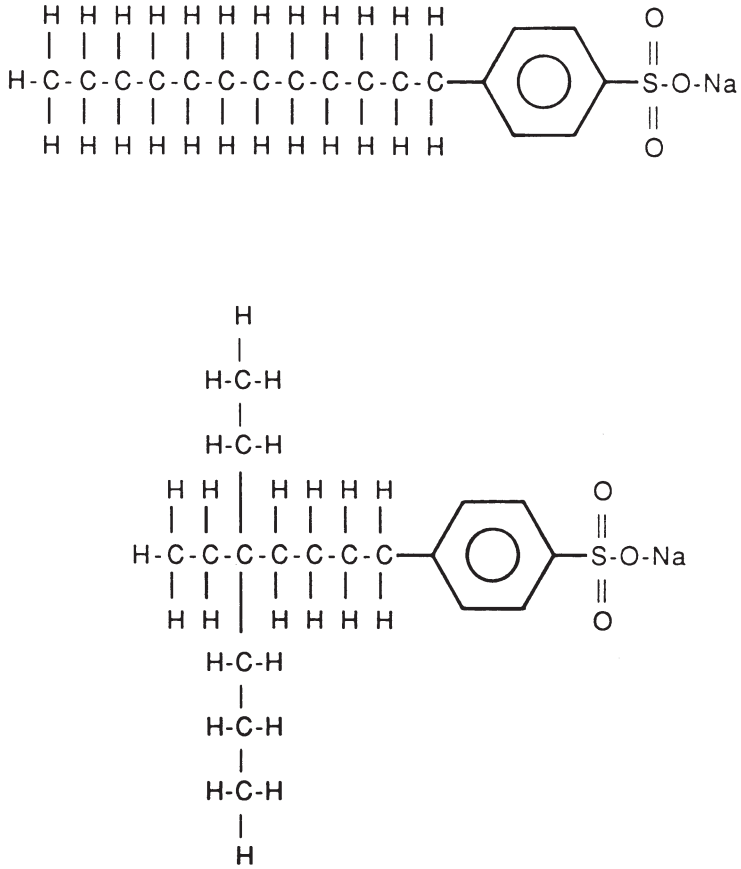



**Figure 2.10** Molecular structure of (above) a typical 'soft' (biodegradable) linear alkylate sulphonate detergent and (below) a 'hard' alkylbenzene sulphonate detergent

In areas where industrial usage of detergents was pronounced (for example, in textile-processing industries) whole towns were frequently covered in detergent foam; in waste treatment works, a number of serious accidents occurred through, for example, operatives falling into sedimentation tanks which were concealed under a thick layer of foam. Consultation between regulatory authorities and the detergent manufacturers led to research which showed that modifications to the manufacturing process could produce *linear alkylate sulphonate* (LAS) detergents (Figure 2.10), which were rapidly degraded in conventional waste treatment plants. From 1965 onwards, 'soft' or biodegradable detergents were introduced for domestic use, and although these are generally more toxic to aquatic organisms, their unbranched hydrocarbon chain is more readily broken

down in treatment processes and in practice, toxicity and foaming problems had largely disappeared by the early 1970s. Synthetic detergents remain significant causes of pollution in some circumstances however. Their toxicity to aquatic organisms is reviewed by Abel (1974). 'Soft' detergents are not suitable for use in certain industrial processes. Detergents are widely used as components of oil-dispersants, particularly in coastal and marine habitats, and are often more toxic to aquatic organisms than the oil itself (see Chapter 7). Finally, some components of detergent formulations exert adverse effects of their own. The best known example is the high level of phosphate found in many formulations (see Section 2.4). Less widely appreciated are the adverse effects of boron, from perborate additives to detergent formulations, which can cause adverse effects on crops if contaminated surface waters are used for irrigation (Lester, 1975; see also Table 2.2).

## 2.11 Oil and Petroleum Products

Oil pollution is commonly perceived as being a problem associated mainly with the marine environment, but in fact oil and related substances account for about one-quarter of reported pollution incidents of fresh waters in Britain (NRA, 1994a); and probably the pattern is not much different in other parts of the world. Also, about one-quarter of all the oil released to the seas by human activity is estimated to enter via rivers. The sources of oil pollution are the usual ones—illegal, negligent or accidental discharges, plant failures and so on—but to these must be added inputs from roads and railways, particularly in the case of traffic accidents, and discharges associated with oil extraction from inland oilfields and processing plants.

The effects of oil discharges vary enormously, because the characteristics of the receiving waters and the various types of oil are themselves very variable. Fortunately many small discharges have relatively trivial effects, but substantial discharges can have severe impacts. Some case studies are reviewed by Green and Trett (1989). Generally, light oils are relatively volatile and disperse quickly, though they are extremely toxic and can cause severe local damage. Fast-flowing waters often recover rapidly, over a few weeks or months, but lentic waters are much more susceptible to long-term damage. Heavy oils are less toxic, but can have marked physical effects in the substratum and the banksides, which of course will be reflected in changes to the biological community. Heavy oils may remain *in situ* for long periods; they do, over time, decompose biologically and chemically, but in so doing impose a high BOD. Frequently, the effects of oil pollution are not dissimilar from those of heavy organic pollution, with varying levels of toxic effects adding to the overall impact. Oils, unlike most forms of water pollution, can also have damaging effects on terrestrial organisms, such as plants, birds and mammals living at the water's edge, since heavy oil contamination usually leads to deposition of oil on the banks of lakes or rivers.

Generally, the use of oil dispersants, which are commonly used in dealing with marine spills, is avoided in freshwater because of their high toxicity. Also, the physical conditions of freshwater habitats are often amenable to containment and recovery of oil by booms, skimmers and/or the use of absorbent materials.

# 3

# Biological Monitoring of Water Quality

## 3.1 The Conceptual Basis of Biological Monitoring

We have by now seen ample evidence that the levels of abundance and patterns of distribution of aquatic organisms may be affected by pollution of the water in which they live. Hellawell (1977, 1978) summarised diagrammatically the changes which may occur in a community subject to pollution (Figure 3.1). Which, if any, of these responses occur will depend upon the nature and severity of the pollution, and upon the relative susceptibility of the species within the community to specific kinds of environmental alteration. It follows that given suitable techniques of sampling and data analysis, monitoring of the biological characteristics of waters might indicate the occurrence of ecologically significant environmental changes—including the incidence of pollution—which may otherwise be undetected.

This basic idea has been understood and utilised in monitoring the effects of pollution since the early years of the twentieth century (Cairns and Pratt, 1993), although many biologists have felt that the techniques of biological monitoring have not, at least until quite recently, been accepted and developed as rapidly as they might have been. There are probably several reasons for this. First, chemists and physical scientists are frequently sceptical of biological results which can sometimes appear to them to lack precision, and frequently require expert interpretation. Biologists themselves, perhaps under pressure to produce results quickly and economically, frequently have in fact failed to follow the best practice and have on occasions produced ambiguous, misleading or inaccurate results. In a recent analysis of published studies, Resh and McElravy (1993) found a number of cases where, for various reasons, some aspects of the monitoring programmes reviewed were

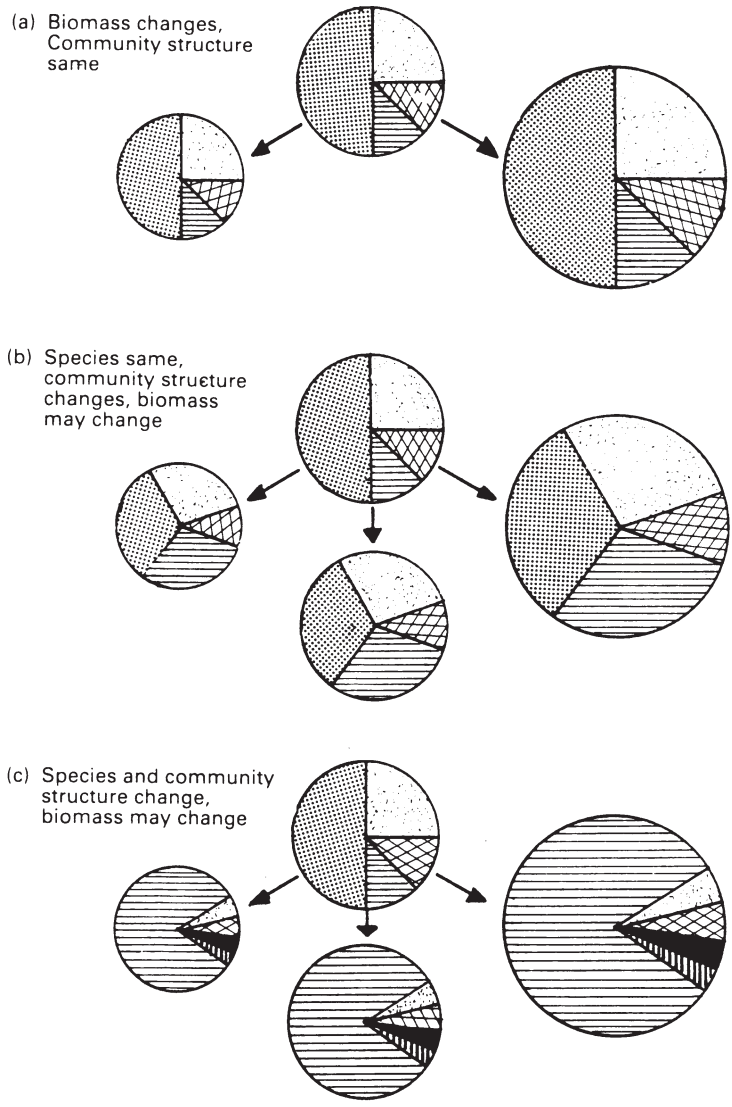



**Figure 3.1** Possible responses of a community to environmental change (from Hellawell, 1978)

inadequate in one way or another, casting doubt on the validity of the results. Insofar as biological monitoring procedures have been adopted in the past by regulatory agencies, biologists have encountered resistance from legislators and administrators who are reluctant to modify regulatory practices, laws and regulations to take account of improvements in techniques, preferring instead to rely on 'established' methodology which more recent research shows can be greatly

improved upon. Finally, there is the stubborn belief that biological monitoring is prohibitively expensive—it certainly is demanding of trained human resources. However, in a very illuminating discussion of this and some of the other points raised above, Brinkhurst (1993) produces evidence to show that the cost-effectiveness of biological monitoring techniques actually compares favourably with physical and chemical methods.

Perhaps optimistically, Rosenberg and Resh (1993) express the view that 'Biomonitoring may have come of age with the recent adoption, by North American and European governments, of national programs of environmental monitoring and assessment that include the use of aquatic biota'. Certainly an impressive consensus has been developing over the last decade or so among actual practitioners of biological monitoring. In fact, this consensus has been in existence among specialists for much longer than that, though it has not always been appreciated by those who wish to utilise their services but who do not wish to pay the necessary price in patience, financial resources or flexibility of attitude. We should also remember that in any field in which new ideas and techniques are being constantly developed, the consensus must always lag a little behind the best approaches, and must itself evolve rather than be fixed in stone. The evolution of this consensus is perhaps best traced through the literature of biomonitoring during the last 20 years (see, for example, Hellawell (1977, 1986); Metcalfe-Smith, (1994); Rosenberg and Resh, (1993)). Now that techniques of biological monitoring are becoming more widely accepted, there are also specific recommendations published in accessible form by reputable agencies, which are a useful source of guidance to the relatively inexperienced. These include APHA (1995), ISO (1985, 1991) and DIN (1983a, b, 1987, 1990). The purpose of this chapter is to give a general introduction to the best current practice. Biological monitoring is a powerful tool, but a very dangerous one if not used correctly.

Biological monitoring programmes are carried out for a variety of reasons. For example, most regulatory agencies, such as water authorities, routinely monitor the biota of the waters for which they are responsible. Typically, sampling stations are surveyed at intervals of between one and six months. Information is gathered on the presence and relative abundance of species, and may be used to derive numerical values, for example of species diversity or biotic index (see Section 3.4), to facilitate spatial or temporal comparisons. Significant alterations from previously-established conditions may indicate the need for further investigation, and lead to action designed to preserve or improve the existing water quality. Sites known to be at special risk from existing pollution sources, and sites of special commercial or conservation value, for example salmon rivers, may receive special attention such as more frequent or more extensive surveillance. Proposed new developments, such as the siting of new effluent discharges, abstraction from or canalisation of watercourses, may be preceded by programmes of biological surveillance to establish 'baseline' biological conditions of the affected water. After implementation, the biota

may continue to be monitored to determine the biological effects of the development and to assist in minimising them. Biological monitoring of receiving waters may also, of course, be used to establish the effectiveness of effluent treatment processes and of the often expensive improvements to them which may be undertaken. Lemlin (1980) describes a good example of this approach. The effectiveness of a ten-year programme of improvement in the effluent treatment plant of an oil refinery was monitored by studying the distribution and abundance of saltmarsh flora in the area receiving the effluent. Since the cost of the alterations to the treatment plan was measurable in millions of pounds, confirmation that this cost was justified in terms of a measurable improvement in the receiving environment was of value to those responsible for the management of the refinery.

Living organisms can provide useful indications of the chemical quality of water. (The term 'sentinel organism' is frequently used to describe the use of living organisms in this way.) For example, chemical analysis of biological material has been widely used to indicate contamination of the environment with levels of contaminants which may be potentially damaging in the long term, but which may not be otherwise detected. Biological monitoring, in conjunction with physical and chemical surveillance of water quality, generates information which is potentially useful in establishing water quality standards. To determine the level of a pollutant which is 'acceptable' (however that may be defined) is a frequently-recurring problem, and many toxicological and ecophysiological investigations are designed to provide information on this point. Complementary information may be provided by analysis of biological and chemical survey data, which may indicate directly the association between particular chemical conditions and the biological characteristics of the water.

Using modern techniques, chemical and physical analyses of water quality can be made with great precision. As will be seen, biological monitoring can be expensive of time and of human and physical resources. Its justification, however, is clear when some of the limitations of purely physical and chemical monitoring are considered. For example, a chemical analysis will only reveal the presence of the substance which the analysis is specifically designed to detect. The presence of a hitherto unsuspected pollutant would therefore not be detected by routine chemical surveillance, since it is uneconomic routinely to analyse water for large numbers of pollutants which might occur only very rarely. Biological surveillance, however, if properly carried out, might reveal the occurrence of ecologically-significant environmental changes and call attention to the need for further investigation.

Second, the concentration of pollutants in receiving waters fluctuates widely and rapidly. Even if samples are taken very frequently, it is highly probable that peak concentrations, which may be of greater biological significance than the 'background' levels, will be missed entirely (Figure 3.2). Unlike a chemical analysis, a biological survey does not simply indicate the

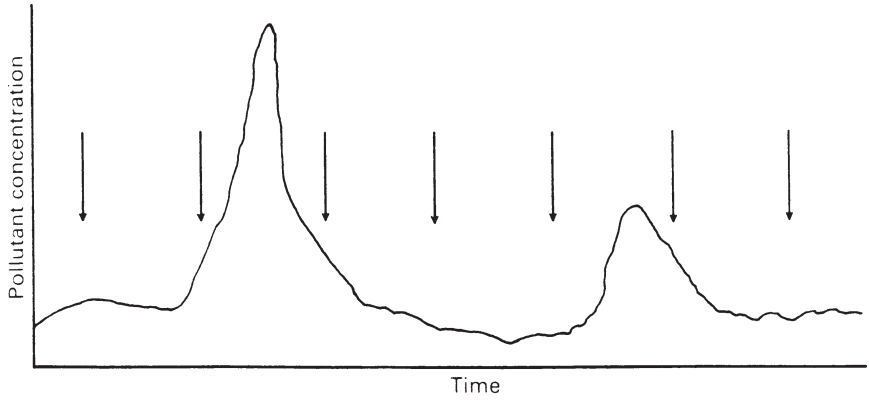



**Figure 3.2** Chemical sampling, even at frequent intervals (arrows) can miss biologically-significant peak concentrations

conditions prevailing at the instant of sampling. The organisms which live in the water respond to the totality of the environmental conditions which they have experienced throughout their lives. This is also important because pollutants usually occur in unpredictable mixtures rather than singly, and their action on aquatic organisms is subject to the modifying effects of environmental conditions (e.g. dissolved oxygen, temperature, water hardness) which themselves fluctuate and interact. The results of experimental studies are therefore difficult to apply to field situations (e.g. in terms of formulating water quality standards) in the absence of reliable information concerning the performance of species in polluted conditions in real rivers. Good biological monitoring programmes are thus complementary to toxicological and ecophysiological studies—each facilitates the interpretation and application of the results of the other.

Finally, biological monitoring data can not only be used to obtain information about the existing quality of the water, or to ensure compliance with pollution control measures, or to check on the efficiency of waste treatment processes; it can also be used to formulate water management strategies, notably through the identification of technically and economically feasible water quality objectives. Indeed, this arguably most advanced strategy of water quality management absolutely requires extensive and accurate biological (and physico-chemical) monitoring data, and is further discussed in Chapter 6.

## 3.2 Indicator Organisms

It is fundamental to ecology that an organism cannot survive indefinitely in an environment that does not provide its physical, chemical and nutritional requirements. Thus the presence of a particular species, especially if it is reasonably abundant, indicates that its environmental requirements are being

met. Its absence, however, does not *necessarily* indicate the converse—one species may, for example, be competitively excluded from a particular habitat by another. Nevertheless, within certain limitations the presence, absence or relative abundance of species may be used as indicators of environmental quality. Changes in the presence, absence or relative abundance of species, whether sudden or gradual, may therefore imply a corresponding change in environmental conditions. In its broadest sense, then, the term *indicator organism* can be used of any member of the fauna or flora of a habitat, and any species may be considered as a potential indicator organism.

Some organisms have such wide tolerances of different environmental conditions that their patterns of distribution or abundance are only slightly affected by quite wide variations in environmental quality. These species may tell us little about their environment which is not readily apparent by other means. The term *indicator organism* is therefore sometimes reserved for those species which have narrow and specific environmental tolerances, so that they will show a marked response to quite small changes in environmental quality. If the environmental factors which are most commonly limiting to the species concerned are known, its presence will be indicative of a specific environmental condition. Thus, nymphs of Plecoptera (stoneflies) are not found in waters where the dissolved oxygen concentration falls substantially below its saturation value for appreciable periods of time. Hence their presence indicates that the water is well oxygenated; and although their absence does not *necessarily* indicate the converse, their absence from waters wherein they might normally be expected at least suggests a possibility for further investigation. In practice, however, it is rare that enough is known of the ecology and physiology of a species for its presence or absence to be indicative of a specific environmental condition. There are, furthermore, very few organisms whose presence specifically indicates that the water is polluted. One exception may be, for example, the presence of coliform bacteria, indicating faecal contamination. Although several organisms are known frequently to be associated with polluting conditions—for example Tubificid worms, and the 'sewage fungus' *Sphaerotilus natans*—they do occur widely in environments which are not polluted. In the present discussion, indicator organisms are considered to be 'those which, by their presence and abundance, provide some indication, either qualitatively or quantitatively or both, of the prevailing environmental conditions' (Hellawell, 1978). The use of the term 'indicator organism' in this sense should be distinguished from the use of the same term to apply to organisms whose tissue levels of contaminants are measured in order to infer the possible extent of chemical contamination of the environment ('sentinel organisms'). The term is widely used in both senses and it is important to distinguish between them.

Ideally all members of a community should be considered as potential indicators of water quality and included in biological monitoring programmes. In practice groups such as the bacteria, algae, protozoa and macroinvertebrates require such different sampling methods and taxonomic

skills that most investigators choose only one such group. Normally this is found satisfactory except for the most extensive and detailed research programmes. The group most commonly employed as indicators is the macroinvertebrate fauna. These possess many of the characteristics required of indicator organisms, including:

1 They are a diverse group in which some hundreds of common species from several different phyla are represented, so there is a reasonable expectation that at least some will respond to a given environmental change.
2 They have relatively limited mobility and relatively long life cycles, so may sensibly be used for temporal and spatial analyses.
3 Good identification keys are available for most macroinvertebrate groups, and it is possible to achieve a reasonable level of taxonomic competence quite quickly.
4 Their high levels of abundance under favourable conditions facilitate quantitative analysis. Sampling techniques are fairly simple and well developed.

The disadvantages of macroinvertebrates as indicators, like their advantages, are common to some other groups. Seasonal variations in presence or abundance are a normal feature of many life cycles, so results need cautious interpretation. Many species drift passively downstream in substantial numbers, and may therefore be found in areas in which they cannot survive indefinitely. Finally, although it is relatively easy to obtain good qualitative samples of macroinvertebrates, reliable quantitative sampling is difficult owing to the physical characteristics of aquatic habitats and the complex horizontal and vertical distribution patterns of at least some species.

Although much of this chapter will be concerned specifically with invertebrates, many of the points discussed are of general relevance and it is undoubtedly true that other groups could, with advantage, be more widely used as indicators. The use of algae as indicators is discussed by Whitton (1979) and Shubert (1984). A scheme for the use of macrophytes as indicators of water quality has been proposed by Haslam (1982). The case for protozoa as indicators is put by Cairns (1974, 1979). The advantages and disadvantages of bacteria, protozoa, algae, macroinvertebrates, macrophytes and fish have been summarised by Hellawell (1978, 1986). More detailed discussions of several of the commonly-used groups of indicator organisms may be found in the volumes edited by Hart and Fuller (1974), James and Evison (1979), Hellawell (1986) and Rosenberg and Resh (1993).

## 3.3 Sampling Methods

The validity of any ecological investigation depends crucially upon the sampling technique and strategy adopted at its outset. Many factors influence the design of a sampling programme. These include: the objectives of the investigation and the type of analysis to which the data are to be subjected; the physical characteristics

of the habitat to be sampled; the characteristics of the organisms to be sampled—their size, habits, abundance and patterns of distribution; and constraints on the human, physical and financial resources available for the investigation. Frequently, a sampling programme is devised on the basis of a series of compromises between what the investigator would like to do, and what it is possible to do. In order to make sensible decisions about sampling—in particular, how to maximise the quantity and *quality* of the information obtained for a given sampling effort—it is necessary to understand something of the characteristics of the sampling techniques available. In the present discussion, some of the more commonly-used methods will be briefly described and their performance as qualitative and quantitative techniques examined.

Good general descriptions of the various methods are given by Southwood (1978), Hynes (1970), Green (1979) and Hellawell (1978, 1986). Elliot (1977) and Hellawell (1978) discuss the statistical considerations underlying the choice of sampling strategies and the analysis of data. Many experienced practitioners tend to make judgements about sampling strategy more or less intuitively; but in a very stimulating discussion of the considerations underlying the development of monitoring programmes, Parr (1994) persuasively argues the case for a more considered approach. His discussion offers useful advice on designing monitoring programmes for various purposes, covering points such as the location of sample sites, frequency of sampling, and choice of data analytical methods. These considerations are all mutually interactive, and decisions and choices should always be made with this in mind, and with a clear idea of the purpose of the investigation. In view of the increasing importance of accurate biological sampling in the overall management of aquatic habitats, several national and international agencies have recently offered specific guidance and recommendations which are designed to be of assistance to inexperienced investigators and also to promote standardisation of techniques. These include APHA (1995), ISO (1985, 1991) and DIN (1983a, b, 1987, 1990).

Probably the most widely-used macroinvertebrate sampling method is the so-called ‘kick sample’. A standard pond-net is held facing upstream, while the operator or a partner disturbs the substratum, preferably by hand rather than by actual kicking. Disturbed animals are washed into the net. Some operators, by sampling from a known area or for a standard period of time, attempt to introduce a quantitative element into the technique. However, it is very difficult in practice adequately to standardise either the area sampled or the duration of a sampling period, and almost impossible to standardise on the actual sampling effort, that is the degree of vigour or enthusiasm with which the sampling operation is carried out! Consequently large variation between samples and between operators is to be expected, and although it

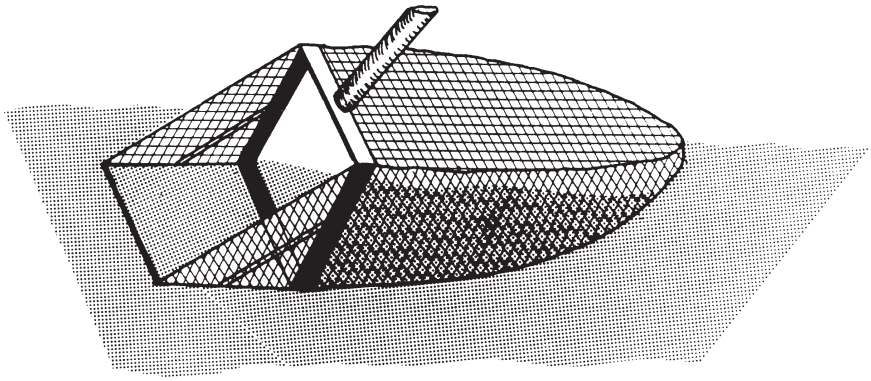


**Figure 3.3** A Surber sampler (from Hellawell, 1978)

is possible to obtain good qualitative samples, the technique is rarely acceptable as a quantitative one.

The Surber sampler (Figure 3.3) is designed to reduce some of the problems associated with operator variability and the standardisation of sampling effort and sampling area. A quadrat (usually 0.1 $m^2$) is attached to the frame of the collecting net in such a way that it can be placed on the substratum. The substratum within the quadrat is disturbed and animals are washed by the current into the net. Triangular vanes of netting at the sides of the sampler are designed to reduce the loss of material around the sides of the net. Box or cylinder samplers (Figure 3.4) of various designs operate on a similar principle. Here the objective is to isolate as completely as possible the area to be sampled by pushing the sampler into the substratum. Both Surber and box/cylinder samplers can be used quantitatively, but both types can only be used in fairly shallow water.

For deeper water, grabs or dredges must be used. Both are available in a wide variety of designs (Figures 3.5, 3.6). Grabs are the preferred method for fine substrata, but their efficiency is often low. Elliot and Drake (1981a) compared the performance of seven commonly-used designs. In one series of trials, the grabs were used to catch plastic beads (representing animals) from tanks containing known densities of beads in gravel of various sizes. The efficiencies of the grabs, in terms of the numbers caught as a percentage of the number available to be caught, are summarised in Table 3.1. The efficiency of all grabs was low when the modal particle size exceeded 16 mm and some performed poorly under all conditions. Furthermore, the grabs in general operated inefficiently when the 'animals' were buried 3 cm below the surface.

For sampling deep water with coarse substratum, dredges (Figure 3.5) may be used. These are dragged along the bed collecting substratum and animals

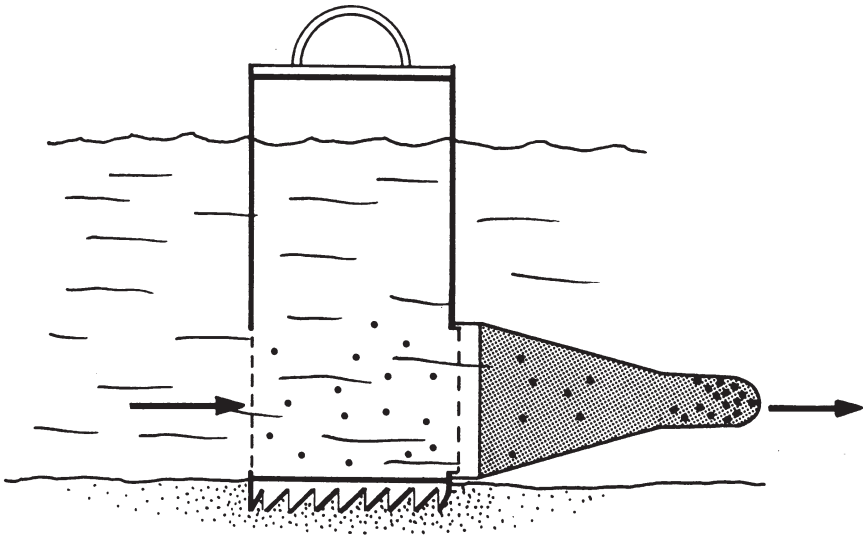


**Figure 3.4** A cylinder sampler

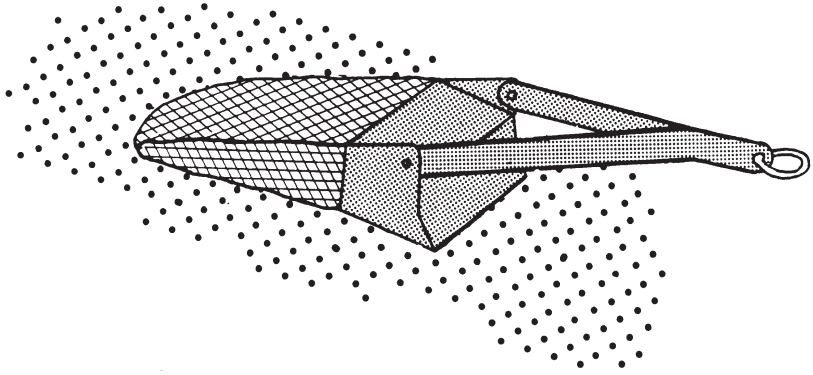


**Figure 3.5** A dredge sampler (from Hellawell, 1978)

in the collecting net. Again, it is impossible in practice to standardise the area sampled and consequently to use dredges as reliable quantitative samplers. Elliot and Drake (1981b) assessed in field trials the efficiencies of four dredges as qualitative samplers. For each sample, a dredge was dragged along the river bed for five metres. The qualitative efficiency of each dredge was determined by expressing the number of taxa caught in five samples as a percentage of the total number of taxa caught at each site by all four dredges. Maximum values ranged from 76% for the most efficient to under 40% for the least efficient. These values were biased by the fact that the larger

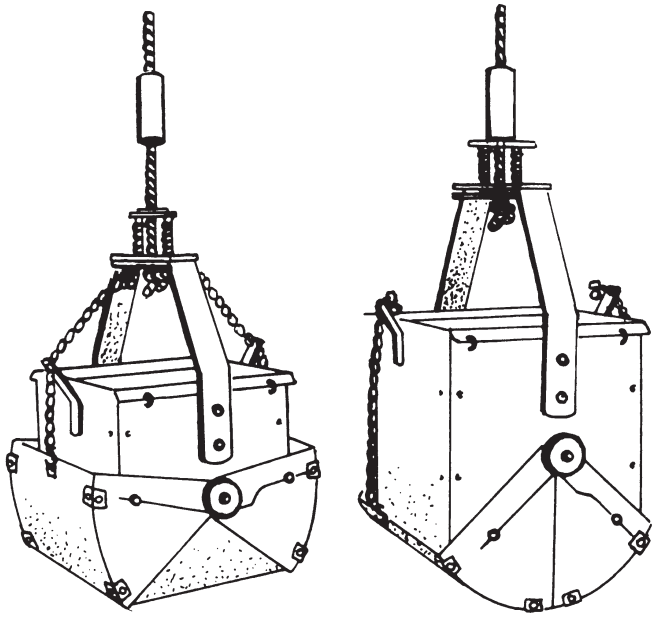


**Figure 3.6** An Ekman grab in its open (left) and closed (right) positions

**Table 3.1** Efficiencies of seven grabs at recovering animals from the surface and below the surface of two different substrata. Data from Elliot and Drake (1981a)

| | % Efficiency | | | |
|---|---|---|---|---|
| | Substratum 2–4mm | | Substratum 8–16 mm | |
| Grab | Surface | 3 cm below surface | Surface | 3 cm below surface |
| Ponar | 100 | 70 | 88 | 50 |
| Weighted Ponar | 100 | 70 | 100 | 50 |
| Van-Veen | 87 | 56 | 50 | 50 |
| Birge–Ekman | 73 | 37 | 30 | 7 |
| Allan | 51 | 36 | 25 | 7 |
| Friedinger | 59 | 7 | 30 | 7 |
| Dietz–La Fond | 22 | 26 | 25 | 20 |

dredges caught more individuals; a more reliable indicator of the comparative performance of the dredges is that the least efficient design would need to be used 150 times to take a sample comparable with that obtained by using the most efficient dredge only five times.

A completely different sampling technique is to place artificial substrates

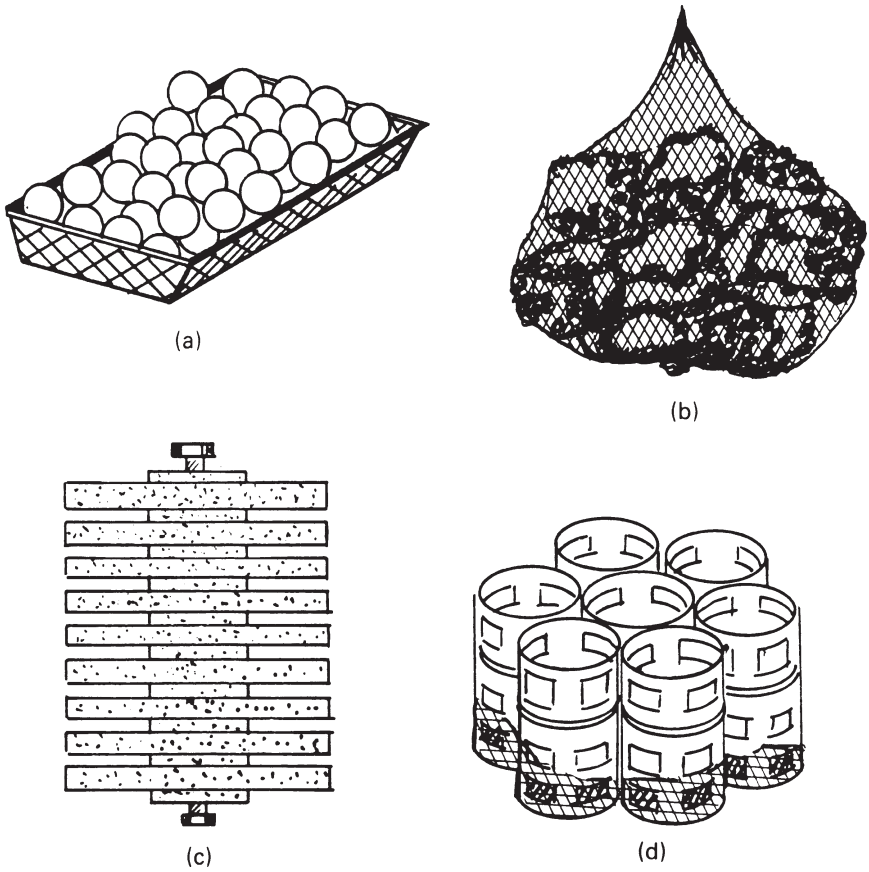



**Figure 3.7** Some designs of artificial substrate samplers: (a) Wire tray or basket filled with stones; (b) Slag bag; (c) Multiplate sampler; (d) SAUFU (Standard Aufwuchs Unit)

in the water. These may take various forms (Figure 3.7) for example baskets or trays filled with stones, porcelain spheres, etc.; 'multiplate' samplers; or bundles of plastic material. Two designs which have been extensively evaluated in Britain are the slag bag, and the SAUFU (Standard Aufwuchs Unit) (HMSO, 1985). The slag bag consists of standardised quantities of graded slag (as used in sewage filter-beds) contained in a bag of plastic mesh. The SAUFU is constructed from a number of units of synthetic plastic material, again originally designed for use in sewage filter beds and similar processes, arranged in a circular configuration. After a period of time they become colonised by plants and animals and can be recovered from the water and the biota extracted. Sampling units may be standardised, for example by using graded stones or completely artificial material. The advantages of standardisation, however, must be weighed against the possibility that some

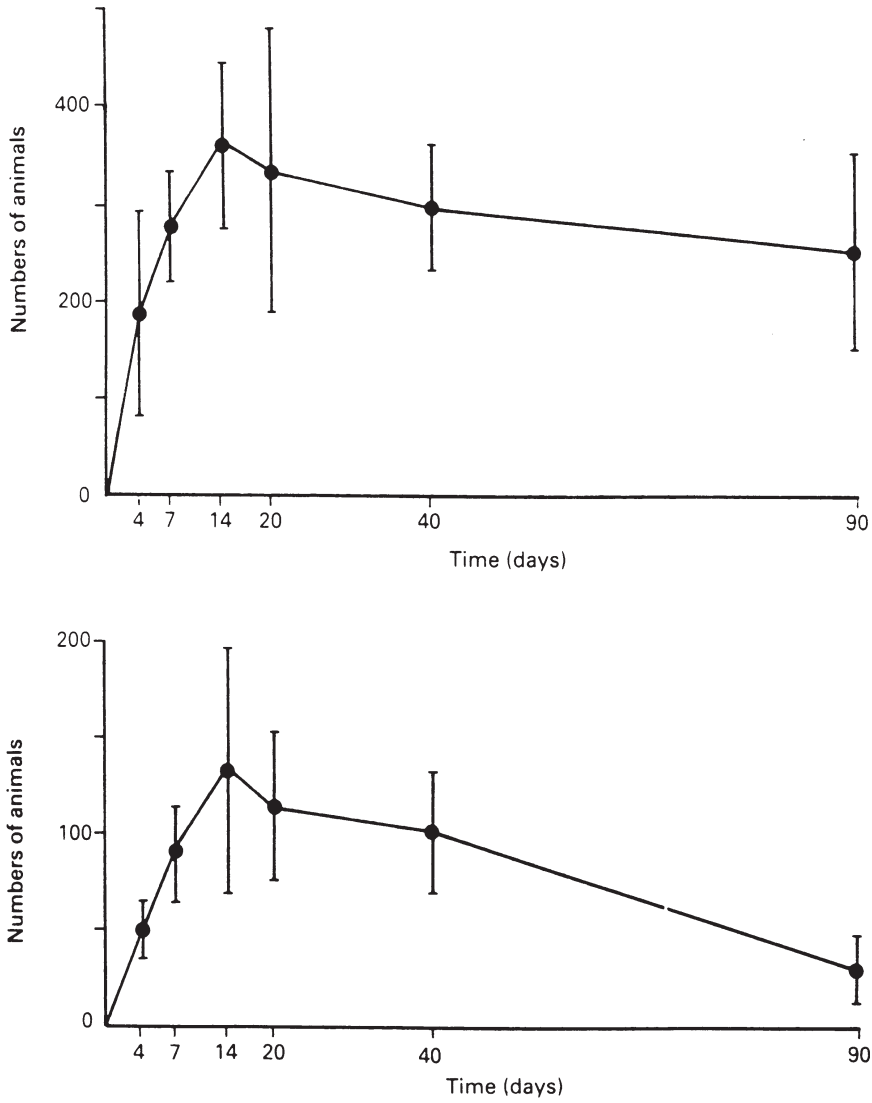



**Figure 3.8** Colonisation of artificial substrate samplers exposed on a gravel river bed (upper graph) and a muddy river bed (lower graph) (from Hellawell, 1978, based on data given by Pearson and Jones, 1975)

designs are likely to be selective, that is the colonising fauna may be unrepresentative of the community sampled. Samplers may be embedded in the substratum, rested upon it, or suspended in mid-water. Typically it may take several weeks for samplers to be fully colonised, and frequently the numbers of taxa and numbers of individuals

recovered reach a peak and then decline (Figure 3.8). Thus it is important to standardise the colonisation period. APHA (1995) recommends six weeks as the optimum period, though this may vary from one location to another, and also depending upon the purpose of the survey; whether, for example, it is to determine the maximum number of species present or to obtain a representative sample of the benthic fauna.

### *3.3.1 Evaluating Sampling Techniques*

An important characteristic of a qualitative sampling technique is the rate of taxon accretion, that is the relationship between sampling effort and the number of taxa caught. Obviously it is important to ensure that a qualitative sample contains a reasonably high proportion of the species actually present. To determine the extent of the sampling effort required to achieve this, a taxon accretion curve (Figure 3.9) should be plotted. The correct procedure is to take a large number of samples, and determine the mean number of taxa recovered, with confidence limits. Samples are then considered together in randomly-chosen pairs, threes, fours and so on. (A simple computer program can be used to calculate the values for all possible permutations of two, three, four, etc.) A simpler method is to plot the cumulative number of taxa recovered against the number of samples taken, but this method can give misleading results owing to the possibility that the earlier samples may, by chance, contain abnormally high or abnormally low numbers of taxa. The resulting curve (Figure 3.9), shows the point at which large increments in sampling effort yield few additional taxa, and the optimum sampling effort can thus be determined. As a guide, general experience shows that about five Surber samples, or 10–20 minutes of kick sampling, yields about 80% of the total number of taxa, though obviously this depends upon the characteristics of the habitat, and the competence with which the sampling is undertaken. Experienced operators tend to recover about 40–50% of the available taxa in one three-minute kick sample, whereas inexperienced operators achieve only about half this level of efficiency. Although it is rarely feasible to recover all the rarer taxa, it should be remembered that when making spatial or temporal comparisons between communities it is often the presence or absence of the rarer species that provides the most useful information.

Kick sampling is in practice the basis of most routine monitoring programmes, and an evaluation of the technique by Furse *et al.* (1981) yielded some interesting results. Four physically-different sites on a river were sampled by three experienced operators, each operator sampling each site for two periods of three minutes. The mean number of taxa caught per sample varied from 31.9 to 37.5 families, and from 48.3 to 58.3 species. Analysis of variance of the data showed that differences in the number of taxa per sample were significant with respect both to sites and to operators. The

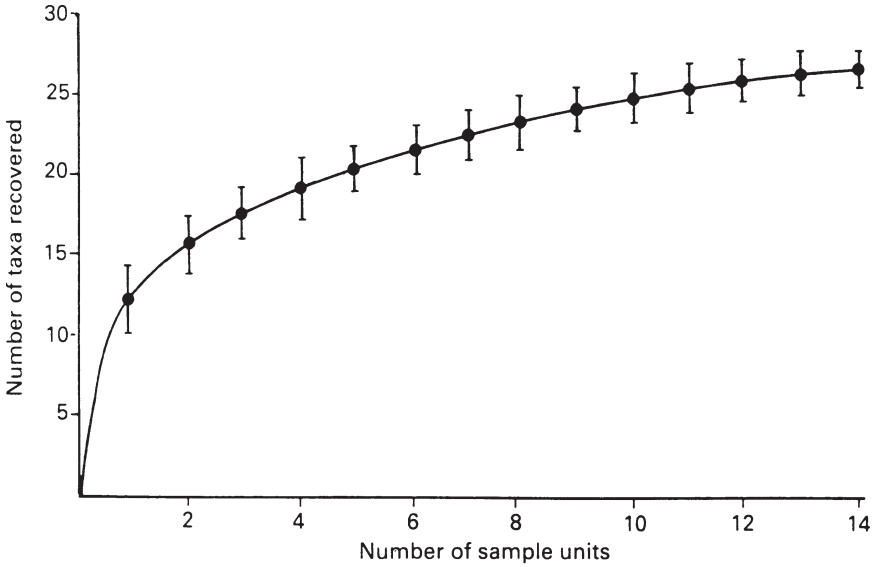



**Figure 3.9** A taxon accretion curve

question then arises of whether the inter-operator differences are so large as to outweigh inter-site differences. If this were the case, kick sampling would be inadequate even as a qualitative technique since most monitoring programmes involve several different operators. To test this, the authors subjected their data to ordination and average-linkage cluster analyses. Six samples were taken at each site, and if inter-site differences were sufficiently large to outweigh inter-operator differences, the result of these analyses should be that each group of six samples should group together and be distinct from each other such group. (The principles of cluster analysis are described in Section 3.4.)

Samples were first classified using similarity coefficients (see Section 3.4) based on the presence or absence of *families*. This resulted in several 'misclassifications', that is samples which grouped with those from sites other than their own. The similarity coefficients were then recalculated to take into account the relative abundance of each family, rather than simply its presence or absence (the advantages of doing this are described in Section 3.4). The resulting plots showed a marked reduction in the number of misclassifications. Perfect inter-site segregation could, however, be achieved by using data on the presence or absence of *species*. These results indicate that in addition to sampling technique, the methods of data analysis and the level of taxonomic skill employed are important contributors to the accuracy and validity of biological monitoring. An intrinsically weak sampling technique may yield biologically meaningful results if adequate taxonomic skills and analytical methods are brought to bear. In connection with the latter, however, caution must be exercised. It is a common weakness of many ecological investigations to use

sophisticated analytical techniques on inherently poor data, as if mathematical manipulation could compensate for bad experimental design or inadequate sampling effort. A good test is whether the data analysis employed is biologically sensible. In the present case, if one site contains 99 individuals of taxon A and one of B, and another contains one of A and 99 of B, they are clearly biologically different. To work in terms of species presence and absence, therefore, is wasting valuable information—since both contain A and B, we might erroneously conclude that they are both the same. Thus the use of a more sophisticated coefficient of similarity is biologically justified.

Quantitative sampling is obviously more demanding than qualitative sampling. Estimates of relative population density are often useful in biological monitoring. It may be valuable to know, for example, that the abundance of a species has halved, or doubled, over time, or that the ratio of abundance of two species has altered. Provided that the data are interpreted cautiously, most sampling methods will yield some information on the relative abundance of the taxa recovered. However, there are several factors other than the actual population density which influence the catch per unit sampling effort. Southwood (1978) offered a very accessible explanation of some of these, and urged the adoption of a more careful approach to the interpretation of relative abundance data than is often shown. Many of the methodological texts referred to earlier in this chapter provide guidance on how these principles need to be applied in the specific context of biological monitoring.

The estimation of absolute population density is extremely difficult. The number of samples required to estimate population density is given approximately by the formula:

$$n = \left( \frac{st}{D\bar{x}} \right)^2$$

where $n$=the number of samples required; $\bar{x}$ and $s$ are the mean, and its standard deviation, respectively of the number of individuals per sample caught in a pilot survey; $t$=the value of Student's $t$ for the required level of confidence; and $D$=the index of precision, that is the ratio of the standard error to the arithmetic mean expressed as a decimal.

Thus for example, if the mean number of individuals per sample is 10 and the standard deviation is 5, to obtain an estimate of the population density which lies within 10% of the true value with 95% confidence,

$$n = \left( \frac{5 \times 2}{0.1 \times 10} \right)^2 = 100 \text{ samples.}$$

The numbers chosen for this example are typical of values found in real investigations. Unfortunately, the pattern of distribution of benthic invertebrates

(and of many other kinds of organism) seems to be such that very large numbers of samples are required for reliable estimates of population density. Even to estimate population densities to within ±20% or ±40% of their true values may require several hundred samples (Elliot and Drake, 1981a; Hellawell, 1977, 1978).

A further difficulty is that nearly all commonly-used sampling techniques are very superficial, in that only the top few centimetres of the substratum are sampled. That this can lead to serious errors is shown by the results of Coleman and Hynes (1970). They used artificial substrate samplers embedded 30 cm into the substratum and divided horizontally into four equal layers. Only 20% of the total catch was found in the top 7.5 cm and 26% was found between 22.5 and 30 cm deep. Clearly animals burrow deep within the substratum and only a small proportion of the population is recovered by normal sampling techniques. Radford and Hartland-Rowe (1971) compared a Surber sampler with an embedded sampler of 17.5 cm depth. Although the samplers produced similar qualitative results, the Surber took only 10% of the numbers and 53% of the biomass taken by an equivalent area of embedded sampler. Even allowing for the greater volume of substratum sampled by the embedded sampler their data suggest that smaller individuals, which may comprise a substantial proportion both numerically and in terms of biomass, are not adequately sampled by the usual techniques. The limitations of sampling techniques are to a large extent a reflection of the characteristics both of the habitats under investigation and of the organisms which live in them. An appreciation of these limitations is essential at all stages of an investigation—in its design, in the analysis of the data, and in the formulation of its conclusions.

## 3.4 Data Analysis and Interpretation

The interpretation of biological survey data is essentially a series of comparisons—spatial, temporal, or both—and a variety of methods of data analysis are available to facilitate the process. At this point, however, it must be stressed that the following discussion is primarily based around *current practice* in biological monitoring of water quality. Over the last ten or 15 years, a body of opinion has been growing that current practices are generally flawed because inadequate consideration has been given to the basic requirements of design and analysis of data from field surveys. This view has recently been eloquently summarised by Underwood (1994). The basic argument is that most monitoring programmes are ill-designed in that they do not involve a testable hypothesis, they are incapable of distinguishing an anthropogenic impact from coincidental and confounding factors and that they are therefore naive and unreliable, if not a complete waste of time. The implications of this are profound, given that current legislation all over the world leads major environmental decisions to be made on the basis of what may be completely false information. On the other hand, proper methodology could lead to monitoring techniques which are more accurate, more simple and more cost-effective of

resources. It will probably take two more decades before these ideas are reflected in practice, but in order to increase the rate at which this occurs, or at least to promote the evaluation of their validity, the present description of practices and procedures currently in use should perhaps be compared with the propositions put forward in Underwood's (1994) discussion.

Consider a single sampling station at a single point in time. Our sample or samples have yielded data in the form of a list of species, and perhaps an indication of their relative abundance. Assuming that the sample is reasonably representative of the community from which it was drawn, that it has been obtained by an appropriate sampling method, we wish to infer from the sample data whether that community is affected by pollution. To know if pollution is affecting the community, we need to know whether that community is 'normal' or not. This can only be done by making temporal or spatial comparisons—is the community at this site the same as it was on some previous occasion? Or is it similar to physically comparable sites at different locations? Clearly in order to answer these questions we need to know the range of temporal and spatial variation in community characteristics which may be considered 'normal'. To the extent that this knowledge is lacking, no matter how sophisticated our analytical techniques, a reasonable interpretation is impossible. Therefore a major need is for basic knowledge of the range of temporal and spatial variation in the community characteristics of *unpolluted* habitats.

In practice such information is available to varying degrees in particular cases, and the comparisons made will be determined by the extent of 'baseline' information available, and by the purposes of the survey. For example, comparisons may be made between different sites along the length of a river, or between samples taken from the same site at different times. Hellawell (1978, 1986) provides a very full discussion, with examples, of the methods which may be used to analyse and present the results of biological surveys. The results of such comparisons may be simply expressed as in Figure 3.10, by plotting some characteristic of the community (in this case, numbers of species) against distance along the river. Such simple procedures may show a clear and comprehensible pattern, but obviously much of the information obtained from the survey is not being used. For example, there may be changes not only in the numbers of species present, but also in their relative abundance. Further, the species present at some sites may be completely different from those present at others. Such valuable biological information should not be wasted. One solution to this problem is to use one or more of the indices available (see below) as the variable which describes the communities. In Figure 3.10 it can be seen that the Chandler Biotic Score more clearly demonstrates the biological impact of the effluents

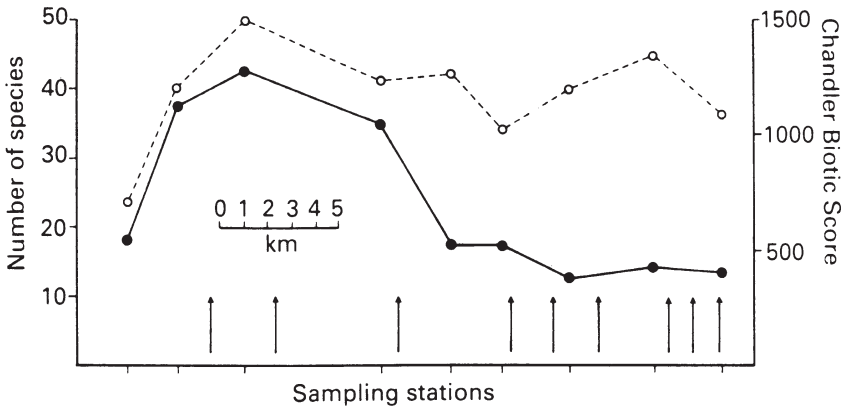



**Figure 3.10** Number of invertebrate species (dotted line) and Chandler Biotic Score (solid line) at stations along the length of a polluted river. Arrows indicate inputs of pollution. Adapted from Hellawell (1978)

than does the number of species present at each station. However, it is not always the case that such derived values are more useful than the raw data, and it is always advisable to compare stations directly in terms, for example, of species numbers, numbers of individuals, and total biomass. Also, it is frequently informative simply to display the relative abundance of taxa (Figures 2.2, 3.11), which may clearly demonstrate differences between the various communities under comparison.

More sophisticated methods of analysis are frequently useful. Given that the data from a single site at a single point in time typically take the form of a list of species and their relative abundance, and that comparisons between data sets may have to be made in both space and time, some means of condensing the data to manageable proportions is often essential. It is also important because although an experienced biologist may be capable of interpreting complex sets of ecological data by inspection, other specialists with whom he/she must co-operate (chemists, engineers, administrators) cannot be expected to do so. Therefore the raw data are frequently condensed by using them to compute one or more of several numerical indices or coefficients, of which four types can be distinguished: pollution indices; diversity indices; biotic indices; and similarity indices or coefficients. The applications of each of these will now be considered in turn.

### *3.4.1 Pollution Indices*

Pollution indices have reached a high degree of sophistication in many central and Eastern European countries (Sladecek, 1979). They are essentially developments of the descriptive Saprobien system of Kolkwitz and Marsson

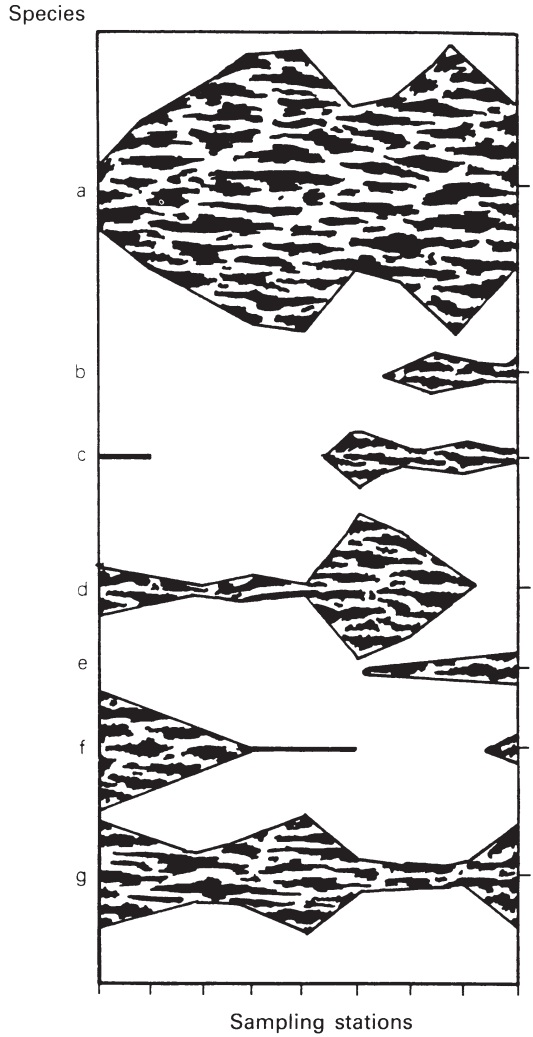



**Figure 3.11** The use of 'kite' diagrams to represent the abundance of species at different sites. The width of the 'kites' represents the abundance of each species

(1909) and are based on the fact that in rivers subject to organic pollution, communities downstream of the pollutant input show a regular and more or less predictable sequence of changes in the presence and abundance of indicator species, such as were described in Chapter 2 (see Figure 2.2).

A typical pollution index is that of Pantle and Buck (1955). Organisms present in a sample are given an s-score and an h-score. The h-score is an index of relative abundance: organisms are scored 1, 3 or 5 depending upon whether they are rare,

frequent or abundant, respectively. The *s*-score relates to the saprobic zone of which the species are characteristic; a score of between 1 and 4 is assigned to each species, depending upon whether it is typically found in oligosaprobic, *a*-mesosaprobic, *β*-mesosaprobic or polysaprobic zones, respectively. Central European workers have compiled extensive catalogues of the saprobic scores of invertebrate species, which are regularly updated as new information on distribution patterns is obtained. A good example is that of Wegl (1983). The saprobity index is given by

$$S = \frac{\Sigma sh}{\Sigma h}$$

and typically varies from less than 1.0 for unpolluted waters to 4.0 for heavily organically polluted waters. Hellawell (1978, 1986) and Washington (1984) give several other examples of pollution indices. In Germany, the Biological Effective Organic Loading method (Persoone and Pauw, 1979) and in the Netherlands the K-Index (briefly described by Metcalfe-Smith, 1994) represent other examples of saprobic-based pollution indices. Although they are widely used in continental Europe, they are less favoured in Britain and North America.

### *3.4.2 Diversity Indices*

Diversity indices were developed by theoretical ecologists who were interested in such questions as the relationship between stability and diversity in ecosystems. Washington (1984) lists no fewer than 18 different diversity indices which have been used in water pollution studies (Table 3.2). Such a variety of diversity indices has arisen because ecosystem diversity is not easily defined, and therefore can be measured in several different ways, depending on how the concept is defined. Some simple examples will illustrate the point.

A simple measure of diversity is the number of species present in the community. However, it could be argued that this is an inadequate measure. For example, consider two communities of 1000 organisms. Community A contains one individual each of species a, b and c, and 997 of d; community B contains 250 individuals each of a, b, c and d. Although each community contains four species, nearly all the individuals in A belong to the same species. Arguably, therefore, community B is a more diverse one than A. A numerical value derived both from the number of species in the community, and from the distribution of individuals between those species, is therefore a better index of community diversity. The application of diversity indices in water quality monitoring is discussed by Wilhm and Dorris (1968), Hellawell (1977, 1978, 1986), Kaesler *et al.* (1978) and Washington (1984). Diversity indices differ from one another in, for example, the relative

**Table 3.2** Diversity indices which are widely used in aquatic ecosystem studies, arranged in eight groups according to their derivation. After Washington (1984)

(1) *Simpson's Index*
Simpson's $D$

where $D = \dfrac{\sum_{i=1}^{s} n_i(n_i - 1)}{n(n-1)}$ (1949)

(2) *Relatives of species number*
Kothé's species deficit

$$\frac{A_1 - A_x}{A_1} \times 100 \qquad (1962)$$

Odum's species per thousand individuals (Odum *et al.*, 1960)

(3) *Guesses by data fitting*

Gleason's index $D = \dfrac{S}{\ln N}$ (1922)

Margalef's index $D = \dfrac{S-1}{\ln N}$ (1958)

Menhinick's index $D = \dfrac{S}{\sqrt{N}}$ (1964)

(4) *Curve fitting approach*
Motomura's geometric series,

$y = Ac^{(x-1)}$ (Whittaker, 1965)

Fisher's $\alpha$,

where $S_1 = \alpha \ln\left(1 + \dfrac{N}{\alpha}\right)$ (Fisher *et al.*, 1943)

The modified Yule 'characteristic'

$$\frac{M_1^2}{M_2 - M_1} = \frac{n^2}{\Sigma n(n-1)} \qquad \text{(Williams, 1964)}$$

Preston's log-normal '$a$'

where $y = y_0 \exp(-aR)^2$ (1948)

(5) *Information theory*

Brillouin's $H = \dfrac{1}{N} \ln \dfrac{N!}{\prod_{i=1}^{s} N_i!}$ (1951)

**Table 3.2** *continued*

Shannon's $H = -\sum_{i=1}^{s} \frac{n_i}{n} \ln \frac{n_i}{n}$ (Shannon and Weaver, 1949)

Evenness[†] $E = \frac{H'}{H'_{\max}}$

Redundancy $R = \frac{H'_{\max} - H'}{H'_{\max} - H'_{\min}}$ (Patten, 1962)

(6) Hurlbert's PIE $= \left(\frac{N}{N-1}\right)\left(1 - \sum_{i=1}^{s} p_i^2\right)$ (1971)

(7) McIntosh's 'Ecological distance' relative

McIntosh's $M = \frac{n - \sqrt{\sum_{i=1}^{s} n_i^2}}{n - \sqrt{n}}$ (1967)

(8) *Theory of runs*

Cairns' SCI $= \overline{Dl_1} \times$ No. taxa (Cairns *et al.*, 1968)

$$\overline{Dl_1} = \frac{\sum \frac{\text{no. runs}}{\text{no. specimens}}}{\text{no. times done to be statistically significant}}$$

Keefe's TU $= 1 - \left(\frac{n}{n-1}\right)\left\{\sum_{i=1}^{K} p_i^2 - \frac{1}{n}\right\}$ (Keefe and Bergerson, 1977)

[†]See Washington (1984) for a discussion of Evenness.

*List of terms*

$S$ = the number of species in either a 'sample' or a 'population';
$K$ = the number of taxa in either a 'sample' or a 'population';
$N$ = the number of individuals in a population or community;
$N_1$ = the number of individuals in species $i$ of a population or community;
$n$ = the number of individuals in a sample from a population;
$n_1$ = the number of individuals in a species $i$ of a sample from a population;
$p_1 = n_1/n$ = the fraction of a sample of individuals belonging to species $i$;
$\Pi_1 = N_1/N$ = the fraction of a population of individuals belonging to species $i$.

Symbols of indices from the literature have been changed to conform to the above.

weighting given to the number of species and to the distribution of individuals between species (the 'evenness' component). Some indices are based on specific assumptions of community structure, and many are not independent of sample

size. Washington (1984) divides diversity indices into eight groups, according to the basis on which they are derived (Table 3.2). Having considered each in detail, he concluded that all were unsuitable for application to aquatic ecosystems except for Simpson's *D* (1949), Hurlbert's PIE (1971), indices based on the theory of runs (Cairns *et al.,* 1968; Keefe and Bergerson, 1976) and McIntosh's *M* (1967). The most widely used indices in practice are those based on information theory, such as the Shannon-Weaver index (Shannon and Weaver, 1949). However, as Washington (1984) points out, the biological relevance of such indices has been widely doubted in recent years.

Diversity indices seem to be particularly favoured by American workers, but are widely used throughout the world. A practical advantage of diversity indices is that it is not necessary to identify specimens. It is necessary only to recognise whether a particular specimen is of the same species, or of a different species, as previously-encountered individuals. Thus it is a useful method of analysis in circumstances where taxonomic skills are lacking, where it is necessary to work with unfamiliar groups of organisms, or where the fauna of an area has been inadequately described and no taxonomic keys exist.

### *3.4.3 Biotic Indices*

In Britain, empirically-derived biotic indices of water quality are generally preferred. These indices are based on two observed characteristics of the communities inhabiting polluted waters. First, they generally contain fewer species than communities from comparable unpolluted waters; and second, as the degree of pollution increases species will tend to be selectively removed in order of their relative susceptibility to that form of pollution. The Trent Biotic Index (Woodiwiss, 1964) has been widely used, and is reproduced in Table 3.3. To derive the index value, each specimen in the sample is identified to the level at which it can be assigned to one of the groups listed in the lower part of the table. It can be seen that in most cases fairly limited taxonomic expertise is required. When the number of groups in the sample is known, the table is entered at the appropriate column (e.g. if seven groups are present, enter the table at column 5). The line in the table at which the correct index value is given is determined by the presence of the highest-ranking indicator group (column 1). Thus if seven groups are present, of which the highest-ranking member is *Gammarus,* the TBI value is V. If seven groups are present, but one is a species of Plecoptera, the TBI score is VII.

The Trent Biotic Index, originally devised for use in the River Trent catchment in England, has been widely used throughout the UK and some European countries, sometimes in modified form. It is a simple system requiring minimal sampling and taxonomic expertise, and the values it yields show a consistent relationship with some chemical variables such as BOD and permanganate values (Woodiwiss, 1964). However, it does not work well

**Table 3.3** The Trent Biotic Index (Woodiwiss, 1964)

| Key indicator groups | Diversity of fauna | Total number of groups (see Part 2) present | | | | |
|---|---|---|---|---|---|---|
| | | 0–1 | 2–5 | 6–10 | 11–15 | 16+ |
| Column No: 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | | Biotic Index | | | | |
| Plecoptera nymphs present | More than one species | — | VII | VIII | IX | X |
| | One species only | — | VI | VII | VII | IX |
| Ephemeroptera nymphs present | More than one species[a] | — | VI | VII | VIII | IX |
| | One species only[a] | — | V | VI | VII | VIII |
| Trichoptera larvae present | More than one species[b] | — | V | VI | VII | VIII |
| | One species only[b] | IV | IV | V | VI | VII |
| *Gammarus* present | All above species absent | III | IV | V | VI | VII |
| *Asellus* present | All above species absent | II | III | IV | V | VI |
| Tubificid worms and/or Red Chironomid larvae present | All above species | I | II | III | IV | — |
| All above types absent | Some organisms such as *Eristalis tenax* not requiring dissolved oxygen may be present | 0 | I | II | — | — |

[a] *Baetis rhodani* excluded.

[b] *Baetis rhodani* (Ephem.) is counted in this section for the purpose of classification. The term 'Group used for purpose of the biotic index' means any one of the species included in the following list of organisms or sets of organisms:
Each known species of Plathyhelminthes (flatworms); Annelid worms (excluding genus *Nais*); genus *Nais* (worms); each known species of Hirudinea (leeches); each known species of Mollusca (snails); each known species of Crustacea (hog louse, shrimps); each known species of Plecoptera (stonefly); each known genus of Ephemeroptera (mayfly) excluding *Baetis rhodani*; *Baetis rhodani* (mayfly); each family of Trichoptera (caddis-fly); each species of Neuroptera larvae (alder fly); family Chironomidae (midge larvae) except *Chironomus thummi*; Chironomus thummi (blood worms); family Simulidae (black-fly larvae); each known species of other fly larvae; each known species of Coleoptera (beetles and beetle larvae); each known species of Hydracarina (water mites).

in all polluted waters. Since the ranking of key indicator species reflects their tolerance to organic pollution (or low dissolved oxygen levels), the system gives anomalous results when used with communities subject to some kinds of toxic pollution. Consider, for example, the data shown in Table 3.4. In this simple example, invertebrate samples were taken from three stations on a river heavily polluted with zinc, and from an unpolluted tributary. The marked faunal impoverishment of the polluted sites 7 and 6+ caused by the zinc is evident from the raw data, but is not reflected in the Trent Biotic Index values (Table 3.4). Site 7, for example, has a score of 9, which might normally be taken to indicate good water quality. The reasons for this anomalously high index value are threefold, and may be used to illustrate some important defects in many biotic indices. First, the index takes no account of abundance:

**Table 3.4** Invertebrates collected by kick sampling from three sites on the zinc-polluted river West Allen and an unpolluted tributary (see text). Levels of dissolved zinc, and Trent, Chandler and BMWP biotic index values are also shown

| | Site | | | |
|---|---|---|---|---|
| | 7 | 6a | 6+ | 4 |
| Dissolved Zn, $mg\,l^{-1}$ | 1.28 | 0.04 | 0.76 | 0.38 |
| Invertebrate taxa: | | | | |
| Plecoptera | | | | |
| *Protonemoura meyeri* | 2 | 7 | 16 | 5 |
| *Isoperla grammatica* | 1 | 0 | 0 | 3 |
| *Amphinemoura sulcicollis* | 3 | 0 | 0 | 8 |
| *Leuctra fusca* | 0 | 3 | 2 | 24 |
| *Taeniopteryx nebulosa* | 0 | 0 | 0 | 2 |
| *Chloroperla tripunctata* | 0 | 0 | 0 | 1 |
| *Perla bipunctata* | 0 | 1 | 0 | 0 |
| *Perlodes microcephala* | 0 | 2 | 0 | 0 |
| *Brachyptera risi* | 0 | 0 | 3 | 0 |
| Ephmeroptera | | | | |
| *Baetis rhodani* | 2 | 9 | 7 | 3 |
| *Ecdyonurus venosus* | 0 | 67 | 7 | 131 |
| *Rhithrogena semicolorata* | 0 | 7 | 5 | 13 |
| Trichoptera | | | | |
| *Polycentropus flavomaculatus* | 1 | 0 | 2 | 1 |
| *Agapetus* sp. | 1 | 2 | 0 | 0 |
| *Rhyacophila dorsalis* | 0 | 1 | 0 | 2 |
| *Hydropsyche* sp. | 0 | 0 | 0 | 5 |
| *Philopotamus* sp. | 0 | 0 | 2 | 0 |
| Coleoptera | | | | |
| *Limnius volckmari* | 1 | 16 | 0 | 0 |
| *Elmis aenea* | 0 | 1 | 0 | 0 |
| Diptera | | | | |
| Chironomidae | 0 | 1 | 0 | 1 |
| Simulidae | 1 | 0 | 0 | 0 |
| *Dicranota* | 1 | 0 | 0 | 0 |
| *Tipula* | 1 | 10 | 1 | 0 |
| Neuroptera | | | | |
| *Sialis lutaria* | 0 | 1 | 0 | 0 |
| Mollusca | | | | |
| *Ancylastrum fluviatilis* | 0 | 11 | 0 | 0 |
| Other taxa | | | | |
| *Gammarus pulex* | 1 | 35 | 0 | 2 |
| Hydracarina | 0 | 0 | 0 | 1 |
| Hirudinea | 0 | 0 | 0 | 1 |
| Oligochaeta | 0 | 0 | 0 | 1 |
| Total no. of taxa | 11 | 16 | 10 | 17 |
| Total no. of individuals | 15 | 174 | 46 | 204 |
| Trent Biotic Index value | IX | X | VIII | X |
| Chandler Biotic Score value | 588 | 1001 | 618 | 998 |
| BMWP Score | 59 | 79 | 61 | 95 |

one individual of a taxon contributes as much to the index value as one thousand individuals. Second, because the index is designed to assess the biological quality of waters subject to organic pollution, it gives a high score to stoneflies. Since stoneflies are not particularly sensitive to zinc, the result is unduly influenced by the presence of stoneflies. Third, because there are only 11 possible Trent Biotic Index scores (0–10), the enormous variety of biological communities is made to conform to one of these 11 categories. A score of 7, for example, can be achieved in eight different ways. A sample consisting of two different stoneflies is scored identically with one containing no stoneflies or mayflies, one caddis larva and ten other groups. Consequently the index is often insensitive to major differences in community characteristics. In the present example, both sites 6a and 4 are assigned a score of 10, but inspection of the raw data indicates that they sustain different biological communities. For this reason, the system has been subjected to various modifications designed to overcome some of these difficulties. In one of these, the 'extended' Trent Biotic Index (Table 3.5), the range of scores is increased so that scores between 0 and 15 are possible.

Some of these difficulties are overcome by the more sophisticated Biotic Score System of Chandler (1970). This system demands a higher level of taxonomic competence and also takes into account the abundance of the taxa present in the sample (Table 3.6). To determine the biotic score value, each taxon present is accorded a score according to its abundance in a standard sample and to its position in the ranking order of sensitivity to pollution. Note also that for pollution-tolerant taxa, increased abundance results in a *lower* score being assigned. The sum of the scores for the individual taxa gives the overall biotic score for the sample. Theoretically there is no upper limit to the score value, but in practice the scale is from 0 to 2000. The Chandler Biotic Score system is superior, in principle, to the simpler Trent Biotic Index. It has a wider range of possible values, it takes abundance into account, and it is less likely to be seriously influenced by the fortuitous presence of small numbers of unrepresentative taxa. Consequently it is able to distinguish more readily between different communities, and is more sensitive than the Trent Index to slight alterations in water quality. Table 3.4 shows Biotic Score values for the four West Allen sites. The difference between the polluted sites 7 and 6+ and the cleaner sites 6a and 4 is more clearly emphasised by the Chandler Biotic Score. Although the system fails to distinguish between sites 6a and 4, an experienced biologist would perhaps recognise that while a score of about 1000 is good for a small upland beck, it is not particularly high for a stream with physical characteristics of site 4. A discussion of the advantages and limitations of the various kinds of biotic index is given by Washington (1984) and Metcalfe-Smith (1994). The Trent Biotic Index has been widely modified for use in different countries in Europe (Metcalfe, 1989) and the United States (Hilsenhoff, 1987).

More recently in regions where biotic indices are widely used, a scheme known as the BMWP score (Armitage *et al.,* 1983; ISO/BMWP, 1979) has

**Table 3.5** The extended version of the Trent Biotic Index. Groups are defined as shown in Table 3.3

| | | Total number of groups present | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0–1 | 2–5 | 6–10 | 11–15 | 16–20 | 21–25 | 26–30 | 30–35 | 36–40 | 41–45 |
| Biogeographical region: Midlands, England | | Biotic indices | | | | | | | | | |
| Plecoptera nymphs present | More than one species | — | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| | One species only | — | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Ephemeroptera nymphs present | More than one species[a] | — | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| | One species only[a] | — | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Trichoptera larvae present | More than one species[b] | — | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| | One species only[b] | 4 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| *Gammarus* present | All above species absent | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| *Asellus* present | All above species absent | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| Tubificid worms and/or Red Chironomid larvae present | All above species absent | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| All above types absent | Some organisms such as *Eristalis tenax* not requiring dissolved oxygen may be present | 0 | 1 | 2 | — | — | — | — | — | — | — |

[a] *Baetis rhodani* excluded.
[b] *Baetis rhodani* (Ephem.) is counted in this section for the purpose of classification.

largely superseded earlier indices (the initials stand for Biological Monitoring Working Party). Like other schemes, it is largely derived empirically, and represents a compromise between ecological validity and practical constraints. For example the taxonomic requirement is limited to the need to identify specimens to the family level. In the BMWP scheme, specimens in a sample are identified to family and each family present assigned a score according to Table 3.7. The BMWP score is the sum of the individual scores of the families recorded at each site. No account is taken of abundance, or of the fact that a family may be represented in the sample by more than one species. These features of the scheme could, in some respects, be considered regressive; comparison of the performance of earlier indices tends to show that more discriminating conclusions are obtained from those indices which rely on more detailed identification of specimens, and which take account of the abundance of taxa. However, these apparent disadvantages are counterbalanced by other considerations. Unlike earlier schemes, the BMWP score system was not devised for any single catchment or geographical area; it is specifically intended to apply equally well to all areas of Britain, and in principle to biogeographical zones on a continental scale. It therefore includes a number of taxonomic groups (e.g. Odonata and Hemiptera) which do not feature prominently in previous systems. This overcomes a major disadvantage of earlier indices, which tended to perform badly outside a relatively small geographic area, and were generally only appropriate for lotic waters. Thus the BMWP index opens up the possibility of a common scoring system for biological monitoring of a wider range of habitat types on a national or even international scale. As biological monitoring becomes incorporated more widely into strategies for environmental management and pollution control, it is increasingly obvious that a major constraint on the number of locations which can be monitored and the frequency with which they can be surveyed is the time-consuming nature of the sorting and identification stages of the whole process, which is expensive of skilled human resources. The relatively simple input data required by the BMWP system offers enormous economies in this respect which in many circumstances compensate for its potential shortcomings. In addition, the BMWP system now forms the basis of a potentially powerful tool for the monitoring and management of water quality, the RIVPACS system, which is described in Section 3.4.6. In the example shown in Table 3.4, the BMWP score ranks the sites in a very similar pattern to the Chandler score, but is much simpler taxonomically.

### *3.4.4 Similarity Indices*

Calculation of the degree of similarity between samples, like the calculation of diversity, can be done in various ways depending upon how similarity is defined. Thus there are a variety of similarity coefficients available for use.

**Table 3.6** The Chandler Biotic Score System (Chandler, 1970)

| | | Abundance in standard sample | | | | |
|---|---|---|---|---|---|---|
| | | Present 1–2 | Few 3–10 | Common 11–50 | Abundant 51–100 | Very abundant 100 + |
| Groups present in sample | | Points scored | | | | |
| Each species of | *Planaria alpina* Taenopterygidae, Perlidae, Perlodidae, Isoperlidae,Chloroperlidae | 90 | 94 | 98 | 99 | 100 |
| Each species of | Leuctridae, Capniidae, Nemouridae (excluding *Amphinemoura*) | 84 | 89 | 94 | 97 | 98 |
| Each species of | Ephemeroptera (excluding *Baetis*) | 79 | 84 | 90 | 94 | 97 |
| Each species of | Cased caddis, Megaloptera | 75 | 80 | 86 | 91 | 94 |
| Each species of | *Ancylus* | 70 | 75 | 82 | 87 | 91 |
| — | *Rhyacophilia* (Trichoptera) | 65 | 70 | 77 | 83 | 88 |
| Genera | *Dicranota, Limnophora* | 60 | 65 | 72 | 78 | 84 |
| Genus | *Simulium* | 56 | 61 | 67 | 73 | 75 |
| Genera of | Coleoptera, Nematoda | 51 | 55 | 61 | 66 | 72 |

| | | | | | | |
|---|---|---|---|---|---|---|
| — | *Amphinemoura* (Plecoptera) | 47 | 50 | 54 | 58 | 63 |
| — | *Baetis* (Ephemeroptera) | 44 | 46 | 48 | 50 | 52 |
| — | *Gammarus* | 40 | 40 | 40 | 40 | 40 |
| Each species of | Uncased caddis (exc. *Rhyacophila*) | 38 | 36 | 35 | 33 | 31 |
| Each species of | Tricladida (excluding *P. alpina*) | 35 | 33 | 31 | 29 | 25 |
| Genera of | Hydracarina | 32 | 30 | 28 | 25 | 21 |
| Each species of | Mollusca (excluding *Ancylus* | 30 | 28 | 25 | 22 | 18 |
| — | Chironomids (excl. *C. riparius*) | 28 | 25 | 21 | 18 | 15 |
| Each species of | *Glossiphonia* | 26 | 23 | 20 | 16 | 13 |
| Each species of | *Asellus* | 25 | 22 | 18 | 14 | 10 |
| Each species of | Leech (excl. *Glossiphonia,* | | | | | |
| — | *Haemopsis*) | 24 | 20 | 16 | 12 | 8 |
| — | *Haemopsis* | 23 | 19 | 15 | 10 | 7 |
| — | *Tubifex* sp. | 22 | 18 | 13 | 12 | 9 |
| — | *Chironomus riparius* | 21 | 17 | 12 | 7 | 4 |
| — | *Nais* | 20 | 16 | 10 | 6 | 2 |
| Each species of | Air breathing species | 19 | 15 | 9 | 5 | 1 |
| | No animal life | | | 0 | | |

**Table 3.7** The BMWP score system

| Allocation of biological scores: Families | Score |
|---|---|
| Siphlonuridae Heptageniidae Leptophlebiidae Ephemerellidae Potamanthidae Ephemeridae<br>Taeniopterygidae Leuctridae Capniidae Perlodidae Perlidae Chloroperlidae Aphelocheiridae<br>Phryganeidae Molannidae Beraeidae Odontoceridae Leptoceridae Goeridae Lepidostomatidae Brachycentridae Sericostomatidae | 10 |
| Astacidae<br>Lestidae Agriidae Gomphidae Cordulegasteridae Aeshnidae Corduliidae Libellulidae<br>Psychomyiidae Philopotamidae | 8 |
| Caenidae<br>Nemouridae<br>Rhyacophilidae Polycentropidae Limnephilidae | 7 |
| Neritidae Viviparidae Ancylidae<br>Hydroptilidae<br>Unionidae<br>Corophiidae Gammaridae<br>Platycnemididae Coenagriidae | 6 |
| Mesovelidae Hydrometridae Gerridae Nepidae Naucoridae Notonectidae Pleidae Corixidae<br>Haliplidae Hygrobiidae Dytiscidae Gyrinidae Hydrophilidae Clambidae Helodidae<br>Dryopidae Elminthidae Chrysomelidae Curculionidae<br>Hydropsychidae<br>Tipulidae Simuliidae<br>Planariidae Dendrocoelidae | 5 |
| Baetidae<br>Sialidae<br>Piscicolidae | 4 |
| Valvatidae Hydrobiidae Lymnaeidae Physidae Planorbidae Sphaeriidae Glossiphoniidae Hirudidae Erpobdellidae<br>Asellidae | 3 |
| Chironomidae | 2 |
| Oligochaeta (whole class) | 1 |

One measure of similarity is the number of species common to samples or communities, as in Sorensen's (1948) coefficient:

$$S = \frac{2c}{a + b}$$

where $a$=the number of taxa in community a, $b$=the number of taxa in community b, and $c$=the number of taxa common to both.

If we have two samples, A and B, each containing two species, x and y, then according to Sorensen's coefficient they are 100% similar. However, if sample A contains 99 individuals of x and one of y, and sample B contains one of x and 99 of y, they are clearly biologically different, and Sorensen's coefficient is a misleading figure. Clearly there are advantages in using coefficients which take into account the relative abundance of species in the sample, and this can be done in various ways. Sneath and Sokal (1973) give a comprehensive list of similarity indices. Of the large number available, relatively few have been applied in aquatic studies and it is not yet clear which indices are generally preferable (Washington, 1984). They have been more widely used by terrestrial ecologists, but their applications in biological monitoring of aquatic habitats are becoming more widely appreciated.

Analysis of survey data by similarity indices offers some important advantages, and may in fact eventually be found superior to analysis by diversity indices. Comparisons may be made simultaneously in space and time, each site or sample being compared in turn with every other site or sample. The following simple example, based on unpublished data obtained by the author and Dr G.Chabrzyk, illustrates the technique.

Samples of benthic invertebrates were taken from each of nine consecutive stretches of a river in northern England. Sites AA, B, D, F and H are riffles (stretches of turbulent water) and sites A, C, E and G are pools. Two similarity coefficients were used, Sorensen's (1948) and Raabe's (1952). The latter is one which takes into account the relative abundance of the species in the sample. Every possible pair of sites was compared, and the results displayed as a matrix (Figure 3.12). Boxes in the matrix can be shaded differentially, each pattern of shading corresponding to a different level of similarity. In some cases, visual inspection of the matrix allows any patterns to be detected. For example, in Figure 3.12 it is immediately obvious that site G has a low similarity with any other site, which suggests that there is some unusual feature of this site which deserves further investigation. More detailed examination of the matrix reveals other interesting features.

There is no doubt that this approach is a very promising one. Its principal disadvantage is that the calculations involved are very tedious, particularly for large data sets. However, they can easily be programmed into a modern microcomputer of modest capacity. In fact, further treatment of the data greatly assists interpretation. The process of average-linkage cluster analysis (Pielou, 1984; Williams, 1971) allows a dendrogram to be constructed which displays visually the varying degrees of affinity between the sample stations

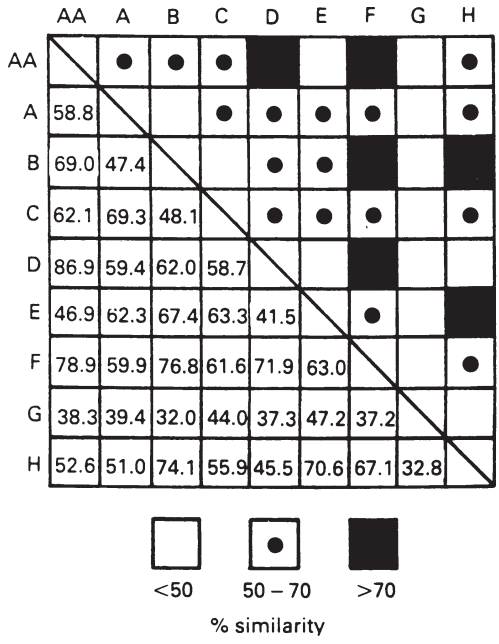



**Figure 3.12** Matrix of Raabe's similarity coefficients for nine alternating pool and riffle stretches on the River East Allen

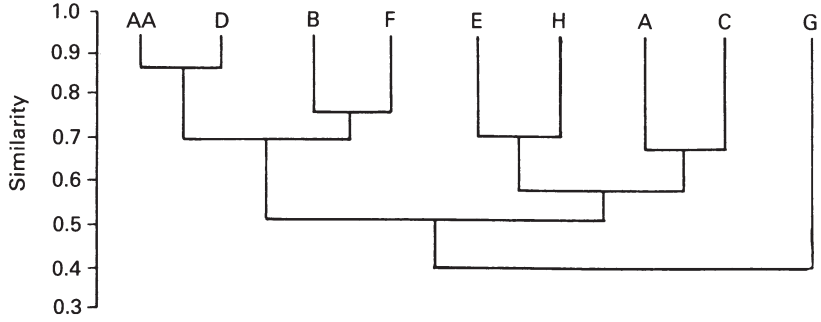



**Figure 3.13** Dendrogram derived by average-linkage cluster analysis of the matrix shown in Figure 3.12

(Figure 3.13). Site G is now clearly seen to be dissimilar to all other sites, with a similarity to them of less than 40%. Two major groupings are now apparent—sites AA, D, B and F, with a similarity of at least 70%, and sites E, H, A and C with a similarity of just under 60%. All the sites on the first group are riffles, and would be expected to have a high similarity. However,

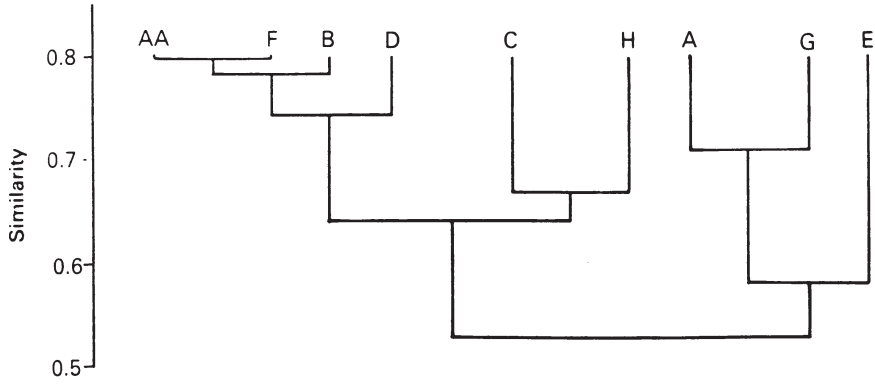



**Figure 3.14** Dendrogram derived by average-linkage cluster analysis of a matrix of Sorensen's similarity coefficients, based on the same raw data used to derive Figure 3.13

another interesting result is that riffle site H has a high affinity with the pool sites E, A and C. Thus the analysis has drawn attention to two stations, out of the nine samples, which have unusual or unexpected biological characteristics, and which perhaps require further investigation.

Figure 3.14 shows the corresponding dendrogram based on the Sorensen coefficient of similarity. Here the pattern is somewhat different. Again, riffle sites AA, B, D and F form a group of high affinity, but the relationship of the 'anomalous' sites G and H with the remaining sites is less obvious. The reason for this is clear from examination of some of the raw data (Table 3.8). Sampling stations A and G are faunistically poor, but have a number of taxa in common. However, site A is dominated by Chironomidae and Limoniinae in roughly equal numbers, whereas site G is dominated by oligochaetes with a much smaller number of chironomids, and other taxa present only in very small numbers. Thus with Sorensen's coefficient, but not with Raabe's, A and G appear very similar.

As argued above, the Sorensen coefficient is a purely qualitative one and arguably inferior to the Raabe one. The example is included to demonstrate the fact that the choice of coefficient can greatly influence the outcome of the analysis, and that sophisticated data analysis is not a substitute for sound biological reasoning, but an adjunct to it. Ideally, several coefficients should be used and the results compared before any definite conclusion is reached. Also, there are several different methods of cluster analysis (Pielou, 1984; Williams, 1971). Hellawell (1978) gives step-by-step descriptions of methods for constructing dendrograms. Many standard computer software packages are readily available which will carry out various forms of cluster analysis.

A further level of sophistication is to estimate the degree of probability

**Table 3.8** Numbers of individuals of various taxa recovered from artificial substrate samples at two sites on the River East Allen (see text and Figures 3.13 and 3.14)

| Taxon | Nos. of individuals | |
|---|---|---|
| | Site A | Site G |
| Oligochaeta | 6 | 51 |
| Chironomidae | 36 | 20 |
| Limoniinae | 32 | 4 |
| Empididae | 1 | — |
| *Limnius volckmari* | 1 | 1 |
| *Esolus parallelepipedus* | 1 | 1 |
| Elmis aenea | 1 | — |
| *Ancylastrum fluviatilis* | 6 | — |
| *Pisidium* sp. | 2 | — |
| *Polycentropus flavomaculatus* | 8 | 1 |
| *Baetis rhodani* | — | 3 |

with which the sites are similar (or different). This is necessary because most samples do not include all the species present in the habitat. Thus two sites which are in fact identical may produce a similarity coefficient of 90%, 80%, 70%, 60%, or even less, depending upon the efficiency of sampling. At what level of similarity, therefore, do we conclude that two sites are significantly different? The question can be answered objectively by using, for example, Kendall's rank correlation coefficient (Kendall, 1962) in place of the more conventional indices of similarity. Examples are given by Hellawell (1978). This approach has been used in Figures 3.15 and 3.16, which relate to data on the invertebrate communities of 23 sampling stations on the rivers East and West Allen (Green, 1984), and which indicate the application of cluster analysis in the study of a polluted river system. In these figures, stations numbered with Roman numerals are situated on the unpolluted River East Allen. Stations numbered with Arabic numerals are situated on the West Allen, which is heavily polluted with zinc in certain stretches. The similarity matrix (Figure 3.15) immediately indicates certain stations which have low similarity to the remaining stations. Construction of the dendrogram (Figure 3.16) allows a more detailed interpretation. In interpreting the dendrogram, we are particularly interested in those sampling stations which appear in an unexpected position. It must be remembered, however, that the appearance of a station in an unexpected position does not in itself indicate that the station is polluted.

At the level of Kendall's *tau* which indicates significant dissimilarity between the stations, seven groups, labelled A-G, are apparent (Figure 3.16). Group A and B each consist of only one station, IX and 10 respectively, which are thus both

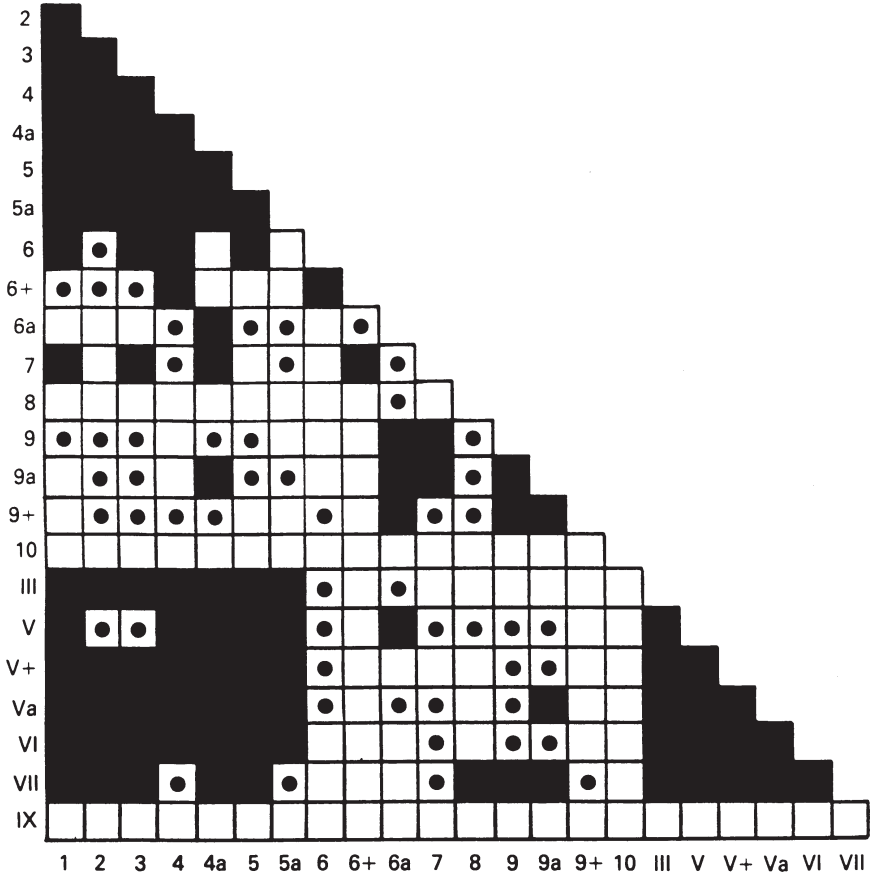



**Figure 3.15** Matrix of Kendall's rank correlation coefficients for pairs of sites on the East and West Allen (Green, 1984). In this matrix, open squares indicate pairs of sites which are significantly different from each other

dissimilar from each other and from any other station. These sites are, in fact, the headwaters of the two rivers, where the physical conditions tend to promote unstable and highly variable communities. This grouping is not, therefore, unexpected. Group D contains four stations: 9, 9a and 9+ are all physically similar and adjacent to one another. The fourth station in the group, 6a, is a small tributary with similar physical properties to 9, 9a and 9+, so this grouping is again in accordance with expectations. Group A comprises 13 stations which include most of the downstream sites on both rivers. Note, however, that within this group two large subgroups occur, comprising stations 1, 2, 3, 4 and 5 and stations III, V, Va, V+ and VI. Thus stations on the same river tend, as expected, to be more closely similar to one another than to stations on another river. The

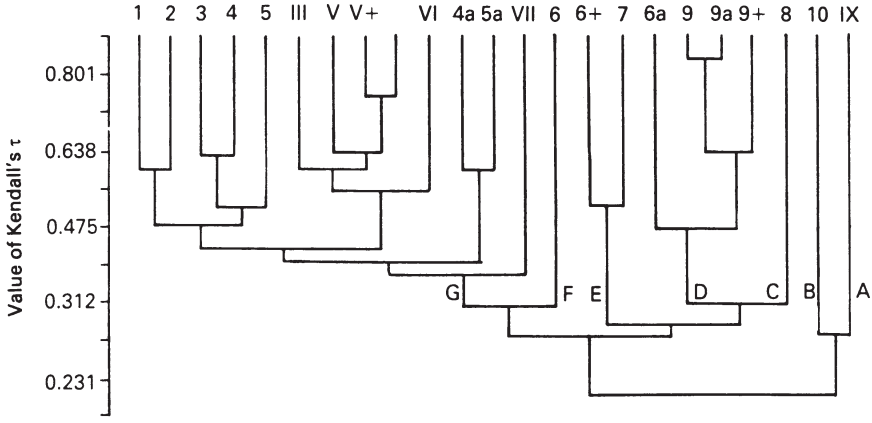



**Figure 3.16** Dendrogram derived by average-linkage cluster analysis of the matrix shown in Figure 3.15. See text for discussion (Green, 1984)

groupings which are anomalous are therefore C, E and F, comprising stations 8, 6+ and 7, and 6 respectively. Chemical analysis of the water at these stations showed that it contained abnormally high levels of zinc (Abel and Green, 1981; Green, 1984). Thus the use of this technique allowed polluted sites to be identified from a large data matrix. The analysis of data by similarity indices, although relatively recent in its application to aquatic ecosystems, is nevertheless a promising approach. It does not in itself allow a particular habitat to be diagnosed as 'polluted' but it does allow a large number of spatial and temporal comparisons to be made simultaneously. It draws attention to habitats which have unusual biological characteristics by comparison with the majority of habitats sampled, and thus allows further research to be directed where it will be most profitable.

### *3.4.5 Other Multivariate Techniques*

Cluster analysis is one of a variety of so-called multivariate analytical techniques which are becoming increasingly popular in the analysis of large and complex sets of ecological data, and lend themselves well to the analysis of biological monitoring data. Though the principles are, in general, not particularly new, they were little used until in recent years the widespread availability of microcomputers made it feasible routinely to carry out the long, complex and tedious calculations which these techniques require. Commonlyused forms of multivariate analysis include, in addition to the various forms of cluster analysis, principal components analysis (PCA); correspondence analysis; discriminant function analysis (DFA); and time-series analysis. It is beyond the scope of this book to provide detailed descriptions

of these processes, but a useful introduction to their applications in biological monitoring of aquatic habitats is given by Norris and Georges (1993). More detailed coverage of these types of analysis in ecological investigations is given by Gauch (1982), Pielou (1984) and Jolliffe (1986).

Many commercially-available software packages now offer the facility to utilise these advanced forms of data analysis routinely. It is important to remember, however, that the accessibility of such techniques can lead the inexperienced investigator into serious error. Most data analytical techniques make certain assumptions about the nature or quality of the input data, and many data sets violate the assumptions or preconditions of the method. Probably the most common error is to subject data sets to a whole range of different analytical processes until the 'right' result (i.e. what the investigator wants or expects) is obtained. There is therefore a great danger that misleading or erroneous results, leading to false conclusions, can readily be obtained. It is therefore important that the operator ensures that he or she is familiar with the assumptions and restrictions of the analytical method used, and develops at least sufficient understanding of the particular analytical process to enable the output of the computer to be interpreted critically and sensibly.

### *3.4.6 RIVPACS*

In the UK, since the early 1980s, the approach of using multivariate techniques to explore the factors governing the distribution of river invertebrates on a national basis has been under development. It has now evolved into RIVPACS (River Invertebrate Prediction and Classification System) and is in use by the National Rivers Authority—now part of the Environment Agency, the main regulatory agency concerned with monitoring of water pollution—to assist in the analysis and interpretation of survey data. A brief history of the development of this system is given by Metcalfe-Smith (1994).

In most polluted rivers, the pollution existed long before any accurate biological records were taken. It is therefore difficult to judge what may have been the ecological status of a particular river stretch in the absence of pollution. If we could predict the nature of the biological community at a given location, assuming the absence of pollution, this knowledge would provide a benchmark against which could be judged the effectiveness of measures to reduce pollution or ameliorate water quality. Without this knowledge, the best information available can only come from contemporaneous studies of similar river stretches in the immediate locality. At best, however, this provides only a crude indication, since quite small variations in local conditions are readily observed to produce significant differences in river communities; and in many areas, suitable unpolluted sites for comparison do not exist.

The factors which govern the distribution and abundance of invertebrate species in rivers are partly local—current speed, water depth and width, nature of the substratum, chemical characteristics of the water, and so on—and partly related to broader parameters such as latitude, longitude, altitude and climate. In theory, if the influence of these factors on the distribution of a particular organism was known, it should be possible to predict the nature of the community which we should expect to find in any particular location.

The first stage in developing a predictive model for river invertebrate communities was an extensive survey of over 400 sites throughout Britain, on rivers of all physical types, using a rigorously standardised sampling technique. All sampling stations chosen were situated on rivers which were known, from previously obtained monitoring data, to be free of significant pollution. At each sampling site, values were also recorded of a range of abiotic factors such as altitude, latitude, longitude, temperature, current speed, width, depth, and of a range of chemical determinands. The taxa present in the biological samples were identified, and the raw data took the form of a list of taxa present at each site, together with the physical and chemical data for each site.

A cluster analysis of the kind described in Section 3.4.4 produced a dendrogram of all the sites, similar in form (but much larger!) to that shown in Figure 3.16. This revealed a number of groupings and subgroupings of sites, which could be interpreted as described earlier, each group or subgroup being characterised by certain common taxa and, to some extent, by common physical and chemical characteristics. In order to investigate the degree to which particular physical and chemical factors influenced the presence or absence of taxa, a principal components analysis was undertaken, which identified a number of key factors which influenced, to varying degrees, the observed pattern of distribution of taxa. From this outcome it was possible to develop a model which would predict, for any given location, the taxa likely to be present at any location within mainland Britain, assuming the absence of pollution or other disturbing factors. To obtain the prediction, it is necessary to input a minimum of eight physical parameters for the site under investigation: latitude, longitude, altitude, distance from source, discharge, width, depth and substratum composition. Additional determinands may be input to improve the accuracy of the prediction, such as slope, alkalinity, mean air temperature, annual air temperature range, chloride concentration.

A typical output from the program for one site is shown in Figure 3.17.

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**Figure 3.17** Part of the output of a RIVPACS prediction for a river site in the North of England. The probability of incidence of invertebrate families is shown, as is the predicted BMWP score based on 1000 virtual samples

**RIVPACS**

**PREDICTION OF FAUNA**

**WEAR BELMONT** **'Autumn'**

Probability of group membership

| | |
|---|---|
| 4b | 60.3% |
| 3d | 25.9% |
| 3c | 4.4% |
| 4c | 3.3% |
| 3b | 2.0% |
| 3a | 2.0% |

Predicted BMWP families in decreasing order of probability

| | |
|---|---|
| 99.8% | Elmidae |
| 99.2% | Chironomidae |
| 97.9% | Hydropsychidae |
| 94.5% | Oligochaeta |
| 93.2% | Baetidae |
| 86.7% | Gammaridae (incl. Crangonyctidae & Niphargidae) |
| 85.1% | Rhyacophilidae (incl. Glossosomatidae) |
| 83.1% | Ancylidae (incl. Acroloxidae) |
| 82.2% | Simuliidae |
| 80.2% | Heptageniidae |
| 79.3% | Caenidae |
| 76.9% | Erpobdellidae |
| 73.1% | Sphaeriidae |
| 72.8% | Hydrobiidae (incl. Bithyniidae) |
| 71.4% | Tipulidae |
| 62.4% | Lymnaeidae |
| 58.2% | Polycentropidae |
| 55.4% | Gyrinidae |
| 54.0% | Glossiphoniidae |
| 48.2% | Taeniopterygidae |
| 48.1% | Hydroptilidae |
| 47.7% | Leptoceridae |
| 46.8% | Perlodidae |
| 46.4% | Asellidae |
| 41.6% | Lepidostomatidae |
| 38.0% | Nemouridae |
| 36.0% | Hydrophilidae (incl. Hydraenidae) |
| 35.5% | Sericostomatidae |
| 34.3% | Planariidae (incl. Dugesiidae) |
| 33.6% | Ephemerellidae |
| 33.0% | Planorbidae |
| 25.7% | Ephemeridae |
| 21.5% | Brachycentridae |

Predicted BMWP score, number of taxa and ASPT

| | n | mean | sd | lcl | ucl |
|---|---|---|---|---|---|
| BMWP score | 1000 | 127.9 | 20.202 | 88.27 | 167.47 |
| No. taxa | 1000 | 22.5 | 2.844 | 16.97 | 28.12 |
| ASPT | 1000 | 5.7 | 0.376 | 4.92 | 6.39 |

First, the site is assigned to one of the groups identified in the cluster analysis. Where the data are insufficient to assign a site with certainty, a range of possibilities is shown with associated degrees of probability. Second, the program lists those families included in the BMWP score index (see Section 3.4.3) which are expected to be present. Again, each family listed is assigned the level of probability with which its presence is predicted. At this stage, it is possible to compare the prediction of the model with data obtained from field surveys. To take a simple example, if several families are shown as having a high probability of presence, say 80% or more, but are rarely or never found at that site, there is an immediate indication that there may be some extraneous influence on the site concerned. Note, however, that in any comparison between observed and predicted values, one vital precondition *must* be met: that the sampling technique utilised to obtain the real samples must be exactly the same as that used in the original survey which gave rise to the model. Obviously, if the sampling effort or efficiency is different, the comparison between observed and predicted values is completely invalidated. This emphasises the importance of standardising sampling techniques (see Section 3.3).

The next stage is to estimate the predicted BMWP score for the site. In real life, single samples tend, even with standardised techniques, to produce variable results (sampling error) due to random variations. We therefore tend to take as large a number of samples as is feasible and average the results, which smoothes out the random variations and allows us to determine the extent of sampling error, and the degree of confidence we may place in our results. RIVPACS, in order to obtain a reasonable prediction of the BMWP score for the site in question, generates 1000 'virtual' samples from the list of predicted families present, taking into account their probability of presence, and produces a mean BMWP score and mean number of families present, together with the confidence limits of these means. These provide another means of comparison between actual and predicted values. Finally, the program computes the average score per taxon (ASPT) for the BMWP index, i.e. the total score for the site divided by the number of taxa present. This is sometimes useful to distinguish between sites which have similar scores but very different taxa present; for example, a site which had just three families of Plecoptera present would have an ASPT of 10 from a total score of 30. Another site could have the same total score, but a completely different community—say six families of molluscs, three families of leeches and one family of Crustacea. In this case (see Table 3.7) the average score per taxon would be only 3 (although the number of taxa present would be 10!).

It is possible also to display graphically the expected BMWP against a series of observed values (Figure 3.18), thus showing whether there are any consistent trends towards improvement or deterioration. If, on any particular occasion, the observed BMWP is unusually lower or higher than the expected value, or if it deviates in either direction from the established pattern, it may be possible to associate this deviation with a particular event or set of

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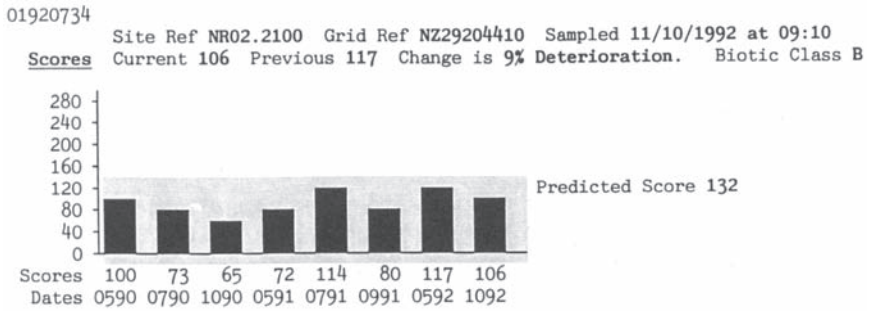


**Figure 3.18** Part of the RIVPACS output for a river site, showing the predicted BMWP score (shaded area) compared with observed scores (vertical bars) on a number of occasions. Observed scores are consistently below the predicted value, which may indicate sub-optimal water quality

circumstances, and thereby obtain valuable information about factors influencing the site under investigation. Some groups of rivers (as defined in the cluster analysis) consistently yield underestimates of BMWP, presumably because they are influenced by factors peculiar to them which the system does not consider. Consistent overestimates could, of course, also occur in theory, though they would tend to be interpreted as evidence of extraneous influences on the river. As the system develops, it is likely that these inconsistencies will be reduced or eliminated.

### *3.4.7 Some General Comments about Data Analysis and Interpretation*

Though the examples used in the preceding discussion have been deliberately simplified, in reality biological monitoring generates large and complex data sets. Even for the specialist, the detection of consistent patterns among the data requires some form of data processing to reduce its bulk and to highlight consistent trends. For specialists in other disciplines, these trends need to be explained in clear terms, though there is an obvious danger that in the process of simplifying the data to aid comprehension, misleading conclusions may be drawn. The simple examples used above show clearly how different forms of data processing may lead to completely different conclusions.

There is probably no single form of data analysis which can be recommended for all circumstances. Several authors have attempted to compare the performance of pollution, diversity and biotic indices by using them to analyse sets of data (e.g. Balloch *et al.*, 1976; Cook, 1976; Hellawell, 1978; Norris and Georges, 1993; Nuttall and Purves, 1974; Watton and Hawkes, 1984). Generally, the simpler indices tend to suffer to a greater degree from one or more of several disadvantages: insensitivity to major differences between communities; the production of anomalous or misleading results when non-organic pollution is involved; or undue

bias in the result owing to the fortuitous presence or absence of small numbers of particular taxa. In view of the great variety of index methods available, and of the fact that none are universally applicable, it is important that no single method be relied upon. There is, unfortunately, a tendency to invest indices with a scientific validity which they do not necessarily possess, and it is necessary to emphasise some of their limitations.

First, it must be realised that the process of calculating an index results in the loss of most of the information represented by the raw data. The data contained in one sample typically consist of a list of species, tabulated to show the relative abundance of each. Such a table clearly contains many separate pieces of information. To reduce this to a single number, by calculating an index value, results in just one piece of information, the index value itself. Thus to rely solely on the index value is to reject most of the information which has been collected. That the consequences of this lead to error is easily demonstrated. Consider the Trent Biotic Index (Table 3.3). It has been seen that a score of VII can be obtained in at least eight different ways. So a sampling station which yields the same score on two different occasions may, in fact, have changed dramatically, and over-reliance on index values in the analysis of the data will actually obscure this fact. Similarly, a particular value of diversity index can be obtained from any of a large number of quite different samples. Two samples which have equal diversity index values may have quite a different composition, indeed they may be so different biologically that they have no species in common at all.

Second, comparison of index values, particularly diversity index values, from different sources (i.e. different laboratories) is extremely dangerous. Consider the widely-used Shannon-Weaver index:

$$H = \sum_{i=1}^{s} P_i \log P_i$$

where $P_i$ is the proportion of the total population belonging to the *i*th species and *s* is the total number of species in the sample. Some authors use $\log_{10}$, some use natural logarithms, and some use $\log_2$. Provided the same base is always used, no problem arises. However, most authors do not even state which base they have used, and therefore comparisons between results from different laboratories are meaningless.

A further difficulty arises through the use of different levels of taxonomic analysis. In theory every individual specimen is identified to the species. In practice this is rare, either because adequate keys do not exist or because individual laboratories lack suitably trained personnel. The extent of simple taxonomic error is, of course, unknown, but it is clear that different laboratories indulge to a varying, and usually undisclosed, extent in taxonomic 'lumping', which again renders inter-laboratory comparisons meaningless. Even within a single laboratory, during a single research programme, personnel changes can result in differing taxonomic standards being applied during a single investigation. Over-reliance on index

methods, particularly those which require only identification to the family level, can cause major community changes to be completely overlooked. For example, the replacement of one species by another which belongs to the same family or genus is a biological change of potentially large significance, and precisely the sort of change which biological monitoring is intended to detect. Preoccupation with indices as methods of data analysis could cause an investigator completely to overlook even such a conspicuous alteration in community structure.

This does not mean that index values are useless in pollution studies. It does mean, however, that the index value is *not* the end-point of the data analysis, as many people seem to think. Index values themselves require biological interpretation. They are simply an aid to the analysis of the raw data, and should always be interpreted by referring back to the raw data *before* any conclusion is reached.

Multivariate methods, particularly in view of the more or less routine use of sophisticated computer software packages, equally need to be treated with caution. Again, the test is whether the analysis aids the detection and interpretation of any patterns which may be present in the data, and whether the conclusions drawn are biologically sensible. It is essential that the operator understands the assumptions and limitations of the analysis, and where different analyses produce different results, the investigator should not simply choose the analysis which produces the result which is wanted or expected, but seeks to understand by further analysis *why* the different methods have produced different outcomes. Uncritical acceptance of computer output, no matter how sophisticated, is a recipe for serious error. Any form of data analysis is designed to be an aid to biological understanding, not a substitute for it.

# 4

# The Toxicity of Pollutants to Aquatic Organisms

There are many circumstances in which the need to measure toxicity may arise. Many thousands of chemical substances are used for industrial, agricultural and domestic purposes, and their numbers increase annually. The toxicity of these chemicals and of their by-products and degradation products to aquatic animals needs to be determined, since any compound manufactured and used in substantial quantities is likely to become a contaminant of watercourses. In the case of novel compounds or formulations, toxicity testing may precede large-scale manufacture and form part of the research into the feasibility of its commercial application. Toxicity tests may be incorporated into effluent monitoring schemes. Identification of the more toxic components of complex effluents may be a prerequisite for the development and improvement of effective treatment processes. The measurement of toxicity is essential in the formulation of quality standards for receiving waters. Finally, compliance with a toxicity standard may be a legal requirement for consent to discharge an effluent. In this chapter we shall consider the basic principles of toxicology in relation to water pollutants and aquatic organisms, and the methods by which the toxicity and toxic effects of pollutants to aquatic life may be studied. The application of toxicological data in water pollution control, and their use in the formulation of water quality standards will also be considered, both in this chapter and in Chapter 6.

Toxic pollutants may exert their effects in several ways, depending upon the characteristics of the poison, of the receiving water, and of the biological community the water sustains. In extreme cases, animals may be killed by the poison. In some circumstances, poisons—insecticides, herbicides, molluscides, piscicides—are applied to water with the express purpose of killing some species and in the hope

that others will be unaffected. Lower concentrations of poison may exert sublethal toxic effects. Some poisons appear to accumulate in the tissues of organisms during their lifetime, and exert toxic effects after prolonged exposure to concentrations which are barely measurable by chemical means. It is widely suspected that some of these may pass from prey to predator organisms and achieve high concentrations in species at the top of a food web. Many poisons are known to be mutagenic, teratogenic or carcinogenic, but the study of these phenomena in aquatic organisms is in its infancy.

From a biological point of view, any toxic effect is significant if it influences, or is likely to influence, the physiology or behaviour of the organism in such a way as to alter its capacity for growth, reproduction or mortality, or its pattern of dispersal, since these are the major determinants of the distribution and abundance of species. Species which are not directly affected by a pollutant may nevertheless be indirectly influenced. For example, if a predator is deprived of its normal prey by the action of a pollutant on the prey, it may itself be numerically reduced. Alternatively it may prey upon some other species, which itself may show a numerical response. Where two competing species are unequally affected by the pollutant, both may show a change in distribution and/or abundance. Thus the effects of toxic pollutants can only be fully understood with some knowledge of trophic, competitive and other interspecific relationships.

Since pollutants can exert such a variety of toxic effects at different levels of biological organisation, an enormously wide range of investigative methods has been employed in their study. There is an enormous literature on the subject of pollutant toxicity to aquatic organisms, especially fish and invertebrates. Much of it is, unfortunately, of limited or even doubtful value, for reasons that will be made clear.

For the purposes of this discussion it is necessary at the outset clearly to define four basic terms which are widely misunderstood:

1 *Lethal toxicity:* toxic action resulting in the death of the organism.
2 *Sublethal toxicity:* toxic action resulting in adverse effects in the organism other than its death.
3 *Acute toxicity:* toxic action whose effects manifest themselves quickly (by convention, within a period of a few days).
4 *Chronic toxicity:* toxic action whose effects manifest themselves over a longer period (by convention, within periods measurable in weeks or months rather than days).

A common misunderstanding among the ill-informed is to use the terms ‘acute’ and ‘lethal’ as if they were synonymous, and to do the same with the terms ‘chronic’ and ‘sublethal’. This is clearly wrong. There are numerous examples

of sublethal toxic effects which manifest themselves quickly, and of organisms which die only after prolonged exposure to a poison. Thus it is perfectly correct to talk of acute sublethal toxicity, acute lethal toxicity, chronic sublethal toxicity or chronic lethal toxicity and in many circumstances it is important to distinguish correctly between them.

## 4.1 Lethal Toxicity and its Measurement

Approaches to measuring lethal toxicity vary in their complexity, in terms of the procedures employed, the apparatus required and the methods of collecting and processing the data produced. There are corresponding differences in the amount of information yielded, the degree of confidence which may be placed in the results, and the purposes for which those results may validly be used. There are many different reasons for carrying out toxicity tests, and it is important that the procedure chosen is appropriate to the purpose for which the results are required. Alabaster and Lloyd (1980) have discussed toxicity testing procedures in relation to their various applications. A useful practical guide to toxicity test methods has been published by HMSO (1983a). There are indeed several such useful manuals, not all of which are readily accessible; Abel (1991) refers to several of these. Since the results of toxicity tests may be significant in connection with pollution control legislation, national and international agencies have made some attempts to agree upon standard procedures (e.g. Alabaster and Lloyd, 1980; APHA, 1995; ASTM, 1973; Maki and Duthie, 1978). These and other sources frequently make specific recommendations on many aspects of toxicity testing methodology (see also Axiak and Abel, 1991; Reish and Oshida, 1986; Sprague, 1969, 1970, 1973; UNEP/FAO/IAEA, 1989; Ward and Parrish, 1982). In some circumstances, special procedures are required in order to obtain meaningful results. This can arise where, for example, the properties of the organism under test, or of the poison to be tested, are unusual in some way; or where the pattern of occurrence of the pollutant in water does not follow the usual pattern. Some examples of modified test procedures are described by Axiak and Abel (1991); see also Section 4.1.5. Some of the simpler methods are widely used but provide very limited information, and their results need to be interpreted with more caution than is sometimes exercised. The reasons for this will become clear later in this chapter, but it is first necessary to understand the principles of more rigorous procedures.

### *4.1.1 The Experimental Conditions*

The basic requirement is for groups of animals to be exposed to each of a series of concentrations of poison in suitable containers. A toxicity test does not differ from other types of experiment in that due consideration should be given to matters such as sample size, acclimation of animals to the experimental conditions, and

maintenance of a constant environment. Normally samples of at least ten are used, and the appropriate range of poison concentrations may be estimated on the basis of preliminary experiments so as to cause most of the animals to die over a period ranging from a few hours to a few days. However, it is often necessary to investigate the effects of lower concentrations such that the experiment may last for several weeks or more. The main practical difficulties encountered are in ensuring constant environmental conditions, maintaining the poison concentrations at their nominal levels, and minimising stress to the animals.

The toxicity of many poisons is greatly influenced by environmental conditions such as pH, temperature, water hardness and dissolved oxygen concentration. Clearly such variables should ideally be measured and controlled during any test, particularly since the presence of the animals themselves is likely to cause a gradual deterioration in the initial experimental conditions. This will arise due to utilisation of dissolved oxygen and excretion of carbon dioxide and other toxic metabolites such as ammonia. Poison concentrations may vary during the experiment due to absorption and metabolism by the animals, chemical and microbiological breakdown, by evaporation, or through adsorption onto the sides of the test vessels. These considerations suggest that the test containers should be fairly large and that the test solutions should be replaced regularly. It is generally considered that a minimum test volume of two to three litres per gram of animal tissue, to be renewed at least once daily, is normally required. The preferred arrangement, however, is to construct a 'continuous flow' apparatus in which the test solutions are automatically replenished, usually achieving a total replacement of solution every six to eight hours. The replacement rate may be greater or less depending upon the size of the test containers, the size, activity level and metabolic rate of the animals, and the volatility or degradability of the poison under test. In practice, most investigators use apparatus of their own design and construction, and of varying degrees of complexity.

### *4.1.2 Data Collection and Analysis*

The raw data from a toxicity test take the form of a record of increasing mortality in each test container as time passes. The survival time of each individual in the experiment should be recorded accurately. In practice the series of observation times recommended by Sprague (1973) are usually adequate, namely at 0.25, 0.5, 0.75, 1, 2, 4, 8, 14±2, 24, and 33±3, hours, and thereafter at daily intervals.

The next step is to estimate the median survival time (LT50) of each group of animals, that is the time required for half the animals to die. The most widely-used technique is the rapid graphical method of Litchfield (1949), which is derived from a procedure developed by Bliss (1935, 1937). For each group of animals, a graph is plotted of cumulative percentage mortality

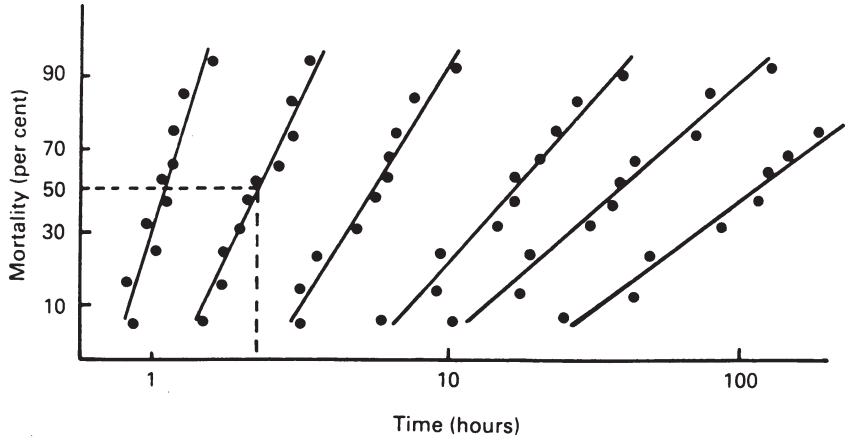


**Figure 4.1** Probit lines resulting from plots of cumulative percentage mortality in each test tank against time, using logarithmic–probability graph paper. The dotted lines show how median survival times are read from each line. Lines on the left are for the higher poison concentrations

against elapsed time. For reasons which will be discussed later, the mortality data are transformed to probits (or plotted on a probability scale) and the time values are transformed to log times. The straight line of best fit is then drawn through each set of points. This line may be computed, but in practice lines fitted by eye are often satisfactory. The result is a set of 'probit lines' or time-mortality curves as shown in Figure 4.1. Values of LT50 can now be read off each line as shown. The time for each percentage response, for example LT10, LT90, can also be read off if required. Normally values for LT16, LT50 and LT84 are required. To estimate confidence limits, the *slope function, S,* for each probit line is determined by

$$S = \frac{\dfrac{\text{LT } 84}{\text{LT } 50} + \dfrac{\text{LT } 50}{\text{LT } 16}}{2} .$$

Using the values of $S$ and $N$ (where N = the number of animals in the test tank), the factor $f$ is computed:

$$f = \text{antilog}\left(\frac{1.96 \log S}{\sqrt{N}}\right) = S^{1.96/\sqrt{N}}.$$

Litchfield (1949) provides a nomograph for the computation of f from values of $S$ and $N$. The procedure is slightly more complex if not all the animals die during the experiment, or if a 'split' probit line (see below) is obtained. The upper and lower confidence limits of the LT50 are given by LT50×$f$ and LT50/$f$ respectively. LT50 values and their confidence limits for each group of animals can be plotted against

poison concentration to give a toxicity curve (Figure 4.2). It is customary to use logarithmic scales on both axes, since the ranges of survival times and poison concentrations to be displayed frequently span several orders of magnitude. This transformation does not, of course, alter the shape of the curve.

An alternative method of plotting the raw data is widely used. This method produces essentially the same toxicity curve but it is arrived at by a different route. For each observation time, a graph is plotted of percentage mortality (transformed to probits) against poison concentration (transformed to log concentration). Instead of a series of survival time-mortality curves (Figure 4.1) we now have a series of concentration-mortality curves (Figure 4.3). For each observation time, the median lethal concentration or LC50 (sometimes termed median tolerance limit, $TL_M$) can be read off. This value is defined as the concentration causing half the animals to die *within a specified period of time*. Its confidence limits can be estimated by a procedure analogous to that described above (Litchfield and Wilcoxon, 1949), based on the original method of Bliss (1935). The resulting toxicity curve (Figure 4.4) of LC50 against observation time differs only in that confidence limits are expressed in terms of concentration rather than time. For certain applications this approach has some advantages; for example, where the test forms part of a research programme designed to establish water quality standards, it is obviously preferable to estimate errors associated with lethal concentrations rather than with survival times. However, the computation of the results is more complex since, among other difficulties, the number of points on each probit line is small. Unless the chosen range of concentrations is narrow, most groups of animals in the test will show either zero or 100% mortality at any single observation time. Thus the lines of best fit must be calculated rather than fitted by eye (Litchfield and Wilcoxon, 1949).

Since these calculations can be tedious, there are obvious advantages in using a computer. Probit analysis is available on several commercially-available statistical software packages, and a purpose-designed program in fact requires a microcomputer of very modest capacity. Inexperienced users should, however, beware of some major problems which can arise with commercial software packages; since they are designed for general purposes, difficulties can arise with the mathematical notations, the format of the input data and in the interpretation of the output, which can lead to erroneous results. The use of 'home-made' programs must be particularly strongly discouraged. Unless they are written with a full understanding of the mathematical and toxicological principles involved, they almost invariably produce ridiculous results. Buhagiar and Abel (1991) provide a full discussion of the subject, and introduced the Toxicologist System, a dedicated and efficient program which carries out probit analysis for toxicity tests with aquatic organisms, plots the graphs and assists in the interpretation of

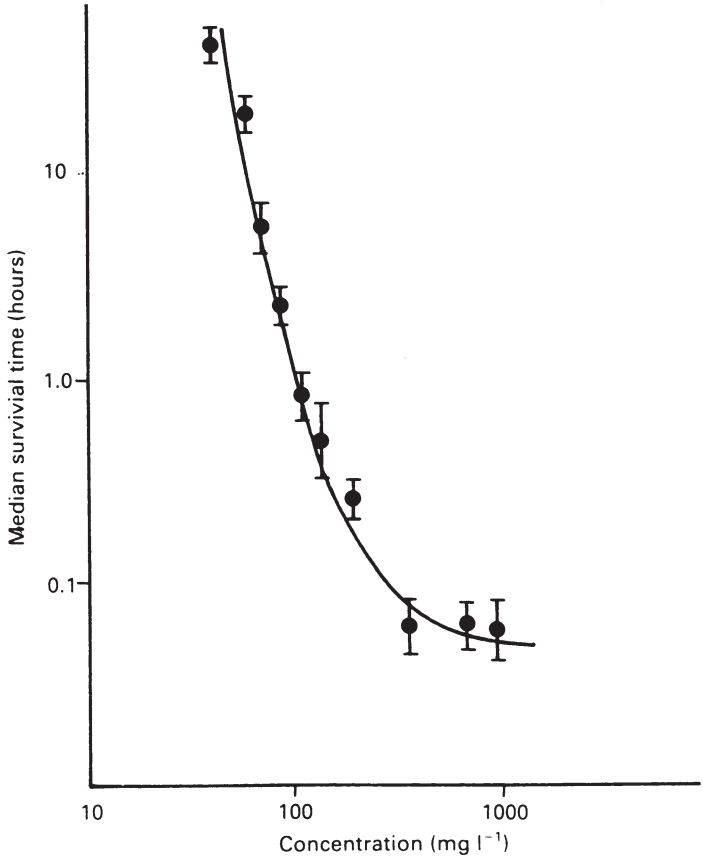


**Figure 4.2** Toxicity curve relating median survival time to poison concentration; rainbow trout (*Salmo gairdneri*) exposed to sodium lauryl sulphate (Abel, 1978). Vertical bars represent 95% confidence limits

unusual probit lines. It also takes advantage of the computer to produce a mathematically exact solution rather than an approximation, while to the operator it appears to follow the familiar graphical procedure.

We may now consider the interpretation of lethal toxicity test results.

### *4.1.3 Probit Lines*

The theoretical basis of the log-probit transformation of mortality data is given by Bliss (1935, 1937) and Finney (1971). This technique of data analysis is widely used in many types of toxicological and pharmacological research. Hewlett and Plackett (1979) provide a lucid and succinct account of the general theory and practice of probit analysis, and Sprague (1969) discusses its application to toxicity tests involving aquatic animals. In practical terms, the

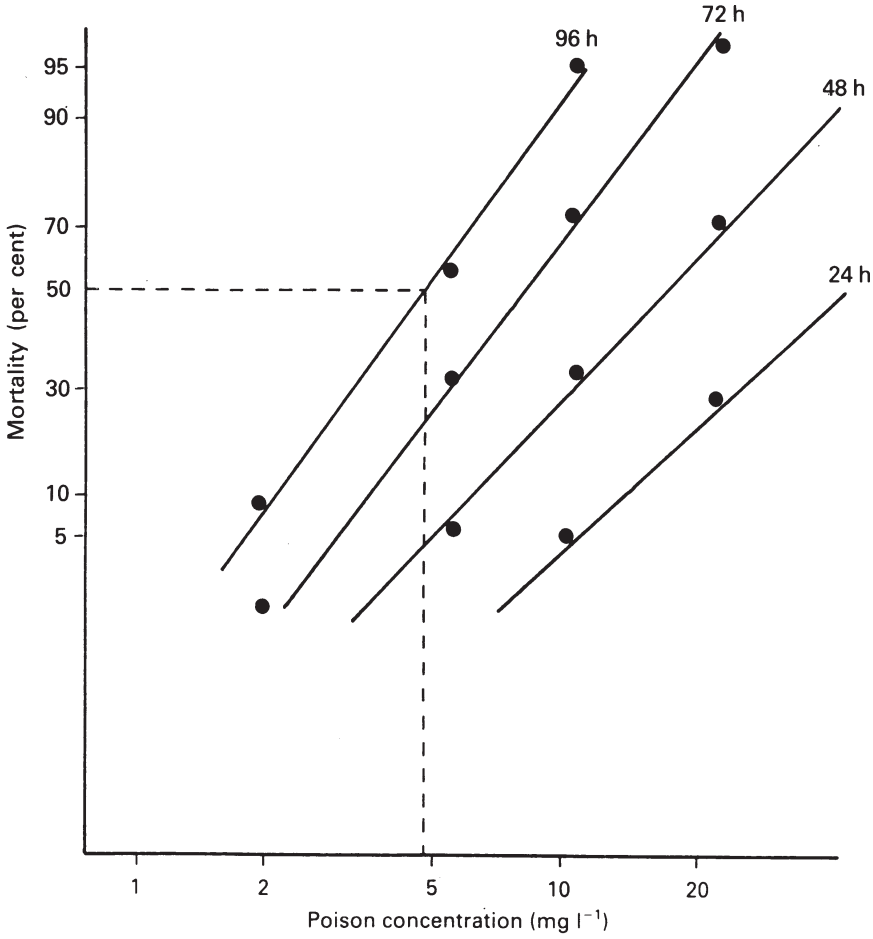


**Figure 4.3** Probit lines resulting from plots of percentage mortality at specified observation times (probability scale) against poison concentration (log scale). The dotted line shows how the 96 h LC50 value may be read off

purpose of the transformation is to allow the estimation of median survival times and/or median lethal concentrations, and their confidence limits, from a relatively small sample. Other transformations have occa sionally been used (Sprague, 1969) and some have been discussed in detail by Stephan (1977), who argues that the log-probit transformation is not necessarily the best. However, it is by far the most widely used, and in practice all appropriate transformations appear to produce very similar results.

Because the probit lines are an essential intermediate stage in the construction of the final toxicity curve, it is often overlooked that they can provide other information of toxicological interest. Useful indications are

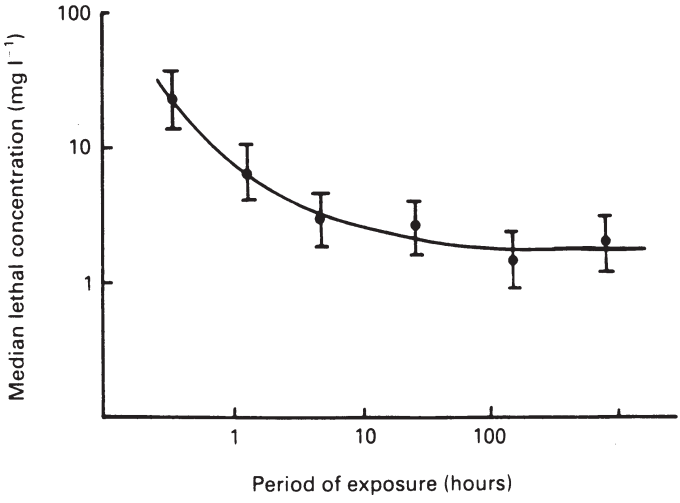


**Figure 4.4** A toxicity curve in which median lethal concentrations and their confidence limits have been plotted against time. See Figure 4.15 for other examples

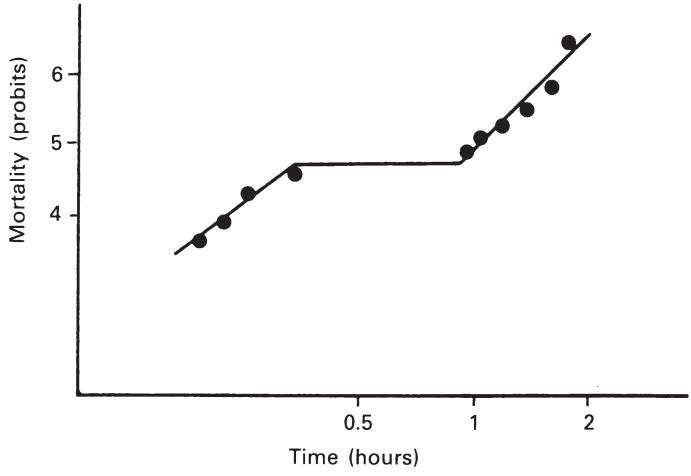


**Figure 4.5** A 'split' probit line from an experiment to determine the lethal toxicity of the detergent sodium lauryl sulphate to rainbow trout, *Salmo gairdneri*. The discontinuity indicated a heterogeneity in the population of test animals, in this case due to the existence of two separate mechanisms of toxic action (Abel, 1978)

provided by examination of probit lines for irregularities of slope or the presence of inflections. 'Split' probit lines (Figure 4.5) occasionally occur, which indicates a heterogeneity of the population from which the sample was drawn (Hewlett and Plackett, 1979). Such heterogeneity may be due to intra-specific variability in

susceptibility to the poison between sexes or age classes, or may indicate the development of resistant strains within the population.

Another possible explanation is that the poison may have two (or more) mechanisms of action, and that animals which are resistant to one mechanism may subsequently succumb to another. Tyler (1965) concluded on the basis of split probit lines that high temperature had three separate mechanisms of lethal action in fish. Confirmation that such an interpretation can be correct is given by studies on the toxicity and toxic effects of an anionic detergent (Abel, 1976, 1978). A concentration-dependent change in the mode of lethal action of the detergent was found to be associated with the occurrence of split probit lines in toxicity tests.

Where the heterogeneity in susceptibility to the poison is not pronounced, it may be manifested by an alteration in the slope of the probit lines rather than in the occurrence of inflections (Hewlett and Plackett, 1979). Simple tests for significant differences in the slope of probit lines are available (e.g. Litchfield, 1949) and have been used to demonstrate the occurrence of more than one mode of lethal action in fish exposed to toxic pollutants (Abel, 1978; Burton *et al.,* 1972).

Ball (1967a) showed that the slope of the probit lines was an important datum in comparing the relative susceptibilities of species to poisons. Ammonia was equally toxic, in terms of the 5-day LC50 value, to roach *(Rutilus rutilus)* and rudd *(Scardinius erythrophthalamus).* However, there was a considerable difference in the slope of the concentration-mortality curves. In practical terms, this may be important in the application of the toxicological data to water quality standards. For example, an ammonia concentration equivalent to two-thirds of the 5-day LC50 may be expected to kill less than 1% of rudd, but 16% of roach.

These examples illustrate the potential importance of a full analysis of probit lines in the investigation of lethal toxicity. Such detailed consideration is rarely given, and it would appear that much useful information from toxicity tests may thereby be lost. Where irregularities of the kind described above are found to occur, they may indicate promising lines of further investigation.

### *4.1.4 Toxicity Curves*

The toxicity curve describes the empirical relationship between poison concentration and survival time of the animals. Some representative curves are shown in Figures 4.2–4.9. There have been several attempts at mathematical descriptions of toxicity curves (reviewed by Sprague, 1969), but the validity and usefulness of this are questionable (Brown, 1973), as is speculation concerning the shape of the curve, and such practices have long been discontinued. As Brown (1973) points out, for any poison there will be a concentration so low that it will never cause the death of half the animals.

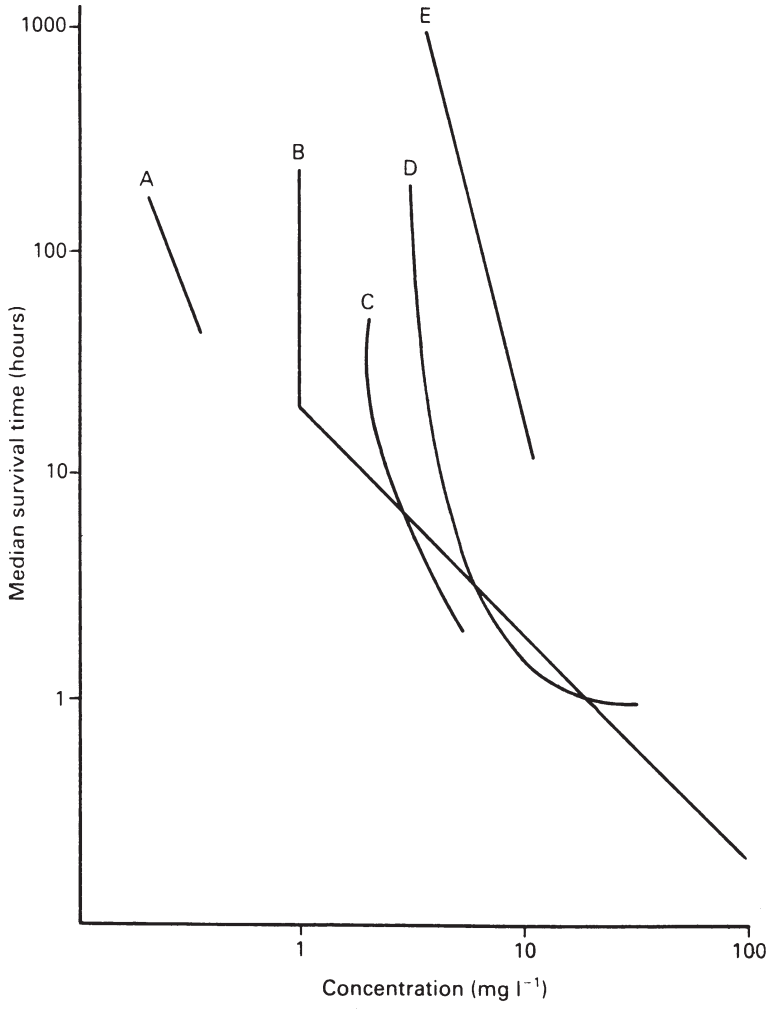


**Figure 4.6** Some toxicity curves for various species of fish exposed to different detergents, redrawn from the originals to the same scale. A, *Salmo gairdneri*, linear alkylate sulphonate (Brown *et al.*, 1968); B, *Salmo salar*, polyoxyethylene lauryl ether ((Wildish, 1972); C, *Lepomis macrochirus*, linear alkylate sulphonate (Hokanson and Smith, 1971); D, *Gadus morrhua*, alkylbenzene sulphonate (Swedmark *et al.*, 1971); E, *Salmo gairdneri*, alkylbenzene sulphonate (Herbert *et al.*, 1957)

Thus the curve will become asymptotic to the time axis. The concentrations at which this occurs may be termed the *threshold median lethal concentration* or threshold LC50. (A synonymous term is *incipient lethal level* or ILL.) Further, even at very high concentrations death will not be instantaneous,

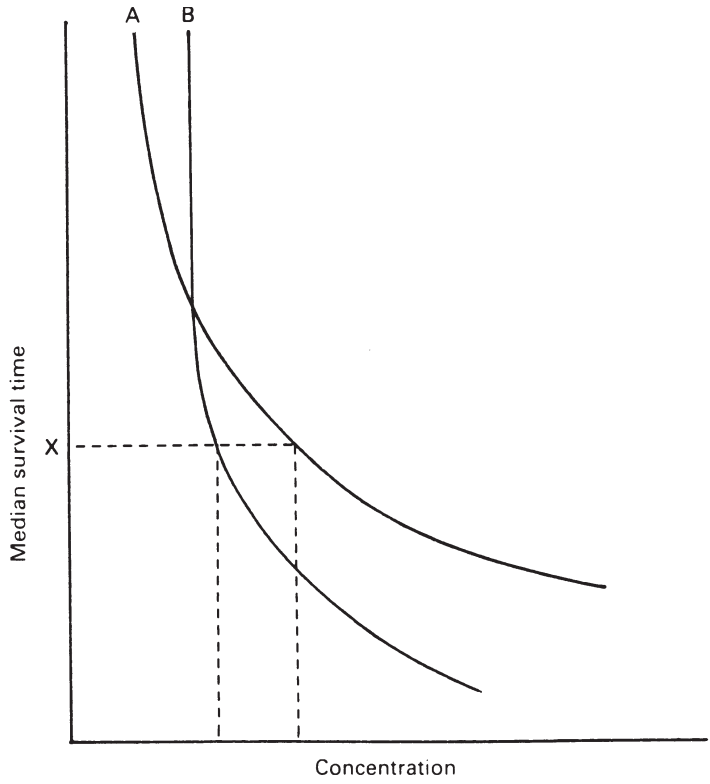


**Figure 4.7** Two hypothetical toxicity curves. Substance A is clearly more toxic than substance B, since the lethal threshold concentration of A is smaller. However, had the test been discontinued at time X, B would have appeared more toxic than A

but will take a finite period of time to occur. Thus the curve will become asymptotic to the concentration axis. Between these two asymptotes, the curve generally will show a decrease in survival time as concentration increases. Whether the toxicity curve is a straight line or a curve is therefore of no significance. Where a straight line is obtained, it may be considered as a segment of a larger curve. This point is illustrated by Brown (1973), and also in Figure 4.6. Here, toxicity curves for several species of fish exposed to some synthetic detergents have been redrawn from the originals to the same scale. Clearly, no significance can be attached to the fact that, for example, curves A and E are rectilinear while C and D are curvilinear. Most of these curves, and A and E in particular, cover only part of the range over which survival time changes with poison concentration. More extensive testing over a wider range of concentration is required to establish the true shape of the curves.

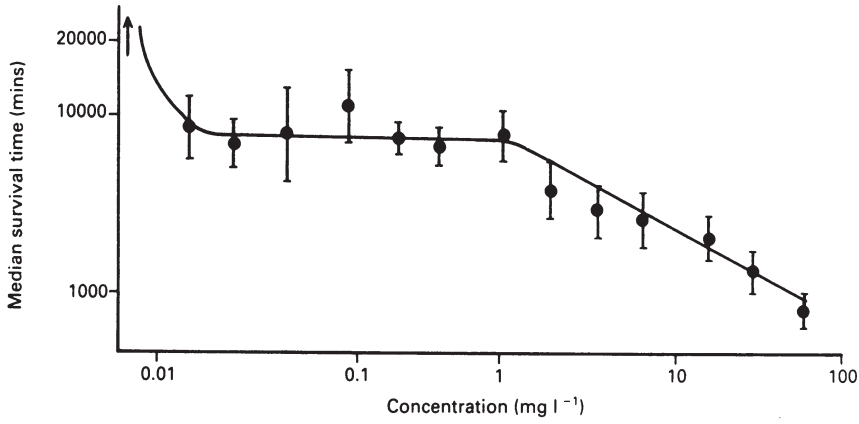


**Figure 4.8** Toxicity curve for rainbow trout (*Salmo gairdneri*) exposed to cadmium (Ball, 1967b)

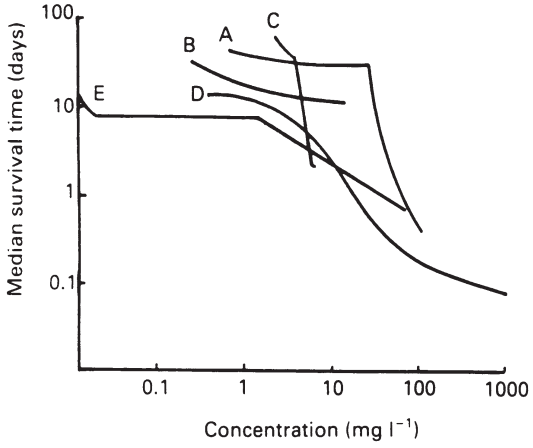


**Figure 4.9** Toxicity curves for several fish species exposed to cadmium, redrawn to the same scale. Note that none of the curves shows a clear threshold concentration, despite the long duration of most of the tests. Inflections in the curves, and segments of curve over which mortality is apparently unrelated to poison concentration, commonly occur. Finally, the curves for different species frequently intersect: which species appears most, or least, sensitive depends upon the duration of the toxicity test. A, *Tilapia aurea* (Abel and Papoutsoglou, 1986); B, *Cyprinus carpio* (Abel and Papoutsoglou, 1986); C, *Noemacheilus barbatulatus* (Solbé and Flook, 1975); D, *Gasterosteus aculeatus* (Pascoe and Cram, 1977); E, *Salmo gairdneri* (Ball, 1967b)

The most important feature of a toxicity curve is the indication it gives of the threshold median lethal concentration, and wherever possible tests should be continued until a lethal threshold is apparent. Figure 4.7 shows clearly the importance of determining lethal threshold concentrations. In this diagram, two toxicity curves are shown. These may represent two poisons tested against the same species, or one poison tested against one species under different environmental conditions. Clearly A is more toxic than B, since the lethal threshold concentration of A is smaller than that of B. If, however, the experiment had been terminated arbitrarily at time X, as shown on the diagram, it would be erroneously concluded that B is more toxic than A. Therefore unless complete toxicity curves are obtained, and lethal thresholds established, the results of any test must be interpreted with caution. This is particularly important where comparisons between species, poisons or environmental conditions are involved. For example, Ball (1967a) found that in tests lasting one day, trout were more sensitive to ammonia than coarse fish. However, when the tests were continued for up to five days, all fish were found to be equally sensitive, the difference between trout and coarse fish being simply that the latter were more slow to react.

Apart from being the only proper basis for comparative studies of lethal toxicity, lethal thresholds are also useful, in conjunction with other information, in setting water quality standards (see Chapter 6). A major criticism of many investigations of lethal toxicity is that experiments are not continued long enough. Sprague (1969) examined 375 published measurements of lethal toxicity and found that only 211 of these showed a lethal threshold within four days. In 122 cases the time required to show a lethal threshold was between four and seven days, and 42 cases required longer than this. Although the results of shorter experiments are by no means invalid, it is nevertheless clear that unless lethal thresholds are clearly established the interpretation which may validly be put on the results is strictly limited. Further, useful toxicological information may be lost. Ball (1967b) reported an example of the advantages of continuing tests as long as is economically or practically feasible (Figure 4.8). The toxicity curve for rainbow trout exposed to cadmium was linear over a concentration range between 1 and 64 mg $l^{-1}$ and a time period of about six days. Continuing the test for 14 days revealed that cadmium continued to act lethally down to 0.01 mg $l^{-1}$ and that the threshold concentration may lie as low as 0.008 mg $l^{-1}$. Fish exposed to concentrations between 0.01 and 1.0 mg $l^{-1}$ continued to die throughout the latter part of the experiment, and between these two concentrations survival time did not increase as the cadmium concentration decreased. These results demonstrated that cadmium was a very slow-acting poison which was considerably more toxic than shorter tests had previously indicated. Toxicity curves for five fish species exposed to cadmium have been compared (Abel and Papoutsoglou, 1986) and show some interesting features (Figure 4.9).

### 4.1.5 Alternative Methods for Measuring Lethal Toxicity

As indicated earlier, there are many different reasons for measuring the toxicity of pollutants to aquatic organisms, and it is important to use a method which is appropriate to the purpose for which the results are required. The procedures described above are unnecessarily elaborate for some purposes, and similarly there are circumstances in which these conventional methods provide inadequate information. It is therefore sometimes necessary to consider alternative methods, on the one hand simpler, and on the other more complicated, than those discussed already. The modifications may be to the experimental apparatus, to the collection of data, to the methods of data analysis, or to the duration of the test. The most common reasons for modifying a test procedure are:

1 *The purpose of the test:* for example, routine screening and monitoring of effluents do not necessarily require the same level of complexity as toxicity measurements designed to contribute to the formulation of a water quality standard. Tests required for legal or quasi-legal purposes such as certification of toxic chemicals for use in water, or for purposes of meeting consent conditions for discharge of wastes, usually must conform to the relevant procedures laid down by the regulatory authority. This type of test is usually kept as simple as possible, consistent with producing useful data, as the results may become subject to legal challenge on the grounds that the procedures were incorrectly followed. However they are usually not suitable for any purpose other than that for which they were designed.
2 *The characteristics of the pollutant:* for example, certain substances have unusual physical or chemical properties, such as low solubility, immiscibility with water, volatility, susceptibility to adsorption or degradation, or other reasons which affect their behaviour in the test and which will require special steps to be taken to ensure that the final result is relevant to the way in which the chemical will behave in the environment. Examples include oil dispersants and certain pesticides. It is also important in some circumstances to consider the pattern of use or discharge of the chemical. For example, a test which assumes continuous exposure of organisms to the chemical will generally produce false results if the organisms are exposed only intermittently. This is particularly relevant to certain forms of pesticide applications, and to estimating the impact of effluent plumes or mixing zones on the receiving water biota (Abel, 1980a, b; Abel and Garner, 1986).
3 *The characteristics of the test organisms:* for example, some organisms have special requirements by virtue of their size, behaviour or environmental requirements which may dictate modifications to the test procedure.

A more detailed discussion of these points is given by Axiak and Abel (1991).

## 4.2 Factors Influencing Toxicity

There is a great deal of published information on the comparative toxicity of pollutants to different species under different conditions, and the present discussion is a selective review of some of the more salient features of the literature. Only influences on lethal toxicity are considered here: factors affecting sublethal toxicity have been little studied. Regrettably, much of the available literature is of limited value, or at least requires cautious interpretation, largely because of the kinds of methodological limitations discussed earlier. For example, we have seen (Figure 4.4) that comparing two LC50 values for a single time period such as 48 or 96 hours can lead to erroneous conclusions. Any biotic or abiotic influence on toxicity may affect the speed of response of the organisms as well as, or instead of, the actual quantity of poison required to kill the animals. Therefore comparisons of toxicity based on measurements of survival times, mortality rates, or fixed-time LC50 values are not adequate firmly to establish the existence or magnitude of an effect on toxicity. The importance of determining lethal threshold values is rarely more evident than when discussing comparative toxicity.

Unfortunately, much of the literature on comparative toxicity takes precisely the form of tabulations of LC50 values, or comparisons of survival times or mortality rates. Therefore the existence and magnitude of many potential influences on toxicity are not well established, despite in many cases the existence of a substantial literature. Indeed, many of the best-known influences on toxicity have been well established for many years, and much of the more recent literature fails to shed further light on the subject.

### *4.2.1 The Toxic Properties of the Pollutant*

The toxicity of a pollutant under any given circumstances must be a function of its chemical structure or configuration, and quite small alterations in the poison molecule can produce major variations in toxicity. Synthetic anionic detergents are a group of compounds which illustrate this well. Modern anionic detergents are of the linear alkylate sulphonate type, consisting of a sulphonated benzene ring with an unbranched alkyl chain containing about 12 carbon atoms (see Figure 2.10). The degradation products of detergents are markedly less toxic to aquatic organisms than the original molecule (Kimerle and Swisher, 1977; Swisher *et al.,* 1964). Numerous studies have shown that the length of the hydrocarbon chain of an anionic detergent molecule exerts a large influence on its toxicity (Abel, 1974; Hirsch, 1963; Lindahl and Cabridenc, 1978; Maki and Bishop, 1979). These studies

are in general agreement that the toxicity of anionic detergents to fish, invertebrates and algae (expressed in terms of 48 h LC50 values) increases by up to one order of magnitude for each increase of 2 alkyl carbons in the detergent molecule, although very long hydrocarbon chains (C16 and above) tend to show reduced toxicity.

The existence of such empirical relationships between chemical structure and toxicity has since been confirmed in other groups of compounds, and has given rise to the idea of *quantitative structure-activity relationships* (QSARs). QSARs may be used to predict toxicity, even of compounds which have not yet been synthesised, and are discussed further in Section 4.6. The toxicity of both organic and inorganic poisons is greatly influenced by the physico-chemical state in which the poison is present. Heavy metal ions, for example, may exist in any of several different oxidation states; in dissolved, colloidal or particulate form; and as simple ions or as inorganic or organo-metal complexes. These various forms may have very different toxic properties. This fact explains, at least in part, the effect of some environmental conditions on toxicity.

The toxicity of copper provides a good example. Pagenkopf *et al.* (1974) recorded from published literature 96-h LC50 values for *Pimephales promelas* ranging over two to three orders of magnitude. From the chemical data provided on the environmental conditions of these experiments, they calculated the equilibrium concentrations of five possible copper species ($CuCO_3$, $Cu(CO_3)^{2-}$, , $CuOH^+$ and $Cu^{2+}$). The concentration of $Cu^{2+}$ required to kill half the fish within 96 h was nearly constant, and it is apparently the major toxic species. $CuOH^+$ was found to be rather less toxic, and the other copper species apparently contributed little to the toxic action of copper. In each case examined, a substantial proportion of the total copper present was complexed with carbonate and hydroxide. It is also known that complexation of copper with dissolved organic material causes a marked reduction in its toxicity. Sewage effluent, glycine, humic substances, and suspended organic matter (Brown *et al.,* 1974) and organic chelating agents such as nitrilotriacetic acid (Shaw and Brown, 1974) markedly reduce the toxic action of copper.

The influence on toxicity of the physico-chemical state, and molecular structure or configuration of pollutants may thus have extensive implications for the conduct of toxicity tests, and for the interpretation of test results, particularly when such results are to be extrapolated in the formulation of water quality standards. Lee (1973), in his review of the topic, pointed out that with the increasing use of chronic, sublethal toxicity testing, concentrations of pollutants which are found deleterious in experimental conditions are sometimes equal to or less than the apparent 'natural' concentrations found in waters which sustain a healthy biota. An obvious possible explanation is that under laboratory conditions the poison may largely be present in a form different from that which predominates in natural waters. While it has

long been standard practice to monitor, control and record the physical environment of toxicity tests, until recently little attention has been paid to the chemistry of the poison under test conditions. Lee (1973) recommended procedures for minimising the problems which may be associated with the influence of the chemical environment on the results of toxicity tests, in particular that in the design and execution of toxicity tests, consideration should be given to the chemistry of the test substance, and to the determination of the precise form of the substance which is responsible for the observed toxic effect. It is surprising, even disappointing, that although the general principle was understood 20 years ago, toxicologists have been slow to incorporate it into routine practice, and to this extent much uncertainty remains about the accuracy of many measurements of toxicity.

### 4.2.2 The Effects of Environmental Conditions

Since the environmental conditions may affect both the poison and the organism under test, it is not surprising that their influence on toxicity can be large. Most of the systematic studies on the effects of environmental conditions on toxicity were carried out in the 1960s and 1970s using fish. More recent work has done little to change the general pattern which is summarised here, except to confirm that the same overall pattern appears to apply also to invertebrates.

*Water hardness* is among the most important environmental influences on toxicity, and its effects are particularly well known in relation to heavy metal toxicity. Lloyd (1960) found that the concentration of zinc which was lethal to rainbow trout *(S.gairdneri)* within two-and-a-half days varied by a factor of eight over the hardness range 12–320 mg $l^{-1}$ as $CaCO_3$. Other metals are similarly influenced, including copper (Howarth and Sprague, 1978), cadmium (Calamari *et al.,* 1980) and silver (Davies *et al.,* 1978). It is now known that this effect is largely due to the effect of water hardness on the distribution of the total available metal ions between each of several inorganic complexes, which are not all equally toxic (see Section 4.2.1). However, the fact that the effect of hardness on toxicity has at least partly a biological basis can be shown by acclimating fish to hard water and exposing them to the metal in soft water. Such fish are more resistant to zinc (Lloyd, 1965) and cadmium (Calamari *et al.,* 1980) than similar fish acclimated and exposed in soft water. Hardness has also been reported to affect the toxicity of poisons other than metals, and should always be considered as a potentially significant modifier of toxicity.

The effects of *temperature* on toxicity may be expected to be complex. Temperature influences the rate of metabolic processes, including the uptake, metabolism and excretion of poisons. Increased temperature will increase the oxygen requirements of aquatic organisms, while decreasing the solubility

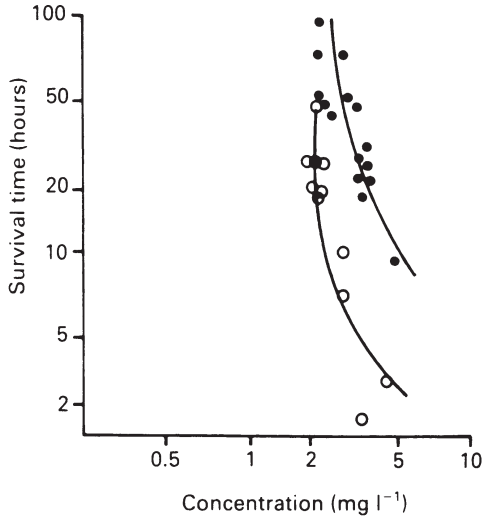


**Figure 4.10** Effect of temperature on the toxicity of linear alkylate sulphonate detergent to *Lepomis macrochirus*. Open circles represent tests at 25°C, closed circles tests at 15°C (Hokanson and Smith, 1971)

of oxygen in water. The properties of the poison itself may, of course, be directly influenced by temperature; for example, through its effect on the equilibrium between molecular and ionised forms. Temperature is itself an important limiting factor to aquatic organisms. Further, as Sprague (1970) points out, it appears to be particularly important in studying temperature effects to determine lethal threshold concentrations, rather than LC50 values at arbitrarily-selected observation times. This is because temperature may influence the rate of reaction of the organism to the poison, but not the actual lethal threshold concentration. For these reasons, the large literature relating to temperature effects on toxicity affords little opportunity for reliable generalisation. It appears to be generally true that at higher temperatures, the time taken for organisms to react to a given concentration of poison is reduced. Cairns *et al.* (1975) cite numerous examples. It follows that results of short-term tests where lethal thresholds are not established, and in particular comparisons based on 48-h or 96-h LC50 values (i.e. the vast majority of reported results), are likely to be misleading and to exaggerate the magnitude of apparent temperature effects on toxicity. Reliable reports of temperature effects on lethal threshold concentrations appear to be very scarce.

These points are well illustrated by the following examples. Hokanson and Smith (1971) showed that the lethal threshold concentrations of an anionic detergent to *Lepomis macrochirus* were similar at 15°C and 25°C. However, at the higher temperature median survival times were reduced and the lethal

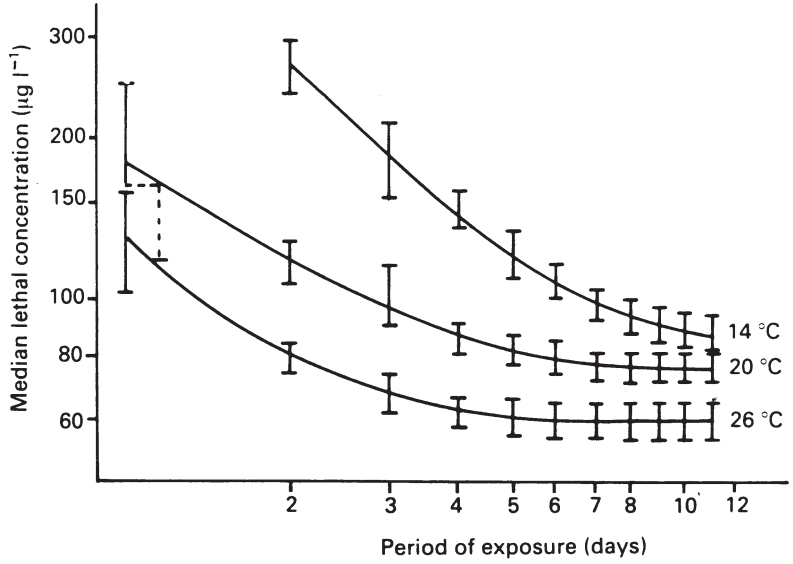


**Figure 4.11** Effects of temperature on the toxicity of hydrogen sulphide to *Carassius auratus* (Adelman and Smith, 1972)

threshold time was 20–24 h at 25°C compared with 48–51 h at 15° (Figure 4.10). Adelman and Smith (1972) studied the effects of temperature on the toxicity of hydrogen sulphide to goldfish, *Carassius auratus,* in tests lasting 11 days. Their results showed that lethal threshold concentrations ranged from approximately 90 $\mu g$ $l^{-1}$ at 14°C to approximately 60 $\mu g$ $l^{-1}$ at 26°C, that is hydrogen sulphide was more toxic at the higher temperature. However, the complete toxicity curves (Figure 4.11) show clearly that the apparent increase in toxicity with temperature was much more pronounced in tests of shorter duration. An example of decreased toxicity with increasing temperature is given by Brown *et al.* (1967a, b). The 48-h LC50 of phenol to *Salmo gairdneri* was 5 mg $l^{-1}$ at 6°C and increased to 9 mg $l^{-1}$ at 18°C. Toxicity curves published by these authors indicate that 48-h LC50 values were in this case close to the lethal threshold concentrations. Interestingly, at all but the lowest concentrations tested the effect of increased temperature was to *reduce* median survival times, that is the toxicity curves intersect in the manner illustrated in Figure 4.7. Thus even when the effect of increased temperature is to reduce toxicity in terms of lethal threshold concentration, comparison of short-term LC50 values would lead to exactly the opposite conclusion. These examples all illustrate the danger of arbitrarily-terminated toxicity tests, and explain why, unfortunately, most of the literature relating to temperature effects on toxicity must be regarded as being of very limited value.

The effects of *dissolved oxygen* on toxicity have been less widely investigated, but in general low dissolved oxygen (DO) concentrations appear to cause an increase in the toxicity of poisons. As with temperature, the majority of reports deal with

toxicity as measured by survival times, mortality rates, or tests of arbitrarily-fixed short duration, and accordingly need cautious interpretation, but there are some clear examples of the effects of oxygen concentration on threshold toxicity values. Hokanson and Smith (1971) found that the lethal threshold concentration of an LAS detergent to *Lepomis macrochirus* increased from 0.4 mg $l^{-1}$ at 2 mg DO $l^{-1}$ to 2.2 mg $l^{-1}$ at 7.5 mg DO $l^{-1}$. Thurston *et al.* (1981) reported lethal threshold concentrations of ammonia to *S.gairdneri* ranging from 0.32mg $NH_3$ $l^{-1}$ at 2.6 mg DO $l^{-1}$ to 0.81 mg $NH_3$ $l^{-1}$ at 8.6 mg DO $l^{-1}$. Acclimation of fish to low dissolved oxygen concentrations prior to testing may influence the results of experiments. Lloyd (1960) found a small increase in the toxicity of zinc to *S. gairdneri* at low DO levels which was abolished if fish were acclimated to low DO before testing. Similar results were obtained by Adelman and Smith (1972) for *Carassius auratus* exposed to hydrogen sulphide.

Although extreme *pH values* are deleterious to aquatic organisms, the pH range 6–9 is generally considered acceptable to most species. Within this range, however, the toxicity of many poisons is influenced by pH. Particularly strongly affected are those poisons which dissociate into ionised and unionised fractions, of which one is markedly more toxic than the other. The best-known example is ammonia (Alabaster and Lloyd, 1980) which is more toxic at high pH values. The reason is that unionised ammonia has high toxicity and the ammonium ion has very low toxicity, and the proportion of unionised ammonia in solution increases rapidly with pH; thus the toxicity of ammonia is about ten times higher at pH 8 than at pH 7. Alabaster and Lloyd (1980) also cite cyanide, nickelo-cyanide complex, sodium sulphide and zinc as examples of poisons whose toxicity is substantially influenced by pH. It is reasonable to assume that pH is potentially an important determinant of toxicity for any poison which ionises in solution.

The effect of *salinity* on toxicity has received some attention since many pollutants are discharged to saline waters. Some poisons appear to be least toxic to fish at salinities corresponding to approximately 30–40% sea water, when the water is roughly isotonic with fish body fluids. Examples include zinc (Herbert and Wakeford, 1964), ammonia (Herbert and Shurben, 1965) and alkylbenzene sulphonate detergent (Eisler, 1965). The effect of salinity on zinc toxicity appears particularly large (Figure 4.12) although in this case the toxicity is expressed as 48-h LC50 and not lethal threshold concentrations. Phenol toxicity to *Salmo gairdneri* increased steadily with salinity (Brown *et al.,* 1967), 48-h LC50 values ranging from 9 mg $l^{-1}$ in fresh water down to 5 mg $l^{-1}$ in 60% sea water. Cadmium is reported to increase in toxicity to the estuarine fish *Fundulus heteroclitus* (Eisler, 1971) as salinity increases. There is no generally accepted hypothesis to explain salinity effects on toxicity. Herbert and Wakeford (1964) suggested that zinc was less toxic to fish in isotonic medium because of the reduced importance of osmoregulatory

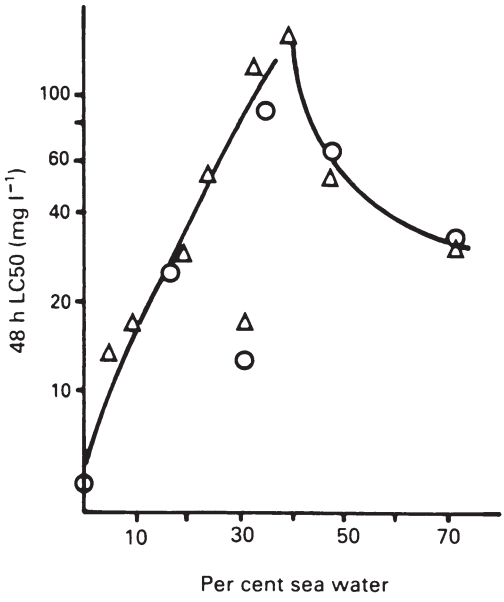


**Figure 4.12** Effect of salinity on the toxicity of zinc to *Salmo gairdneri* (Herbert and Wakeford, 1964)

stress during intoxication. Skidmore (1970) showed that trout poisoned by zinc in fresh water maintained normal osmotic and ionic balance, and that death was due to asphyxiation associated with gill damage. This does not mean, however, that osmoregulatory requirements have no influence in zinc poisoning. It has been suggested (Abel and Skidmore, 1975) that the gill damage associated with poisoning by zinc and other pollutants is a consequence of a rearrangement of the gill epithelium to preserve an osmotic barrier in the face of a rapid loss of viable epithelial cells due to the action of the poison. Thus the gill damage, and consequent tissue hypoxia, may be less extensive for fish in isotonic medium, though this has never been investigated. Poisons which are more toxic in hypertonic medium may be so because teleosts drink copiously in hypertonic medium, and thus may accumulate poison more rapidly. The effect of salinity on chemical speciation of the toxicant (see above) may also be relevant.

### *4.2.3 Combinations of Poisons*

An important environmental variable which may influence the toxicity of a poison is the presence of other poisons. Although most toxicological investigations involve the study of a single pollutant, the biota of polluted waters are usually exposed to several pollutants simultaneously.

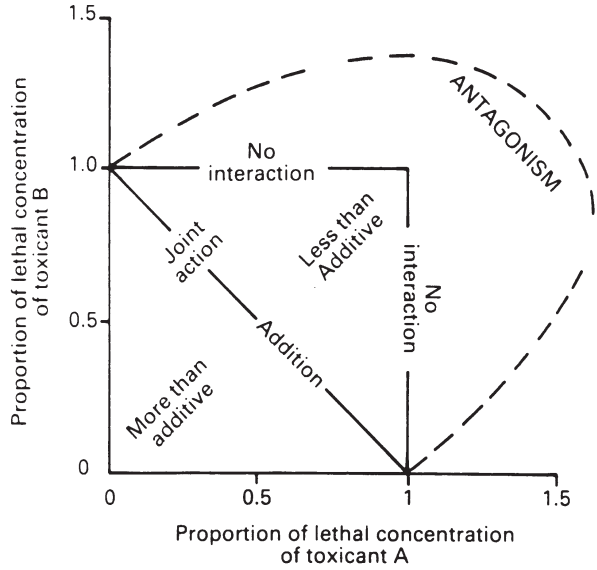


**Figure 4.13** Diagram showing terms used to describe the combined effects of two pollutants (Sprague, 1970). For full explanation, see text

Sprague (1970) has pointed out that the terminology widely used to describe the behaviour of poisons acting simultaneously is confused and potentially misleading. In particular, the terms *synergism, potentiation* and *antagonism* have been defined and used in different ways by different authors, and their use is best avoided. Sprague proposed a system of nomenclature based on that of Gaddum (1948). This scheme is shown in Figure 4.13 and is the one adopted here. Sprague's description of this scheme of nomenclature is as follows.

> "The diagram represents the combination of two toxicants. The axes represent concentrations. The concentration of 1.0 toxic unit of toxicant A produces the response (death in this case) in the absence of toxicant B, and 1.0 unit of B will do the same in the absence of A. If the response is produced by combinations of the two toxicants represented by points inside the square, the toxicants are helping one another; this is called *joint action* which may be further broken down in three special cases, as follows. If the response is just produced by combinations represented by points on the diagonal straight line (e.g. 0.5A+0.5B) the effects are said to be *additive*. If the response is produced by combinations falling in the lower left triangle (e.g. 0.5A+0.2B) the effect is *more-than-additive*. If in the upper right triangle (e.g. 0.8A+0.7B) the toxicants are still working together in joint action but are *less-than-additive*. Those combinations falling exactly in the upper and right boundaries of the square show *no interaction* between the toxicants. For example, if 1.0 unit of A is required to just produce the response, no matter what concentration of B, below

1.0 unit, is present, then A is causing the response and B is neither helping nor hindering. If more than 1.0 unit of A is required to just produce the effect, because of the presence of B, this is *antagonism,* with B antagonising the effect of A. That combination of concentrations would fall on some point to the right of the square (e.g. 1.5A+0.5B). Any combination of concentrations which would fall outside the square would represent antagonism, loosely represented by the broken curved line."

A useful method for measuring the toxicity of poisons in combination was described by Brown (1969) and Sprague (1970). The concentration of a pollutant can be expressed as toxic units, that is as a proportion of its lethal threshold concentration, or of some approximation of the lethal threshold concentration such as 48-h or 96-h LC50. Thus:

$$\text{Toxic units} = \frac{\text{Concentration of poison}}{\text{Lethal threshold concentration}}$$

Thus for any poison, one toxic unit is equal to its lethal threshold concentraton. To test the toxicity of a mixture of poisons, the following procedure may be employed. Assume we wish to test the effect of two poisons acting simultaneously. Having determined the lethal threshold concentration of each poison individually, test organisms are then exposed to a mixture of the poisons which contains, say, 0.5 toxic units of each poison (i.e. half the lethal threshold concentration of each poison). If significantly more than half die, the poisons are more-than-additive. If significantly fewer than half die, the poisons are less-than-additive. Obviously it is feasible, using this technique, to investigate the effects of combinations containing more than two poisons, and of mixtures in other than equal proportions. Marking (1977) proposed a modification of this approach, whereby the toxicity of chemicals in combination may be expressed in terms of a single numerical value. Index values of zero represent additive toxicity; values significantly above, or below, zero represent more-than-additive and less-than-additive toxicity respectively. The method includes a simple test of significance for values near zero. An alternative approach (Calamari and Marchetti, 1973) is to construct complete toxicity curves for the poisons individually and in combination, converting values on the concentration axis of the graph to toxic units (Figure 4.14).

There are several examples in the literature of both additive and more-than-additive toxicity. Mixtures of copper and zinc (Lloyd, 1961; Sprague and Ramsay, 1965); copper and phenol; copper, zinc and phenol; and copper, zinc and nickel (Brown and Dalton, 1970; Marking, 1977) have all been reported to be simply additive. More-than-additive toxicity has been reported for anionic detergents with copper or mercury (Calamari and Marchetti, 1973), the piscicide rotenone with sulphoxide or piperonyl butoxide, and the organophosphate insecticides malathion and Delnav

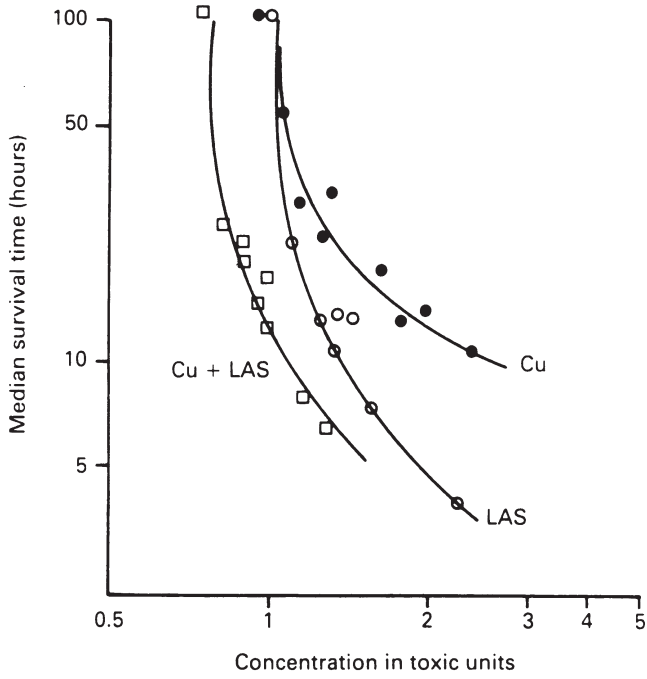


**Figure 4.14** Toxicity of copper (Cu), detergent (LAS) and a copper-detergent mixture (Cu+LAS) to *Salmo gairdneri*. Poison concentrations are expressed in toxic units (Calamari and Marchetti, 1973)

(Marking, 1977). As in other types of toxicological study, it appears that tests of short duration where threshold toxicity values are not established are likely to produce misleading results. Sprague (1970) cites examples which illustrate that while threshold toxicities may be simply additive, survival times in strong mixtures may be shorter than expected on the basis of simple additive toxicity.

Examples of less-than-additive toxicity seem to be rare. Calamari and Marchetti (1973) reported that a mixture of equal proportions (in toxic units) of copper and the non-ionic detergent nonylphenol ethoxylate showed a less-than-additive toxicity to *S. gairdneri,* but this conclusion was based on a comparison of observed and expected survival times. One circumstance in which less-than-additive toxicity sometimes occurs is when a component of a mixture is present as a small fraction of the whole mixture. Thus Brown *et al.* (1969) found that the observed toxicity of a mixture of ammonia, phenol and zinc to rainbow trout was significantly less than the predicted toxicity when zinc contributed about 0.75 toxic units and the remaining 0.25 toxic units was roughly equally distributed between the phenol and the ammonia.

In the case of ammonia, Lloyd and Orr (1969) demonstrated that concentrations below 0.12 toxic units exerted no effect on the water permeability of the fish. If toxic levels of ammonia exert their effect at least partly by increasing water permeability of the fish, as is apparently the case, this finding suggests an explanation for the non-contribution of low concentrations of ammonia to the toxicity of mixtures. For most poisons, however, no such detailed knowledge of their mode of actions exists, and their behaviour in mixtures can be predicted only on the basis of empirical findings without any explanation of the underlying mechanism. Nevertheless a series of attempts by British workers to predict the toxicity of mixtures, and to test such predictions against field observations of the fishery status of polluted rivers, have been reasonably successful. Sprague (1971) reviews some early attempts and more recent examples include those of Alabaster *et al.* (1972), Solbé (1973) and Garland and Rolley (1977). In British rivers the most common toxic pollutants are ammonia, phenols, cyanide, zinc and copper. For those poisons, summation of the fractional toxicities expressed in toxic units as described above gives generally good agreement between predicted and observed toxicities in laboratory experiments. The application of this technique in field conditions was discussed in Chapter 1.

### *4.2.4 Fluctuating Concentrations*

The concentrations of pollutants in receiving waters are rarely constant; they fluctuate, often quite widely and with rapid periodicity. Consequently the effects of fluctuations of poison concentration are of some interest. Additionally, even in the best-designed toxicity tests some fluctuation in the poison concentration is occasionally unavoidable, and information on the response of test animals to fluctuating concentrations would also be of value in this context. Surprisingly, there have been relatively few investigations of the point. Brown *et al.* (1969) determined the 48-h LC50 of ammonia, zinc, and an ammonia-zinc mixture to *Salmo gairdneri*. They then exposed replicate groups of fish to a constant 48-h LC50 of each poison, and to fluctuating concentrations such that the mean concentration was a 48-h LC50 but the actual concentration varied between 0.5 and 1.5 times the 48-h LC50 at intervals of one to four hours. In the majority of cases there was no significant difference between the survival times of fish exposed to constant concentrations and those exposed to fluctuating concentrations, suggesting that fluctuations of ±50% of the nominal concentration do not affect the response of the fish. However, in the case of ammonia, where the periodicity of the fluctuations was two hours rather than one, the median survival time was significantly reduced. Thurston *et al.* (1981) reported similar findings, also using trout exposed to ammonia. In these experiments fish were alternately exposed to ammonia and clean water, the periodicity of the fluctuations being six or 12 hours. On the basis of 96-h LC50 values for stable and fluctuating concentrations, the

fish were more sensitive to the fluctuating concentrations, than to a constant concentration equivalent to the mean value. One explanation of this (Brown *et al.,* 1969) is that when the periodicity of fluctuation is long, fish will suffer irreversible damage during the exposure to the peak concentration which cannot be compensated for during exposure to the lower concentration. An extreme case of fluctuating concentrations is the test designed to determine the effects of a relatively short exposure to poisons, followed by an extended period of recovery in clean water (Abel, 1980b; Abel and Garner, 1986). In this case, tests based on continuous exposure to lethal concentrations were found severely to underestimate the impact of short, high-level exposures as might occur during an accidental discharge.

### *4.2.5 Biotic Factors Influencing Toxicity*

Animals may show both interspecific and intraspecific variation in susceptibility to pollutants. It is beyond the scope of the present discussion to review in detail all the relevant literature. However, it is possible to draw attention to some of the more salient general findings regarding the way in which the biological characteristics of the test organisms influence their response to toxic pollutants.

Among fishes, interspecific variation in susceptibility is probably smaller than was once thought, and may be less important than variation due to environmental conditions. Because of the large influence of environmental conditions on toxicity, it is arguably unwise to place too much reliance on comparisons between the results of different investigators who have measured toxicity under different environmental conditions. In many studies, environmental variables which are known to have a large influence on toxicity have not been measured or specified; and there have been relatively few studies in which several species have been tested under similar conditions.

Thatcher (1966) found that 96-h LC50s of an alkylbenzene sulphonate detergent to 11 species ranged from 7.7 mg $l^{-1}$ to 22 mg $l^{-1}$. For a linear alkylate sulphonate detergent tested against five species, 96-h LC50s varied from 3.3 mg $l^{-1}$ to 6.4 mg $l^{-1}$ (Thatcher and Santner, 1967). Lethal threshold concentrations of the fungicide captan to three fish species ranged from 29 to 64 µg $l^{-1}$ (Hermanutz *et al.,* 1973). The 96-h LC50 of hydrogen cyanide to five species varied from 57 to 191 µg $l^{-1}$ (Smith *et al.,* 1978). Interspecific variations in short-term LC50 values of about threefold to fourfold appear to be typical for many types of poison (Sprague, 1970). Larger differences have occasionally been reported, particularly for pesticides. Eisler (1970) determined the 96-h LC50 of 12 insecticides for seven estuarinespecies. Interspecific variations for endrin were 0.05–3.1 µg $l^{-1;}$ for dieldrin, 0.9–34 µg $l^{-1;}$ for heptachlor, 0.8–194 µg $l^{-1}$; and for malathion, 27–3250 µg $l^{-1}$.

The importance of determining lethal thresholds, rather than relying on short-term LC50 values, is again evident from Ball's (1967a) studies on the relative susceptibility of fish species to ammonia. Although Salmonid fishes are widely

considered to be more sensitive to pollutants than coarse fish, he showed that the lethal threshold concentration of ammonia for *Salmo gairdneri* was the same as that for three Cyprinid species *(Abramis brama, Rutilus rutilus* and *Scardinius erythrophthalmus).* However, the trout reacted far more rapidly to the ammonia than did the cyprinids, so that in tests of short duration they would appear to be more sensitive. Ball (1967c) also measured the toxicity of zinc to trout and four coarse fish species. In this case the trout were markedly more sensitive: the threshold LC50 value for trout was 4.6 mg $l^{-1}$, significantly lower than the corresponding values for *Rutilus rutilus, Abramis brama* and *Perca fluviatilis,* which lay between 14.3 and 17.3 mg $l^{-1}$. The gudgeon, *Gobio gobio,* had a seven-day LC50 (not a threshold value) of 8.4 mg $l^{-1}$. As seen earlier, the toxicity of both zinc and ammonia is greatly influenced by environmental conditions, and it is likely that variation in susceptibility between trout under different environmental conditions is actually greater than that between trout and other fish species. The results of Smith *et al.* (1978) indicate that environmentally-induced variability in the toxicity of cyanide to fishes is of a similar magnitude to interspecific variability.

A further point arising from Ball's (1967a) study has been referred to earlier but is also relevant here. It appears that some species may show much greater individual variation in susceptibility to a poison than others. Thus in one of Ball's experiments, the five-day LC50 values of undissociated ammonia to roach *(Rutilus rutilus)* and rudd *(Scardinius erythrophthalmus)* were identical, but the slopes of the probit lines differed, indicating that roach were more variable in response than rudd. The practical implications relate to the application of toxicity data to field observations, and to the formulation of water quality standards. In an example given by the author, two-thirds of the five-day LC50 of ammonia would kill only 1% of a rudd population, but 16% of a roach population. This example again illustrates the advantages of a full analysis of toxicity test data, and the limitations of comparative studies based on simple determinations of short-term LC50 values.

Several authors have undertaken comparative studies of the susceptibility of invertebrate species to pollutants. In most of these, however, authors have chosen representative species from each of several orders, classes or phyla. Not surprisingly, the susceptibility of such 'representative' species shows some very wide variations, often of two or three orders of magnitude in terms of short-term LC50 values, in comparison with the phylogenetically more uniform fishes (e.g. Bell, 1971; Gaufin *et al.,* 1965; Rye and King, 1976). However, there have been some studies of the comparative toxicity of poisons to fairly closely-related species and it is useful to consider some examples in the present context. For five genera of oligochaetes exposed to four poisons, variation between 96-h LC50 values was generally less than one order of magnitude, although the two marine genera tested appeared highly resistant to the lethal action of cadmium (Chapman *et al.,* 1982). There were four species of *Daphnia* (Winner and Farrell, 1976). Sanders (1970) measured

only small differences between the 72-h LC50 values of copper tested against the toxicity of several herbicides to six crustaceans representing four orders. In general, variations in 48-h LC50 values between species were within one order of magnitude or less, but some species displayed considerable resistance to some or all of the herbicides, and some herbicides elicited interspecific variations in 48-h LC50 values spanning three orders of magnitude. These and similar examples from the literature indicate that it is not easy to draw firm conclusions about the relative susceptibility of invertebrate species to many poisons; there remains a need for continuous review in an accessible form of the data which is available.

### *4.2.6 Intraspecific Variation*

The effects of size and age of fish on their susceptibility to poisons has been the subject of surprisingly few systematic investigations, although the point is important both for the practical application of toxicological data and because laboratories which routinely carry out toxicity tests cannot always obtain animals of standard size and/or age. In most reports, fish of different sizes have also been of different ages, so the separate influences of these two variables remains largely unknown. Nevertheless it is common practice to standardise as far as possible on the age and size of animals, and to restrict the size/age range of specimens in a test as narrowly as is practicable.

Adelman *et al.* (1976) measured the toxicity of pentachlorophenol to goldfish *(Carassius auratus)* and fathead minnows *(Pimephales promelas)* of different sizes and ages. Differences in threshold LC50 values were small and probably of no practical significance. However, the range of sizes and ages tested was small, for example, from four to 14 weeks of age and 13–30 mm in length for the fathead minnow. Kumaguru and Beamish (1981) found a more marked effect of size on the toxicity of the pesticide permethrin to *Salmo gairdneri*. The 96-h LC50 values ranged from 3 $\mu g\ l^{-1}$ for fish weighing 1 g, to 287 $\mu g\ l^{-1}$ for 50-g fish. In contrast Stendahl and Sprague (1972) reported that small rainbow trout (1.5g) were more resistant than larger (12 g) fish to vanadium, although lethal threshold concentrations were not established for the smaller fish and the difference in susceptibility is probably small.

The young of most species show a clear division of their life cycle into distinct stages, and there have been several investigations of the relative susceptibility to poisons of the different life stages of fish. (Toxicity tests spanning whole life cycles are discussed later.) Skidmore (1965) found that zebrafish *(Brachydanio rerio)* were most susceptible to zinc between 4 and 13 days after hatching. Eggs were considerably more resistant than newly-hatched fry, and after 13 days resistance increased rapidly. This seems to be a general pattern, similar results having been reported for species exposed to detergents (Marchetti, 1965), hydrogen sulphide (Smith and Oseid, 1972) and heavy metals (Chapman, 1978).

An important potential source of intraspecific variation in susceptibility to poisons is genetic variation between different strains or populations of a species. Many waters have been subjected to pollution for a period equivalent to many generations of the organisms living there, and it may be expected that natural selection would lead to an increase in the tolerance of populations living in polluted waters. Thus natural populations may in fact be more tolerant of pollutants than those used in laboratory experiments, which are usually inbred strains or acquired from unpolluted habitats. It is important, however, to distinguish increased resistance due to genetic adaptation, from that due to acclimation effects (i.e. long-term exposure to low levels conferring increased resistance to a subsequent high-level exposure) which are not genetically determined and of which a few examples exist in the literature (Sprague, 1970). Also, since such differences in tolerance are likely to be relatively small, short-term LC50 values or median survival times at fairly high concentrations are not really adequate measures of toxicity. Thus examples of genetically-acquired resistance to poisons are few. Brown (1976) reported that Isopod crustaceans *Asellus meridianus* from metal-polluted streams were more resistant to copper and lead than animals from clean streams, and that the resistance persisted to the $F_2$ generation. Rahel (1982) reported that perch *(Perca flavescens)* from acid lakes were more tolerant to low pH than those from alkaline lakes. He did not show that the difference was genetically based, but acclimation experiments failed to produce increased tolerance in fish from alkaline lakes. Swarts *et al.* (1978) measured the resistance of several strains of brook trout *(Salvelinus fontinalis)* to low pH and investigated the effects of genetic and environmental influences on resistance. They found that fish could not be acclimated to low pH, that strain differences in resistance to low pH were detectable, but that an attempt to produce a resistant strain by breeding from fish which had survived exposure to low pH was unsuccessful.

## 4.3 Applications of Lethal Toxicity Measurements

Until fairly recently, the literature on the toxicity of pollutants was dominated by measurements of lethal toxicity, and the relevance of these measurements was widely questioned. There are, however, genuine technical and conceptual difficulties with the measurement of sublethal toxicity (see Section 4.4), and as these difficulties have been resolved there has been much more emphasis on measurements of sublethal toxicity at poison concentrations which more realistically simulate the levels at which organisms are more usually exposed in the field. However, it would be a mistake to think that lethal toxicity testing is of no value, or that it has been altogether superseded by more recently-developed sublethal test methods. On the contrary, lethal toxicity testing continues to make a valuable and very practical

contribution to the control and regulation of pollution, as the following examples may illustrate.

There remain instances (in the UK, about one every day of the year) of illegal, negligent or accidental discharge of polluting material to watercourses which cause observable damage to the receiving environment, frequently involving mortalities of fish. In order to investigate the causes of these, and where appropriate to take legal action against the perpetrator, it is often insufficient to rely on the circumstantial evidence that a certain substance was present at or before the time the fish died. It is obviously preferable to be able to show causation, i.e. that the substance actually caused the death of the fish. There can be circumstances where the true cause of an incident can be obscured by the fortuitous presence of an unrelated but more obvious condition; or where the nature of the suspect substance is not actually known. Therefore some measure of the toxicity of the suspect substance is often required.

In some heavily polluted rivers, the total burden of pollutants can reach, at least at times, levels which are threatening to the endogenous fauna. In order to formulate specific and cost-effective remedial measures to improve the overall water quality, a model based on a careful series of lethal toxicity tests has been found useful. This approach was described in Section 1.3.

A related application of toxicity measurements can be used to assist in improving the quality of effluents. This is particularly important for effluents which are complex and/or very variable in nature. Measuring the toxicity of a series of effluent samples at different times (see Figure 4.15), can yield useful information (Lloyd, 1991a). In this case, effluent samples 4 and 9 are markedly more toxic than the other samples. If some chemical data are available, for example regarding the composition of the effluents or the operating conditions of the plant producing them, then it may be possible to identify particular components of the effluent or particular operating conditions which are associated with increased toxicity, so that structural or process modifications can be implemented to reduce the overall impact of the effluent. In another case (Figure 4.16), an effluent contained a number of components, one of which was thought to be responsible for most of the toxicity. Comparison of the toxicity of the whole effluent with a range of concentrations of the suspect substance equal to those in the effluent samples showed that the toxicities of the two were almost identical, confirming that the suspect substance was indeed the main cause of the problem. This means, of course, that improved effluent treatment can be specifically designed to remove the chlorpyrifos.

In fact, toxicity measurements are increasingly used in the regulation of effluent discharges: permission to discharge is frequently granted or withheld at least partly on the basis of a measurement of effluent toxicity; and measurements of toxicity may be specified in discharge consents to be carried

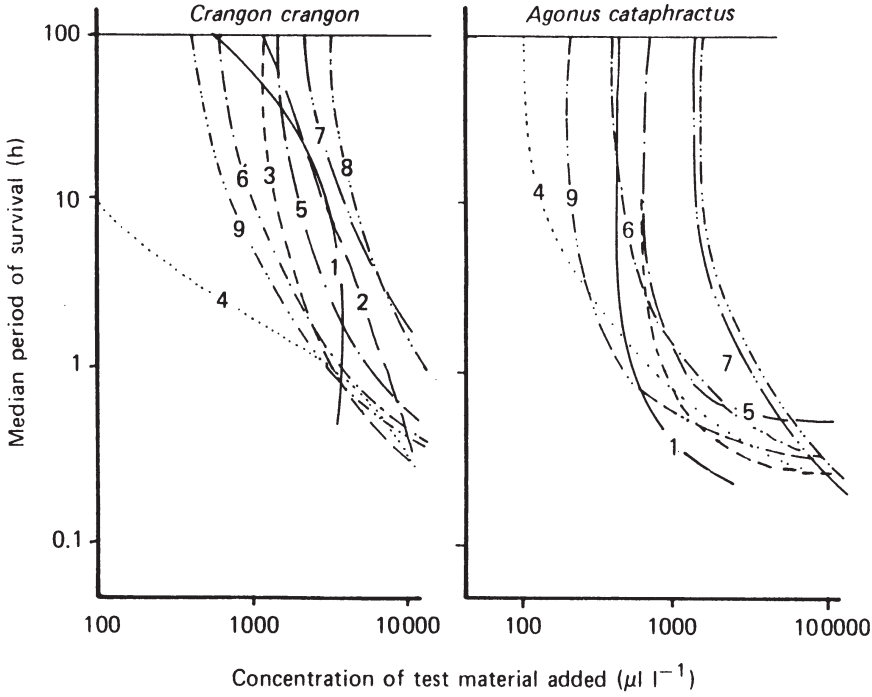


**Figure 4.15** Time-series toxicity curves for a complex effluent tested against two different species under identical conditions at different times. After Lloyd (1991)

out on a regular basis. Even a simple test can influence decisions on whether or not to grant permission to discharge, or whether to carry out further research. For example, an effluent whose toxicity curve shows a distinct lethal threshold within a short time, as do most of the samples in Figure 4.15, will almost certainly be treated more leniently than one which shows little sign of such a threshold, as in Figure 4.16.

Lethal toxicity tests also form an important part of the process known as *sequential hazard assessment* (see Chapter 6). This is a codified sequence of investigations and observations on the properties of poisons or effluents, designed to allow a reasonably informed decision to be made quickly on whether or not a particular substance is environmentally acceptable. Although in an ideal world every poison and effluent should go through an exhaustive testing process, in practice it is neither feasible nor necessary to do so. In the light of all available information, and some reasonably brief toxicity measurements, it is often possible to make a rapid decision in a particular case and concentrate resources on more difficult cases. Finally, some chemicals are released to the environment under such rare and special circumstances that lethal toxicity tests provide all the information

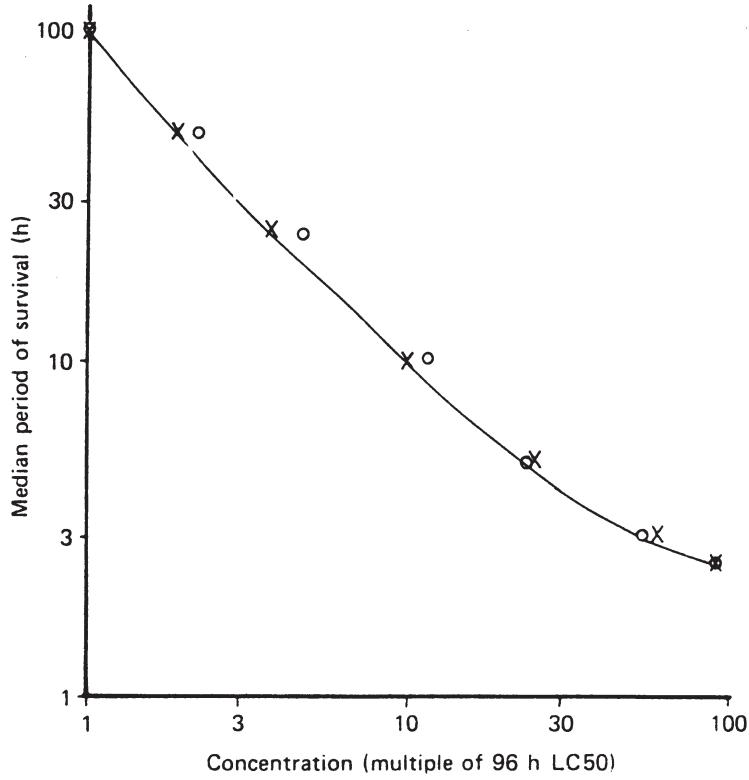


**Figure 4.16** Toxicity curve for a complex effluent containing chlorpyrifos, compared with chlorpyrifos alone. Circles represent effluent containing chlorpyrifos, crosses represent chlorpyrifos alone. After Lloyd (1991)

which is likely to be required to assess their acceptability. Regulatory agencies therefore tend to license them for sale or use on the basis of lethal toxicity tests alone. Oil dispersants provide a good example. They are only used rarely, under circumstances where their effects are likely to be short-lived and localised, and only when a body of water has already been heavily contaminated with oil. However, in many countries their sale or use is forbidden unless their toxicity to living organisms is sufficiently low. By this means, manufacturers are encouraged to develop products and formulations of low toxicity, and the original, more toxic, formulations in use some 30 years ago have gradually been phased out.

## 4.4 Sublethal Toxicity

The fauna of polluted waters is more commonly exposed to relatively low concentrations of poison for long periods, rather than to levels of pollution which will cause rapid mortality. Therefore it is important to study the effects on aquatic

organisms of exposure to sublethal levels of pollution over periods which represent at least a substantial proportion of their life cycle. The historical predominance of acute lethal toxicity studies has therefore often been criticised as being of limited relevance to real situations, but is due less to the failure of toxicologists to appreciate the point than to the many technical and conceptual difficulties involved in the measurement of sublethal toxicity.

Relatively few species can be satisfactorily maintained in the laboratory for long periods and fewer still can complete their life cycle under such conditions since their environmental requirements are complex, unknown or both. Maintenance of constant experimental conditions for periods which may exceed a year is expensive of human and physical resources, and the longer an experiment continues the greater is the chance of failure due to accident or equipment malfunction. Therefore the preferred species for research of this kind are those which can be cultured in the laboratory, are indigenous to the geographical area in which the study takes place, have reasonably short generation times and which display a more or less representative response to a wide variety of poisons; that is they should not be unusually resistant or unusually sensitive to particular poisons or categories of poison. Unfortunately in many regions few, if any, species which meet all these requirements are available.

Assuming that purely technical difficulties can be overcome, the problem remains that whereas the death of an organism is an unequivocal and easily-identifiable response to toxic action, criteria of sublethal toxic effects are less easy to define and, as will be seen, if they are recognised their biological significance is frequently difficult to assess. Nevertheless the meaningful application of data from lethal toxicity studies to many practical pollution problems is difficult, if not impossible, in the absence of some information on sublethal toxicity. Therefore a wide variety of approaches to the measurement of sublethal toxicity has been employed, and some of the more important ones are reviewed here.

### *4.4.1 Single-species Toxicity Tests*

Because of the practical problems of maintaining animals under experimental conditions for very long periods, early studies in sublethal toxicity generally relied on the use of histological, pathological, biochemical, haematological, physiological or behavioural criteria of toxic effect in experiments lasting weeks rather than months. Sprague (1971) has reviewed some examples of these studies. More recently, the ever-increasing range of analytical and diagnostic techniques devised by biochemists and clinical chemists has been widely applied or adapted to the detection of sublethal toxic responses of fish to pollutants. Table 4.1 lists some of the techniques which have been

| Variable | Variable |
|---|---|
| *Cardiovascular physiology* | *Enzyme assays* (various tissues) |
| Heart rate | Lactate dehydrogenase |
| Arterial pO2 | Glutamic-oxaloacetic transaminase |
| Ventilation rate | $\gamma$-Aminolevulinate dehydrase |
| Cough frequency | |
| Opercular and buccal pressure | *Others* |
| Ventilation volume | Urine pyruvic acid content |
| Oxygen consumption | $O_2$ consumption of tissue homogenate |
| Oxygen utilisation | Electrophoretic patterns of serum proteins |
| Ventilation frequency | Body moisture content |
| *Haematology* | Body lipid content |
| Haematocrit | Body protein content |
| Haemoglobin content | Liver glycogen content |
| Methaemoglobin content | Liver phenylalanine content |
| Total blood cell counts | Urine excretion rate |
| Erythrocyte counts | Swimming performance |
| Differential leucocyte counts | Food conversion efficiency |
| Erythrocyte ATP concentration | Locomotor activity |
| *Blood metabolite levels* | |
| Lactate | |
| Glucose | |
| Sodium | |
| Chloride | |
| Cortisol | |
| Serum proteins | |
| Pyruvate | |
| Osmolality | |
| Cholesterol | |
| Other electrolytes | |

used. The list is a representative rather than an exhaustive one, and no attempt is made here to review this aspect of pollution toxicology in detail. Rather, the discussion will focus on some conceptual and practical difficulties raised by the very diversity of criteria which have been employed.

The objective of experiments such as those represented in Table 4.1 is essentially to determine whether animals exposed to sublethal levels of pollutant are healthy or not. It might be thought that with the aid of the

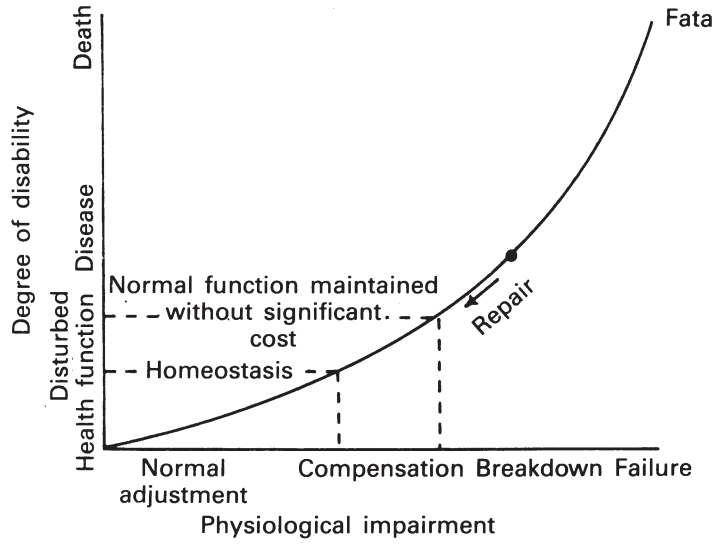


**Figure 4.17** A possible relation between physiological impairment following increasing exposure to pollutants and the consequent disability of the fish (Lloyd, 1972)

wide range of modern diagnostic and analytical techniques, the state of health of the test animals can be relatively easily determined, but this is not necessarily the case. For example, in order to state that a particular value is abnormal it is necessary to know the normal range for that particular variable, and the way it is affected by the physiological status and environmental history of the animal. For aquatic animals, including fish, such detailed knowledge of their biochemistry and physiology is generally lacking. Thus although it is possible to say that a particular value is statistically different from that of the control animals, it cannot readily be inferred that the change has any ecological consequences. The 'abnormal' value may represent not damage to the fish, but a metabolic adjustment well within the animal's ability to compensate for varying environmental conditions, which are a normal feature of aquatic life and to which many aquatic animals have a wide range of tolerance. Mount and Stephan (1967a) succinctly stated the difficulty thus: 'An exposure causing death is obviously significant, but even the best fish physiologist would have difficulty establishing that a 10 per cent reduction in haematocrit would result in an undesirable effect on a population'.

A more detailed exposition of the problem was given by Lloyd (1972) with the aid of the diagram reproduced in Figure 4.17. The diagram shows the hypothetical relationship between physiological impairment following exposure to pollutants, and the consequent disability of the fish. Measured values of physiological or biochemical variables, or alterations in the behaviour of the animal or in the

histological appearance of a tissue, may represent conditions within the areas of the graph marked 'homeostasis' or 'normal function maintained without significant cost', even though they may be statistically different from control values. The toxicologist's problem is to distinguish the point at which the value of a measured variable deviates so far from the control that it falls outside these zones. Unless this is clearly established, any change in the value of a measured variable is not necessarily an indicator of sublethal toxic effect. An example is provided by the report of Grant and Mehrle (1973) on the effect of exposure to sublethal levels of endrin on 19 physiological and biochemical variables in the rainbow trout. Although statistically significant differences occurred in 12 of these, the authors showed that nine out of 16 blood serum variables showed similar changes when the fish were subjected to moderate exercise, thus casting doubt on their usefulness as indicators of toxic effect.

Thus the validity of the approach to sublethal toxicity which is implicit in much of the work published in the last 30 years or so is questionable. Essentially this implicit approach has been to measure as many variables as possible, and to seek to determine the 'no observed effect concentration' (NOEC), that is, the highest concentration which has no observable effect on any of the variables measured. The 'maximum acceptable toxicant concentration' (MATC) is thus determined as lying between the NOEC and the next highest concentration tested. This rationale may be criticised on several grounds. First, as we have seen, a statistically significant difference in a measured variable between exposed and control fish does not imply that sublethal toxicity has occurred, unless it can be shown or at least reasonably expected that the change has actual or potential ecological significance. Second, there is a certain arbitrariness under this protocol in the decision as to whether or not a particular concentration exerts a sublethal toxic effect. If, for example, in an experiment 20 variables are measured, it follows that another 20, or 50, or 100, have not been measured. Any of these might, if they had been measured, have shown a difference from the control value. Thus the NOEC is determined partly by the choice of variables to be measured during the experiment. Further, there is no general agreement on what variables should be measured, so comparisons of results from different sources are difficult. Practising scientists will recognise that some variables are measured because they are easy to measure, some are selected in order to follow precedent, and some because of the availability of equipment or skilled personnel capable of making the measurement. Of course some measurements are made because there is a sound biological reason for making them, and as will be seen later it is becoming possible at least for some pollutants to identify on a rational basis specific and useful indicators of sublethal toxic effect. Finally, the use of the NOEC as the end-point of the experiment is a statistical absurdity since, as Skalski (1981) pointed out, it depends upon the non-falsification of the null hypothesis, a procedure which cannot be carried out with confidence (in the statistical sense). Such criticisms are not to deny the usefulness of the existing

literature and practices in the study of sublethal toxicity. Rather, they are a reflection of the fact that the methodology is in a relatively early stage of development.

It is generally accepted that a pollutant effect on growth, reproduction or development of a species is an unequivocal criterion of sublethal toxic effect, since its ecological significance is reasonably clear. The first successful toxicity tests over a complete life cycle of a fish species appear to be those of a group of American workers (e.g. Mount and Stephan, 1967a, b) using the fathead minnow, *Pimephales promelas*. Since then, tests have been successfully carried out with about half a dozen fish species, mainly North American or small tropical species. It remains true that the number of species with which it is practicable to carry out such tests is, at the present time, a very small proportion indeed of the aquatic fauna as a whole. Apart from reproduction, it is clearly easier to carry out investigations on the effects of pollutants on growth rates, using a wider range of species. However, there are examples in the literature which show that growth rate is not necessarily a very sensitive indicator of sublethal toxic effect (Sprague, 1971), and there are even some examples of growth apparently being stimulated by sublethal concentrations of poison (e.g. McLeay and Brown, 1974).

Obviously experiments conducted over the whole, or a substantial proportion, of the life cycle of a species are both expensive and time-consuming, and it is not feasible to test all poisons, species and environmental conditions using such procedures. Consequently it remains important to develop and evaluate rapid methods for measuring sublethal toxicity. One approach is the so-called 'critical life stage bioassay'. Analysis of the results of a large number of partial- and complete-life-cycle tests with various species and poisons shows that in the majority of cases, the early embryo and larval stages are the most sensitive part of the life cycle, and an estimate of the MATC based solely on the response of the embryo-larval stages generally lies very close to the value obtained when the whole life cycle is considered (Macek and Sleight, 1977; McKim, 1977). Thus the duration and scale of experiments can be considerably reduced, and the critical life stage bioassay (sometimes called the 'embryo-larval test') has been widely used. It also offers the possibility of increasing the range of species available for testing, since there are several species (e.g. many salmonids) whose eggs and early life stages can be maintained in the laboratory but which are difficult or expensive to maintain throughout an entire life cycle. Nevertheless there remain many species of interest which cannot be used, or which are only available during a relatively short period of the year. Thus interest remains strong in alternative criteria of sublethal toxicity.

For the reasons outlined above, such criteria should preferably be specific responses to the pollutant, that is related to the poison's mechanism of toxic action, rather than non-specific responses which may merely represent physiological adjustment to new, but perfectly tolerable, environmental conditions. Ideally, sublethal toxicity tests should also be rapid, sensitive, relevant to actual environmental conditions, and based upon a measurable response which

has, or may reasonably be expected to have, ecological significance; that is, likely to reduce significantly the fitness of the population. As we know relatively little about mechanisms of toxic action in fish and aquatic invertebrates, examples which meet all of these criteria are rare. Nevertheless there appears to have been a distinct change in emphasis in the study of sublethal toxicity over the last 15 years, away from the traditional approaches and towards a novel series of techniques based on improved knowledge of the physiology, biochemistry and cellular biology of aquatic species and of their interactions with toxic substances. Some examples are given below.

Heavy metals being among the most common of pollutants, the discovery of metallothioneins in the 1970s gave rise to interest in their use as 'biomarkers' of toxic effect. Metallothioneins are a group of proteins characterised by their low molecular weight (6000–20000), their high content of amino acids containing sulphydryl groups (especially cysteine), and their ability to bind to heavy metals. They are absent, or present at very low levels, in the tissues of vertebrates and invertebrates, but are produced at high levels when the animal is exposed to heavy metals (Kagi and Nordberg, 1979). They are relatively easy to isolate and identify using standard biochemical techniques. A tissue homogenate is separated into fractions of different molecular weight by gel chromatography, and the optical density of the fractions measured in the UV range at 250 and 280 nm. Fractions of the appropriate molecular weight which show a high absorbance at 250 nm compared to 280 nm (this is due to the sulphydryl groups), may be tentatively identified as containing metallothioneins if they also contain high levels of heavy metal; this last stage is destructive of the sample, as atomic absorption spectrophotometry is usually used to determine the metal content of the fractions. Figure 4.18 shows a typical result obtained from Plecopteran larvae isolated from a metal-contaminated river.

The potential value of metallothioneins is that since they appear only to be produced in quantity in animals under stress from heavy metals, they could be used directly to determine the level of heavy metal which a particular organism found unacceptable. Alternatively, it may be possible to determine whether the level of heavy metal present in a particular environment was above the limits of tolerance of the organisms living there. Some caution, however, is required at the present stage of knowledge. For example, many heavy metals which are toxic at a certain level are normal, even essential, metabolites at lower levels. Therefore organisms must have some means of metabolising them, and the induction of metallothioneins may represent a normal adjustment of the organism, or detoxification, rather than a manifestation of toxic effect. Although there are examples of the use of metallothioneins to assess the extent of metal pollution in field situations (Roch *et al.,* 1982, appear to have reported one of the first examples), there

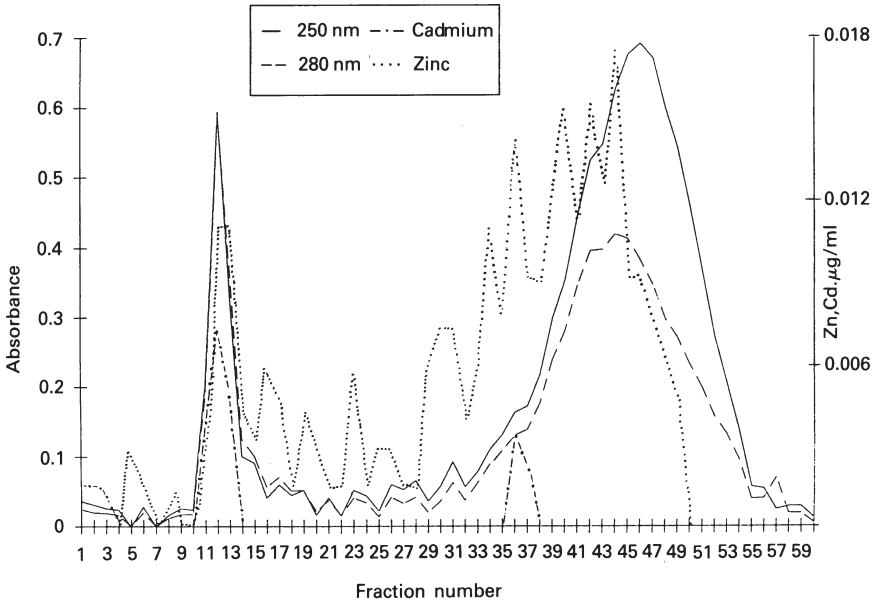


**Figure 4.18** Elution profile of the cytosolic supernatant from homogenised tissue of a stonefly nymph, *Diura bicaudata*, taken from a metal-polluted river. The presence of metallothionein in fractions 11–13 is suggested by the corresponding peaks for zinc, cadmium and absorbance at 250 nm

is as yet no clear link between metallothionein levels and variables of direct ecological significance. Benson and Birge (1985) reported an association between metallothionein levels and metal resistance, in field and laboratory trials. Possibly further experience may allow the determination of levels of metallothionein which can be considered abnormal. Some other proteins of a generally similar nature, often called stress proteins, have been found to be induced by other forms of stress, such as temperature shock, and may be confused with metallothioneins (Sanders, 1990), if indeed they are different entities at all.

Mehrle and Mayer (1980) in a brief review of clinical tests in aquatic toxicology, drew attention to a promising series of investigations involving study of the effects of poisons on biochemical processes related specifically to growth in fish. They argued that growth in fish is the culmination of a series of biochemical processes which should show changes *before* any effect on growth rate is detectable by conventional measurements of weight and length. They showed that several organic toxicants affected the vertebral collagen content of fish, and the proline and hydroxyproline growth rate, and were more sensitive indicators of toxic effect than measurement of growth rate itself.

A group of enzymes known as mixed-function oxidases (MFO) are widely found in vertebrates, including fishes, and have been reported in some invertebrates. They show some potential as indicators of sublethal toxic effect, and possibly as a means of monitoring pollutant effects in field populations (Addison, 1984). The normal function of MFO appears to be associated with the metabolism of steroid hormones, but they have been found to be produced at elevated levels in animals exposed to some pollutants, particularly aromatic hydrocarbons and halogenated hydrocarbons. Metabolites of these important pollutants appear to resemble those of some natural steroids, and to induce MFO synthesis. Thus elevated MFO levels may be used to indicate exposure of the animal to pollutants. The technique and its applications are not, as yet, well developed; for example, the link between MFO synthesis and actual toxic effect is not generally established. Mixed-function oxidase production may again, therefore, represent an adjustment whereby 'normal function is maintained without significant cost' (see Figure 4.17). However, it seems likely that enhanced MFO production can lead to another important consequence, genotoxic effects.

The relationship between MFO and genotoxicity is explained by Zahn (1991), and in Figure 4.19. MFO breaks down toxic organic chemicals but the immediate consequence is the production of carcinogenic compounds which can cause alteration of the DNA of the cells, which can be detected by a variety of techniques (Zahn, 1991). Nearly all DNA alterations can be considered as deleterious; and although there is a repair mechanism for restoring damaged DNA, if it becomes overwhelmed DNA damage will accumulate and have consequences either in the present generation (e.g. through tumour formation), or, of course, in future generations (e.g. through the production of mutations).

Lysosomes in cells appear to be deeply involved in the detoxification of or response to toxic substances, and tests based on lysosomal stability have been developed (Moore, 1991). Physiological measurements to determine the effect of exposure to pollutants on the scope for growth of some animals appear to show promise (Axiak, 1991). These have mainly been applied in the rather different circumstances of the marine environment, where they have particular advantages over more conventional approaches (see Section 7.3). Indeed, the range of methods available for assessing sublethal toxicity appears to be limited only by the ingenuity of investigators, and it increases year by year.

### *4.4.2 Experimental Ecosystems*

An alternative approach to the study of sublethal toxicity is that of the experimental ecosystem. Instead of exposing a population of a single species to the pollutant, populations of two or more species, frequently representing different trophic levels, are maintained and exposed together to the pollutant.

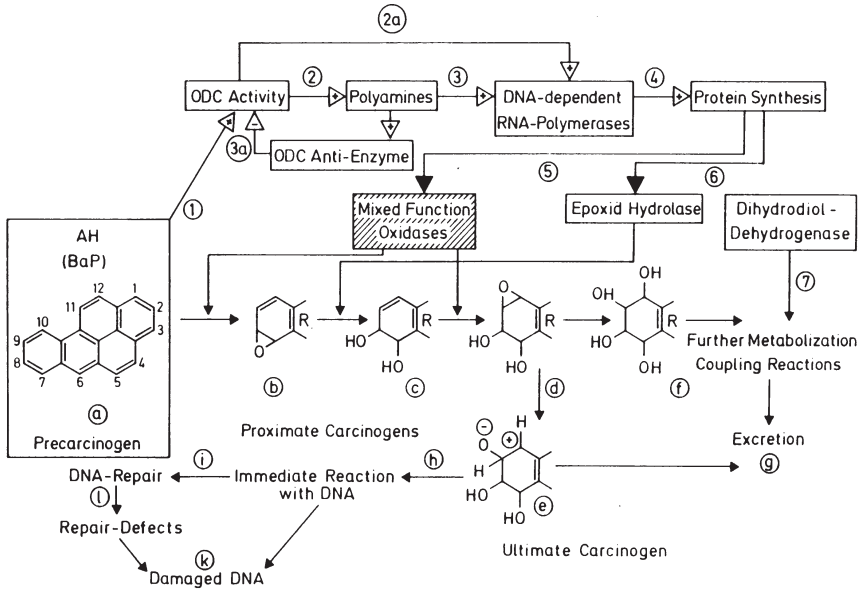


**Figure 4.19** Fate and effects of polycyclic aromatic hydrocarbons (PAH). Sequence of events initiated by contact of an inexperienced vertebrate with benzo(a)pyrene (B(a)P). Only a selection of reactions is given. B(a)P is used as an example (+ arrows mean increase, − arrows mean decrease of enzyme activity).

(1) The precarcinogen form provokes a rise in ornithine decarboxylase (ODC) activity (Byus *et al.*, 1976) leading to (2) an increased polyamine synthesis (Russell, 1973) and RNA synthesis through (3) more RNA polymerase activity (Russell, 1971; Jacob and Rose, 1976). The latter is also enhanced by (2a) direct action of ODC (McEnroe and Healy, 1977). RNA synthesis is followed by (4) protein synthesis (DePierre and Ernster, 1978), which enables (5) synthesis of MFO and (6) epoxide hydratase (DePierre and Ernster, 1978). (3a) ODC-antienzyme is induced by high levels of polyamine (Heller *et al.*, 1976). (7) Dihydrodiol-epoxygenase action.

Out of many PAH, B(a)P is one; and from this, one single pathway to an 'ultimate carcinogen' with the capability as an electrophilic to combine, among many others, with DNA, is depicted. The precarcinogen (a) B(a)P, under the influence of MFO is transformed into the (b) 7,8-epoxide and by epoxide hydrolase into (c) the 7,8-trans-diol, which is another substrate for the MFO, thus yielding two diastereomeric forms of (d) 7,8-diol-9,10-epoxide-B(a)P. These 'proximate carcinogens' give rise to 'ultimate carcinogens', which most likely are (e) carbenium ions with the capability to (h) direct action with nucleophilics (Yang *et al.*, 1977) in specific manner (Deutsch *et al.*, 1978; Meehan and Straub, 1979). (f) Some of the compounds arising from the activity of the MFO, epoxide hydrolase, glutathione-*S*-transferase, UDP-glucuronic acid transferase and sulphotransferase may be transformed and/or coupled, thus being detoxified

In part this approach represents an attempt to make the experimental situation more realistic, and hence more directly applicable to the field situation. For example, in the field the ecology of a species is governed not only by its relationship with the physical and chemical environment and by purely endogenous population processes, but also by its relationship with other species (prey, predators, competitors, parasites) with which it shares its habitat. Thus the ecological effect of a pollutant on a species does not depend only upon the properties of the species itself; its influence may be accentuated or attenuated depending upon the nature of interspecific relationships which exist in the environment. Experimental ecosystems are both useful and necessary for studying these and other phenomena, including the distribution of pollutants between water, sediments and living tissues; the biodegradation of pollutants; and the accumulation of pollutants by living organisms from their environment, and the passage of pollutants through the food chain.

Experimental ecosystems vary in their scale and complexity. Generally the smaller-scale systems offer the advantages of more precisely-controlled experimental conditions, but are at best gross simplifications of real systems. Larger-scale systems more closely represent natural systems, but clearly cannot be so precisely controlled and experiments may be difficult or impossible to replicate. The smallest systems are more-or-less enclosed vessels containing populations of microorganisms or plankton. On a larger scale, experimental aquaria or ponds ranging in size from a few litres to a few cubic metres have been used (Giddings, 1983). Experimental river channels, both on a laboratory scale and outdoors, have been widely used (e.g. Arthur *et al.,* 1982; Watton and Hawkes, 1984). Finally, there have been attempts to isolate large water masses in lakes and coastal marine areas, to study the processes which occur within, as it were, a representative sample of the natural environment (Davies and Gamble, 1979; Steele, 1979). Since they vary widely in their scale, complexity and objectives perhaps the only generalisation which can be made about experimental ecosystems is that they can provide valuable information which bridges the gap between laboratory and field studies. Large-scale systems are, however, expensive to maintain, and their use is likely to be confined to aiding the interpretation of the results of more economical methods of study. There appears to have

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and (g) excreted (Oesch, 1982; Yang *et al.*, 1978). (i) Interaction of DNA with ultimate carcinogens leads to strand scissions (Gamper *et al.*, 1977) and to coupling of PAH derivatives (Miller and Miller, 1981). DNA alterations cause repair to start, which to a certain degree may cause (l) some secondary DNA defects (Hanawalt and Friedberg, 1978). This altogether results, in a time-dependent manner, in DNA showing (k) secondary alterations giving rise to mutagenesis/carcinogenesis (Ames *et al.*, 1975; Huberman and Sachs, 1974, 1977)

been some reduction in interest in experimental ecosystems in recent years, perhaps because their results have been less useful and informative than was once hoped. However, this tendency is to some extent counteracted by recent legislative pressure which is leading to the development of artificial ecosystem techniques for regulatory purposes. This arises because, particularly in Europe and the United States, laws are being promulgated which demand that pollutants discharged to the environment are shown to have no unacceptable environmental effect. Such laws are not necessarily very sensible, and they are certainly not scientifically sensible. First, it is not possible to prove that an effect does not exist; second, there is no definition of what is acceptable; and third, experimental ecosystems do not, as we have seen, necessarily simulate the natural environment much more accurately than properly controlled experiments. Nevertheless, if such laws exist some attempt must be made to conform with them, and the volume edited by Hill *et al.* (1994) provides a very full review of recent developments in the applications of experimental ecosystems. (See further discussion in Section 7.2.3.)

### *4.4.3 Bioaccumulation*

Bioaccumulation is an aspect of sublethal toxicity which has received much attention, though many areas of uncertainty remain. Pollutants may, over long time periods, accumulate in tissues to levels which may be harmful to the organism. Since many aquatic species are utilised for human consumption, the public health significance of toxic substances accumulated in their tissues is obvious. Many national and international agencies set concentration limits for pollutants, particularly heavy metals, in tissues for human consumption, and promote research and monitoring programmes. Study of the uptake, metabolism and excretion of pollutants, and of their distribution in the various body organs and tissues, makes an important contribution to understanding their mechanisms of action. Levels of pollutants in the tissues of living organisms are widely used to indicate the degree of contamination of the waters in which they live, particularly when the pollutants are present only intermittently or in very low concentrations, making chemical analysis of the water difficult. Finally, many poisons, particularly heavy metals and refractory organic compounds such as some pesticides, are widely believed to pass from the tissues of prey organisms into those of predators and to attain concentrations there which are several orders of magnitude higher than those in the tissues of the prey species. This phenomenon poses a specific threat to long-lived organisms at the higher trophic levels.

Studies of bioaccumulation are carried out in the laboratory, in experimental ecosystems and in the field. Laboratory investigations are usually concerned initially with determining the 'bioconcentration factor' (BCF), that is, the ratio between the concentration in the animals and the concentration in the water, when the animals

have been exposed for sufficiently long for an equilibrium or steady state to be achieved. This ratio is generally regarded as a valid indicator of the capacity of a pollutant to accumulate in animal tissues. Such limited data as are available (Davies and Dobbs, 1984; Schnoor, 1982) suggest that laboratory-derived BCF values agree reasonably well with those derived from field observations on the animals of polluted waters, at least for certain groups of organic pollutants and provided certain conditions are met in the laboratory determinations. Under certain conditions, there is a good correlation between log $P$ (where $P$=the octanol-water partition coefficient of the chemical) and the BCF. This offers the possibility that the bioaccumulation potential of a pollutant can be indicated by the result of a relatively simple chemical determination, rather than by the expensive and time-consuming estimation of BCF. However, Davies and Dobbs (1984) in their study of this question, found it necessary to reject determinations of BCF which did not meet certain criteria.

There are many models of bioaccumulation. In the simplest possible model, two compartments are considered: the organism and the environment. Pollutant will enter the organism at a certain rate, which is dependent upon the amount present in the environment. Pollutant will also be lost from the organism, at a rate dependent upon the amount present in the organism. This simple model can be expressed mathematically and predicts that organisms exposed to a constant level of pollutant will eventually reach a 'steady state'; that is, the concentration of pollutant will increase to a certain level and thereafter remain constant. Conversely, the model predicts that in contaminated organisms maintained in clean water, the concentration of pollutant in the organism will decline exponentially. These predictions are generally confirmed by experimental findings.

Such a simple model is of limited practical use, however, and it is not difficult to see why. Most organisms cannot be considered as a single compartment. Studies of the distribution of pollutants in animals invariably show that the pollutant is very unevenly distributed between the various body tissues. Different pollutants behave in different ways. Clearly the animal will begin to suffer harm when the poison concentration in a particular organ reaches a critical level. Thus the concentration of the pollutant in the whole body is not a good indicator of harmful effect. For this reason, models have been derived which treat the organism as a set of interacting compartments. These models treat discrete organs (e.g. liver, kidney, brain) as interacting compartments connected via the blood (itself considered a compartment) with each other and with the external environment. Further, in some models the exchange of pollutants between the compartments is considered as a series of separate processes. For example, uptake of the pollutant from the environment may occur through the body surface, or by ingestion of food or of non-food particulate material. Elimination of the pollutant may occur through outwards diffusion, through renal or gastrointestinal excretion, or by metabolic breakdown. Clearly models which attempt a complete and accurate description of

bioaccumulation soon become extremely complex, and for that reason they are not treated in detail here. Hamelink (1977) and Moriarty (1984) provide good discussions of the strengths and weaknesses of bioconcentration models.

The purpose of such models is twofold. First, if they can be validated by experimental findings (i.e. if the predictions of the models correspond with actual observations), they provide the means for making useful predictions based on comparatively simple experimental measurements. In other words, they can eventually become a substitute for actual experimentation, which may be time-consuming and expensive. Second, they can provide valuable information about the mechanisms of bioaccumulation. If, for example, an experimental finding does not agree with a prediction of a model, it indicates that one or more of the assumptions in the model is wrong, and thus focuses attention on areas which require further investigation. Although it is by no means clear that any existing model is of general application, studies on bioaccumulation and its mechanisms are of great practical importance. For instance, Moriarty (1984) has convincingly argued that the lack of understanding of bioaccumulation processes in single species has serious implications for ideas about the passage of pollutants through food chains.

It is widely believed that many pollutants pass through succeeding trophic levels and accumulate in high concentrations in the tissues of long-lived predators. Although there are a small number of widely-accepted examples of this, it is by no means well established that this is a general phenomenon, even for persistent pollutants like heavy metals and refractory organics. Moriarty (1984) has discussed some of the inadequacies of current knowledge. For example, comparing tissue levels of pollutants in field populations is very likely to produce biased results if, as is usually the case, mean levels of pollutants are compared. This is because of the differences in pollutant concentrations between individuals of the *same* species; frequently, mean values are biased by a very small number of individuals which have very high concentrations; that is, the frequency distribution of pollutant concentration values is highly skewed. Further, although under experimental conditions a steady-state concentration of the pollutant in the tissues generally is eventually achieved, it is not clear that this is the case in the field. Field populations are generally exposed to lower, and more widely-fluctuating, pollutant concentrations in their environment. Clearly a comparison between tissue pollutant concentrations in two different species is invalid if they are not both at their respective steady-state concentrations. It is also true that the interpretation of field observations, and the design of experimental investigations, often rests upon unverified, and unwarranted, assumptions about what animals actually eat. Obviously in a simplified experimental system, predators will feed on prey which may not form their normal or natural diet in the field. A further example is the widespread assumption that large marine predatory fish such as tunas feed primarily

or exclusively on smaller fish such as mackerel, herring or sardine. However, the stomach contents of several hundred tunas caught in the Mediterranean during an international angling contest consisted almost entirely of crustaceans and plankton (Fowler *et al.,* 1979). This should not have been a surprise to anyone who knows anything about the biology of tunas, but this simple observation destroyed some elaborate theoretical models! Thus while the question of bioaccumulation and biomagnification along food chains is an important and interesting one, authoritative answers require the solution of several technical and conceptual problems, and a greater knowledge of the basic ecology and physiology of many species.

## 4.5 Evaluating Toxicological Data

The literature on the toxicity of pollutants to aquatic organisms is enormous, and yet there are several important questions which cannot yet be clearly answered. The range and variety of lethal and sublethal toxicity test methods available are huge, and increasing; as is the recorded occurrence of sublethal toxic effects whose significance can frequently only be guessed at. The number of chemicals requiring some form of risk assessment increases by hundreds each year, the majority of species have yet to be investigated, and every circumstance has its own environmental conditions which we do know will influence toxicity considerably.

Probably few readers of this book will ever themselves be actively engaged in toxicological investigations, yet probably the majority will at some time need to refer to toxicological data and evaluate its reliability and significance. It is unfortunate that a great deal of the information available is of poor quality and likely to be misleading. This section offers some simple guidelines to assist in the evaluation of toxicological data.

Measurements of lethal toxicity are, in principle, the most straightforward because the criterion of toxicity, death of the organism, is usually easily recognisable. However, much published data are of less use than it may at first appear. Probably the commonest mistake is to use simple test methods for purposes for which they are not suitable. For example, tests designed for routine screening and monitoring are of little value in comparing the toxicities of different poisons, the susceptibility of different species or the effects of environmental conditions, for reasons explained in Section 4.1. Similarly, data presented in the form of fixed-time LC50 values are of doubtful significance unless there is some indication of how close that time is to that at which the lethal threshold becomes apparent. It is still disturbingly common to see data presented in the form of cumulative mortality curves—often without even the log-probit transform, which allows median values and their confidence limits to be determined—which are incapable of distinguishing between the speed of reaction of the organisms and the inherent toxicity of the pollutant to which they are exposed. All reports should be routinely examined to see if adequate

information is given on the chemical composition of the dilution water, the volume of the test containers and how frequently the test solutions are changed; on the frequency of observations of the experiment; and whether any steps have been taken to *measure* the actual concentrations of poison in the solutions, and the other experimental variables which are known to have large influences on the final result. To the extent that such information is lacking, the significance which can be attached to the results is diminished. Finally, some checks can often be done, based on internal evidence of the report, on whether the results have been correctly computed. This should not be necessary, but experience unfortunately shows that it often is! For example, if an LC50 value is expressed as, say, 15.56±0.008 mg $l^{-1}$, a rapid back-calculation will probably show that to achieve this result, the experimental concentrations would have to be impossibly closely spaced. Therefore the investigator did not carry out the experiment properly, or does not understand the calculations, or both!

For sublethal toxicity experiments, the same considerations apply, but in addition there is the problem of evaluating the biological significance, if any, of the toxic effect used as the end-point of the experiment. At one extreme, any effect could be considered significant. On the other hand, arguably only those effects which are of ecological significance could be considered as important. This point has yet to be resolved by consensus, but it is important in view of the considerable demand for toxicity tests for regulatory purposes. There is a tendency, whenever a novel toxic effect is discovered, to suggest that it could be used as a toxicity test, particularly if the experimental procedure is relatively quick, simple and sensitive. However, it is important to distinguish between the study of sublethal toxicity *per se,* and the application of sublethal toxic effects as test methods for regulatory purposes. If we wish to know how poisons affect organisms at sublethal levels, what is the sequence of cause and effect at succeeding levels of biological integration from the passage of the poison molecule into the organism, its initial reaction within the cells, and the ultimate consequences of this at the level of the whole organism or the population, then we are legitimately interested in any response we can detect. If, however, we wish to develop a particular biological response into a test method for regulatory purposes, we should be interested in a much smaller range of effects. For this purpose, we require methods which offer economies of time and scale, which are ecologically relevant and which bear some relation to the behaviour of a range of key organisms exposed to a wide range of pollutants as determined by more conventional methods. Thus, since insecticides are designed to be harmful to insects and relatively harmless to other species, any sublethal response of an insect species to an insecticide is most unlikely to be of use as a general test method for regulatory purposes, since it will give little accurate idea of how non-insecticidal poisons might behave. Toxicity testing for regulatory purposes is one important branch of ecotoxicology, but no more than that. Similarly, not all sublethal toxic

effects are suitable for this purpose, though they may be interesting for other reasons; and not all sublethal toxic effects have any particularly clear ecological significance. They may, in fact, be quite trivial.

## 4.6 Quantitative Structure-Activity Relationships (QSARs)

In Section 4.2.1 it was seen that in a homologous series of linear alkylate sulphonate detergents, a regular empirical relationship could be discerned between the lethal toxicity of the detergent and the length of the hydrocarbon chain. This is a simple example of a *quantitative structure-activity relationship,* or QSAR. To determine whether or not a QSAR exists for a particular group of chemicals, it is necessary to plot a graph to show the relationship between some measure of biological activity (such as 96-h LC50, bioconcentration factor, or the concentration causing some sublethal effect) against some structural parameter (chain length, molecular weight) or some value closely related to structural parameters such as octanol-water partition coefficient. If a good correlation is found, using univariate or multivariate linear or non-linear regression techniques, the QSAR for this group of chemicals can be a useful predictive tool.

For example, if measured toxicity data for several of a particular group of chemicals are available, it becomes possible to predict with reasonable accuracy the likely toxicity of any other chemical in the group. This can afford considerable savings of time and resources in the development of novel compounds, or where a chemical is encountered for which no data on biological activity are available. Another possibility is that of predicting the toxicity of chemicals to one species based on data for another species. This situation commonly arises, since the bulk of the available data relates to a relatively small number of 'standard' species, whereas the species under threat from real pollution incidents are usually of another kind. Figure 4.20 shows the basis of this idea. The line is based on the open circles, which are coordinates of a plot of the toxicity of 16 chemicals to *Daphnia* against the toxicity of the same chemicals to the guppy. Note that this line is *not* a QSAR. Superimposed on this line are toxicity *predictions* based upon the QSAR for this group of chemicals for *Daphnia*. The fact that all the data points, whether real or predicted, fall close to the line suggests that for this group of chemicals, the two species share a common QSAR. This does not mean that the toxicity of the chemicals to the two species is the same. However, it does mean that the *Daphnia* QSAR can be easily adapted to make predictions for this group of chemicals against the guppy. In principle, it should then be necessary only to undertake real tests on a new species with a few chemicals in a series, to establish the commonality of the QSAR, in order to be able to make reasonable estimates of toxicity of the whole group to that species (Lloyd, 1991b).

There are still, however, some limitations on the use of QSARs which

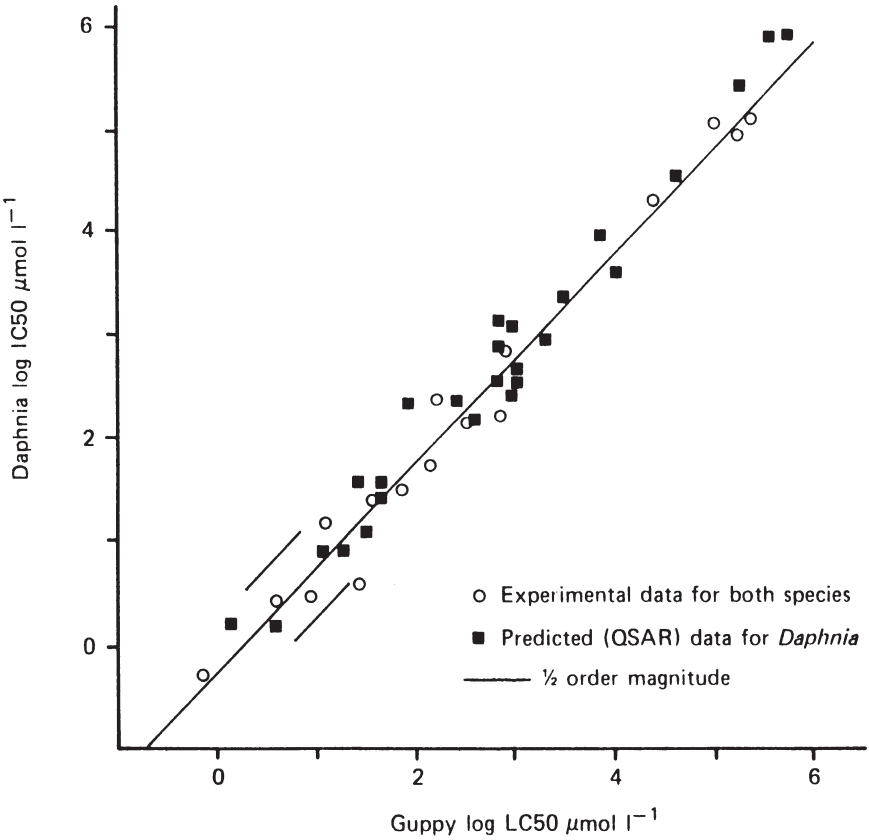


**Figure 4.20** Correlation between the sensitivities of two organisms to 42 industrial organic chemicals. After Lloyd (1991)

means that they cannot yet be considered a substitute for real measurements in risk assessment, but rather as a useful aid. First, QSARs are correlations, not causal relationships. In any group of chemicals, it is not uncommon to find one or more which cause the QSAR to break down. There are several possible reasons for this, but one important one is that the idea of QSARs makes a hidden assumption, that each chemical in the QSAR has basically the same mechanism of action in the living organism. This is not necessarily the case. However, where it can safely be assumed that a group of chemicals does have the same mechanism of action, a further possibility is opened that a group of chemicals in a QSAR do not have to form a homologous series, or be structural isomers, or necessarily to have any such close chemical similarity. Thus the idea of QSARs could be extended to very large groups of chemicals indeed. Of course, the term 'mechanism of action' subsumes a complex series of biological and chemical processes, but some attempt has been made to recognise basic modes of action in terms of the symptoms poisons

produce in fish: so-called *fish acute toxicity syndromes* (FATS). Five toxic syndromes have been recognised so far (Lloyd, 1991b): respiratory uncoupling, respiratory irritation, acetylcholinesterase inhibition, and narcosis types I and II. Any chemical which causes one of these syndromes at acutely-toxic levels in fish appears to form a QSAR with all others which cause the same syndrome. This empirical finding appears greatly to extend the potential of QSARs in the future.

Second, QSARs do not appear to work well with chemicals which are strongly polar; they work best with non-polar organic chemicals. QSAR predictions are also insufficiently accurate for many purposes—in view of the many factors which can influence a measurement of toxicity, predictions to within an order of magnitude are probably the best that can be achieved. Nevertheless use of QSARs is a promising approach which at the very least offers the possibility of a substantial reduction in the number of toxicological experiments which currently need to be carried out. Konemann (1981) and Kaiser (1987) are useful sources of further information.

# 5

# Water Pollution and Public Health

It is widely but erroneously believed that the marked increase in life expectancy which has occurred among the populations of many countries since the end of the nineteenth century was due to advances in medical science. In fact, in the conditions which prevailed in the swelling and poorly-sanitated cities of the nineteenth century, and which still prevail in much of the developing world today, the major hazards to life were epidemic diseases which exacted a heavy toll, particularly among the young. Certainly medical scientists played a major role in establishing the nature and the means of transmission of these diseases. Nevertheless the control of epidemic diseases was achieved largely through the development of proper methods for the treatment and disposal of wastes, and by attention to the provision of clean water supplies, together with education and legislation concerned with general hygiene. These developments preceded by some decades the availability of medical treatments, such as antibiotics, for the cure of waterborne infections. Today, those of us who live in the better-developed parts of the world can be reasonably optimistic, should we catch a waterborne disease, of a successful outcome, since most of these diseases respond well to modern medical treatments. Nevertheless we should not forget that the overwhelming majority of us will never become infected in the first place; and the reason for this is primarily the existence of adequate measures for the monitoring and control of pollution.

All this is of more than historical interest, since in many parts of the world today waterborne diseases remain a major hazard. They are endemic in those countries which have not yet established systems for the sanitary disposal of wastes. It is striking that in times of war or natural catastrophe, when sanitary systems cannot be maintained satisfactorily, waterborne diseases take very little time to spread through the human population. Even in countries where waterborne diseases are not considered endemic, the speed and frequency of international travel and

the magnitude of international trade present a constant threat of the reintroduction and spread of infections. Further, increasing demands upon the water resources even of developed countries present new public health problems. Britain, for example, is not a country which could be considered as suffering from an overall shortage of water. Nevertheless the demands on the country's water resources are such that it is increasingly necessary for water to be reused. The preferred sources of water supply are upland surface waters or ground water from deep wells or boreholes; these are unlikely to be contaminated with noxious chemicals or disease-causing organisms and can be rendered suitable for potable water supply with minimal treatment. However, Evans and Johnson (1984) estimate that already 30% of Britain's water supply is derived from lowland rivers. Such water is likely already to have been used several times over by communities upstream, and to have received waste discharges from domestic, agricultural and industrial sources. The reuse of water poses special problems in relation to the spread of infectious diseases and of other harmful effects caused by chemical contaminants.

Public health measures for the control of infectious and other diseases associated with water take various forms. Examples include good medical services for rapid diagnosis and treatment; a system of rapid reporting of infectious diseases so that epidemics and their sources can be quickly identified and eliminated; education and general public awareness of good hygienic practices; and controls over the processing and handling of food for human consumption. The monitoring and control of water pollution is a central element in the preservation of public health, because water is potentially the means by which many diseases can be spread.

## 5.1 Water Pollution and Pathogens

Pathogenic organisms which are spread by polluted water include bacteria, viruses and parasites. Some common examples are discussed here, but it is important to remember that many infections are never identified as caused by any of the well-known agents of epidemic disease, particularly in the less well-developed areas of the world. In fact, diarrhoea, which may be caused by bacterial, viral or parasitic infections, is responsible, according to some estimates, for six million deaths per year (Slade and Ford, 1983). UNEP (1989a) estimated that in 1988 there were over 1300 million cases of diarrhoeal illness worldwide, causing four million deaths, mainly of infants. Some common means of transmission of enteric diseases are summarised in Figure 5.1.

### *5.1.1 Bacterial Pathogens*

Typhoid fever is a disease of the gastrointestinal tract which frequently gives rise to systemic infections. If diagnosed and treated with antibiotics, it is

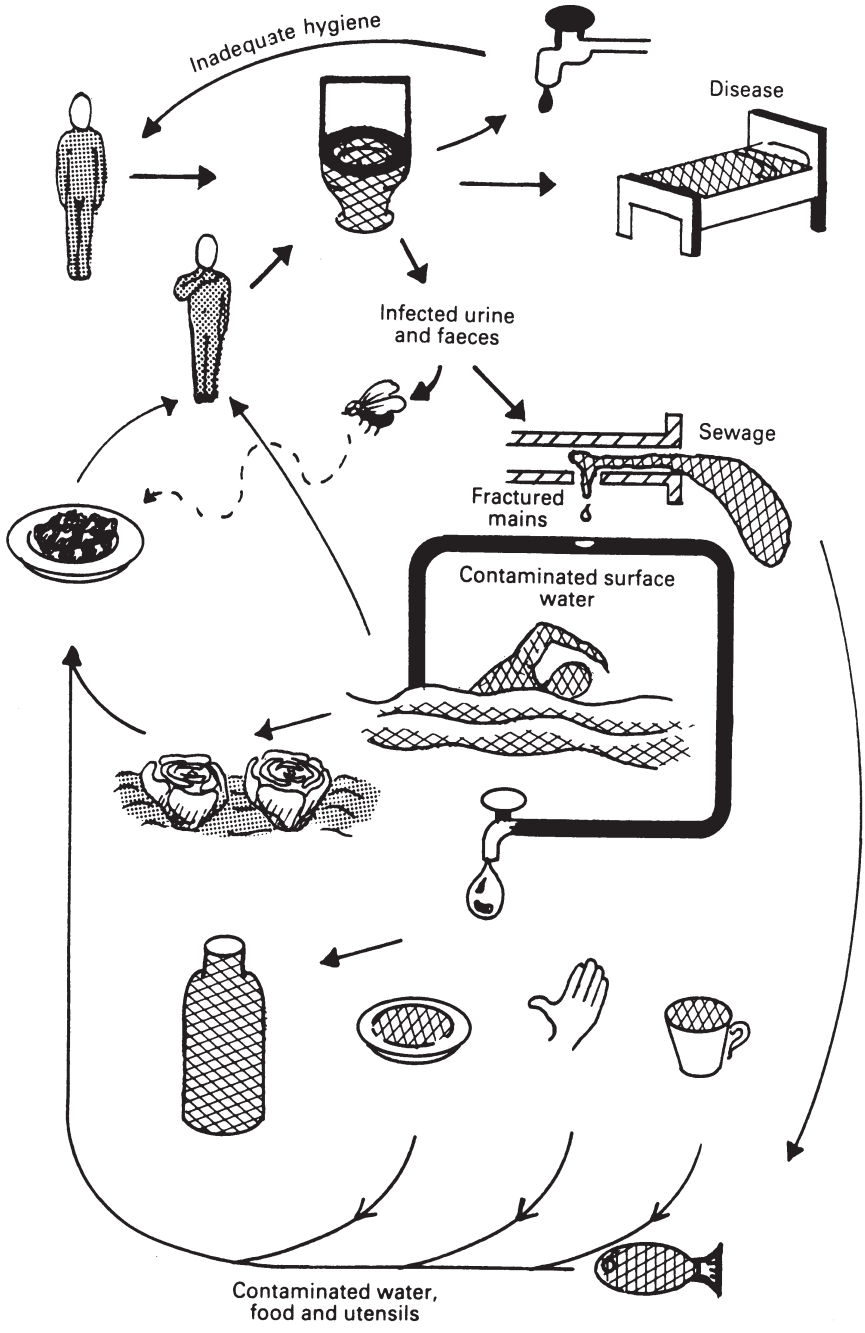


**Figure 5.1** Summary of the modes of transmission of enteric diseases through contaminated water

debilitating but rarely fatal. Without treatment it is fatal in between 12% and 30% of cases (Hornick, 1982). The disease is rare wherever public health and pollution control measures are adequate, but minor outbreaks are not uncommon, particularly among travellers recently returned from less-developed parts of the world. Typhoid infection is usually caused by ingestion of bacteria from faecally-contaminated water or food. In Britain, the most serious epidemic of recent times affected over 500 patients. It was caused by canned meat imported from Argentina. It was discovered that the canned and sterilised meat had been cooled in contaminated water, and that water had leaked through imperfect seals in the cans (Ash *et al.,* 1964). Figures for typhoid outbreaks in more recent years in various regions of the world are given by UNEP (1989a). The causative organism is *Salmonella typhi*. If the bacteria survive passage through the acid of the stomach (a process which may be assisted if the bacteria are ingested with water), they colonise the intestine and enter the epithelial cells of the gut lining. Very few bacteria (between 100 and 1000 cells) are required to establish a *Salmonella typhi* infection; for other *Salmonella* infections, the minimum infective dose is rather higher, approximately $10^5$ to $10^6$ cells. Ulceration of the intestine occurs, and bacteria enter the bloodstream. At this stage, fever occurs and the patient becomes mentally and physically debilitated. Subsequently, the bacteria may become established in various parts of the body, especially in the lymph nodes, gall-bladder, spleen and skin—characteristic haemorrhagic spots on the skin may be seen in some patients. Infected individuals excrete large numbers of bacteria in the faeces, providing a source of infection of further individuals. An interesting and dangerous feature of this disease is that in some cases the symptoms of infection can be mild and undiagnosed, although the patient becomes chronically infected with *Salmonella* without showing symptoms of the disease. These 'carriers' are a persistent source of infection in the community; further, even if diagnosed, the disease in such patients does not readily respond to the normal treatment with antibiotics. It is estimated (Hornick, 1982) that in the USA there are about three or four typhoid carriers per 100000 in those populations which have been studied, although this number seems to be declining. However, it must be assumed that typhoid carriers will be present in any sizeable community.

Bacterial dysentery (shigellosis) and its cause were first recognised in Japan at the end of the nineteenth century during an epidemic involving 90000 cases. It was almost certainly a scourge of the human population long before its nature was understood. Keusch (1982) refers to descriptions of the disease in the Old Testament, and by the ancient Greek writer Thucydides. Today the disease remains common throughout the world. In the USA as recently as 1978, data from the Centre for Disease Control indicated nearly 25 cases per 100000 among infants (Keusch, 1982). In a rural community in Guatemala during the period 1965–1969, the incidence rate among infants was almost 200000 per 100000 per year, that is each infant was attacked by the disease on average twice per year (Mata, 1978).

Shigellosis is caused by the consumption, in faecally-contaminated water or food, of live bacteria of the genus *Shigella;* known pathogenic species include *Sh.dysenteriae, Sh. flexneri* and *Sh. sonnei*. All of these appear to be associated exclusively with humans and some non-human primates, and transmission of the disease is usually by the faecal-oral route. *Shigella sonnei* outbreaks, although less serious than *S. dysenteriae,* are not uncommon in schools and similar establishments. If ingested bacteria survive passage through the stomach, they invade the epithelial cells of the intestine and give rise to ulcerous lesions. It is believed that the bacteria also secrete a toxin which may have pathological effects. Infected patients excrete bacteria with the faeces. Like typhoid, the disease typically takes a few days to manifest itself. The classical symptoms are the frequent passage, in small volume, of stools accompanied by blood and mucus, along with severe abdominal cramps; often, however, watery diarrhoea is the only manifestation and many infected patients may be undiagnosed. Shigellosis is often a seriously debilitating disease which can give rise to chronic infection and periodic recurrence of symptoms. If untreated, death occurs in up to 15% of cases, most fatalities occurring among infants and elderly patients. Complications include haemolytic anaemia and Reiter's syndrome, an arthritic condition. As with typhoid fever, chronically-infected patients can act as carriers of the disease within the community without manifesting clinical symptoms of the disease.

Cholera is perhaps the most devastating of the waterborne bacterial diseases, and well exemplifies the importance to public health of good sanitation and water pollution control. Cholera epidemics are by no means uncommon, especially in countries where adequate waste disposal practices are not well established. Benenson (1982) in his account of the disease, considered that it was possible to identify seven cholera pandemics; the most recent of these he considered to have originated in Indonesia in 1958 and to have spread to South-West Asia, India, the Middle East, Africa and to some countries in Southern Europe by 1976. At the time of writing Benenson (1982) considered this pandemic to be still extant. Earlier epidemics affected Britain and other European countries (Benenson, 1982; Fraser, 1984). The disease appears to be endemic to areas of Asia and to have first been recognised in Europe in the early sixteenth century, almost certainly introduced by a sailor returning from a foreign journey. The frequency of foreign travel in modern times presents a constant threat of the reintroduction and spread of the disease even in countries where good pollution control practices are established. UNEP (1989a) records data showing about 50 000 cases worldwide per year as a background level of morbidity, reaching much higher numbers in certain years when particularly large outbreaks occur.

Cholera is caused by the bacterium *Vibrio cholerae,* and is transmitted by ingestion of live bacteria from polluted water or other material contaminated with faecal matter from an infected individual. The bacteria colonise the intestinal tract and produce a potent toxin which attacks the intestinal mucosa and interferes with

the normal processes of salt and water balance which occur across the gut wall. The patient suffers severe diarrhoea involving sudden and massive dehydration of the body and serious salt imbalance which impairs the normal function of many organs. Death, due to circulatory failure consequent upon dehydration and salt imbalance, can occur within hours of infection. In some cases the bacterium can cause serious infections in other parts of the body.

Bacteria of the genus *Vibrio* have been studied mainly because of the medical importance of *V. cholerae,* which was first recognised about one hundred years ago. More recently, a wider biological perspective upon the genus *Vibrio* has become available, which is important when considering the status of these organisms in relation to water pollution (Colwell, 1984). Vibrios appear to be primarily free-living organisms of soil and water, particularly associated with saline environments such as estuaries and salt marshes. Apart from *V. cholerae,* several other *Vibrio* species are pathogenic to humans, and some are pathogenic in aquatic organisms including crustaceans and molluscs. These animals are primarily detritivores and filter-feeders, and frequently accumulate high concentrations of *Vibrio* bacteria in their bodies. This gives rise to a hazard of human infection, especially in areas where shellfish are cultured, harvested, processed or consumed in significant quantities.

### *5.1.2 Viral Pathogens*

At the time when the relationship between water pollution and the spread of bacterial diseases was first understood, the role of viruses was hardly recognised. Today, the importance of viruses as agents of disease is well known, and it is clear that the pollution of water with human wastes is a major potential source of serious disease.

The viruses of greatest interest in this context are the group known as enteric viruses. The taxonomy of viruses is complex and unfamiliar to many biologists, and is based not only on the morphological and physical characteristics of the organisms but also upon their chemical and immunological properties. The term ‘enteric viruses’ is one of convenience rather than a distinct taxonomic grouping. Enteric viruses include agents from widely different taxonomic groups, but they have in common the fact that the intestine is their primary lodgement site. As they are commonly found also in water, they can potentially be transmitted by ingestion of water or other matter contaminated with faecal waste from an infected individual. Human pathogenic viruses that are commonly found in polluted water are listed in Table 5.1. However, the link water-patient-water-patient has so far been firmly established only for poliovirus and Hepatitis type A virus.

In many cases, viral infections caused by these agents do not cause serious symptoms, but acute gastrointestinal and diarrhoeal illnesses are the commonest waterborne diseases in the more developed countries, and they

| Virus | Disease caused |
|---|---|
| *Enteroviruses* | |
| *Polio (3) | Paralysis, meningitis, fever |
| Echo (34) | Meningitis, respiratory disease, rash, fever, gastroenteritis |
| Coxsackie A (24) | Herpangina, respiratory disease, meningitis, fever, hand, foot and mouth disease |
| Coxsackie B (6) | Myocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory disease, pleurodynia |
| New enteroviruses types 68 to 71 (4) | Meningitis, encephalitis, respiratory disease, rash, fever, acute haemorrhagic conjunctivitis |
| *Hepatitis A (enterovirus 72) | Infectious hepatitis |
| Norwalk (2) | Epidemic vomiting, diarrhoea, fever |
| Rotavirus (4) | Gastroenteritis, diarrhoea |
| Reovirus (3) | Unknown |
| *Parvoviruses* | |
| Adeno-associated (3) | Unknown |
| Adenovirus (30) | Respiratory disease, conjunctivitis, gastroenteritis |
| Cytomegalovirus (1) | Infectious mononucleosis, hepatitis, pneumonitis, immunological deficiency |
| Papovavirus SV 40-like (2) | Immunosuppression, progressive multi-focal leucoencephaly |

may have serious consequences in some patients, particularly in infants, elderly patients and immunologically compromised hosts. In addition, some enteric viruses are capable of causing seriously debilitating or even fatal illness, and can give rise to epidemics. Consequently they are considered as serious hazards to public health, and some examples are discussed briefly here. A fuller account of the subject is given in a concise form by Rao and Melnick (1986).

Poliovirus is the causative organism of poliomyelitis, probably the most familiar of the waterborne viral diseases. In developed countries, public health policy generally dictates that the majority of the population is immunised in childhood against this disease. Poliovirus infection is primarily one of the alimentary tract and may cause symptoms of fever, vomiting and diarrhoea. These symptoms may not be recognised as serious. In a minority of cases (up to approximately 1%) the virus enters the bloodstream of the infected individual and may penetrate the nervous system and give rise to paralysis; this may be permanent and, if vital organs are affected (such as respiratory muscles), fatal. Infected individuals, whether or not showing serious disease symptoms, excrete poliovirus in large quantities in the faeces, giving rise to the risk of infection of further individuals.

Hepatitis A (infectious hepatitis) is clearly transmitted through contaminated water, and numerous outbreaks of the disease have been traced to this source (Rao and Melnick, 1986; Vaughn and Landry, 1983). Viral hepatitis of the non-A, non-B form has also been shown to give rise to waterborne epidemics with fatality rates of up to 40% among susceptible groups of the population (Rao and Melnick, 1986). There is strong circumstantial evidence of the waterborne spread of rotavirus, Norwalk virus and of infections caused by consumption of shellfish from polluted water which contain high concentrations of parvovirus, rotavirus and small round virus (SRV).

### *5.1.3 Parasitic Infections*

Parasitic infections can also be spread through polluted water. Amoebic dysentery, caused by the protozoan *Entamoeba histolytica,* is a typical example. Of the world's population, no less than 4000 million are at risk of infection, and 400 million actually infected (UNEP, 1991). Its life cycle consists of four stages: trophozoite, pre-cyst, cyst and metacyst. Ingestion of the mature cyst from contaminated food or water initiates the infection. In the small intestine the cyst gives rise to the metacyst, which in turn divides to give a number of amoebae. These enter the large intestine, where they may invade the host tissues or give rise to further cysts. Infected patients excrete trophozoites (amoebae) and cysts in the faeces, though only the cysts can survive in the external environment and survive passage through the stomach to initiate a new infection. Within the large intestine, invasion of the host tissues causes ulceration and abscesses, which may spread to other organs such as the liver. The most obvious symptom of acute amoebic dysentery is diarrhoea, accompanied by blood and mucus. Chronic infection can occur giving rise to recurrent episodes of diarrhoea alternating with constipation.

Other parasitic protozoans with relatively simple life cycles are known to spread in a similar manner. Examples include the flagellate *Giardia lamblia,* which causes serious gastrointestinal disorders; major outbreaks continue to be recorded in Europe and the USA (Zaman and Ah Keong, 1982). Another protozoan which has been causing concern recently is *Cryptosporodium parvum* (Badenoch, 1990). Originally thought to be spread to humans directly from animals or by person-to-person contact, it is now increasingly thought to be transmitted via water. Current sewage and water treatment processes do not always remove protozoans effectively, as they were not designed to do so, and there may be a need to modify treatment processes in areas where protozoan parasites pose risks.

Parasitic nematodes (roundworms) are important human parasites, and many have a simple life cycle involving direct transmission by the faecal-oral route. Others require passage through an intermediate host, but completion of the life cycle is facilitated by contamination of water, soil or food with faecal wastes of infected individuals. In many species, the life cycle includes a stage which is specifically adapted for survival in the external environment, so that infective life stages may remain viable in water or soil for long periods. In some species, a period in the external environment is essential for completion of the life cycle. One example of a nematode with a simple life cycle which causes human infection is the whipworm *Trichuris trichiura.* Mild infections produce few serious symptoms, but provide a focus of infection from which more serious cases can arise. Heavy infection produces symptoms of dysentery, and leads to severe damage of the intestinal tract, anaemia and (especially in children) loss of weight. The faeces of infected individuals contain eggs which require about 20 days in the external environment to complete their development; the infective stage can survive for several months in water or soil. Several other nematode species have similar life cycles.

Tapeworms (cestodes) include a number of species which infect humans and whose transmission is aided by contamination of water or soil with human faecal matter. The beef tapeworm, *Taenia saginata,* is a typical example; again, there are a number of other species whose life cycles are broadly similar. The adult *T. saginata* lives in the human intestine and sheds, in the faeces of the host, proglottides which contain eggs. The eggs are ingested by cattle, from polluted water or from pasture contaminated with human faeces. In the cattle, the eggs develop through several stages, migrating through the intestinal wall and via the circulation to the muscles, where the cysticercus stage is formed. This encysted state remains in the muscle, and if ingested by a human in uncooked or partially-cooked beef, develops into an adult tapeworm.

Trematode parasites which infect humans include a number of blood flukes (Schistosomes), liver flukes, intestinal flukes and pulmonary flukes which cause serious disease. They are relatively rare in temperate zones and in countries where pollution control and public health practices are well established, but are major causes of ill health and economic loss in many parts of the world (Zaman and Ah Keong, 1982). Their life cycles are complex, involving one or more intermediate hosts, and all require a period of development within the body of a mollusc, usually an aquatic snail. Invariably, transmission of these parasites involves the contamination of water with human faeces. The life cycle of a typical blood fluke is shown in Figure 5.2.

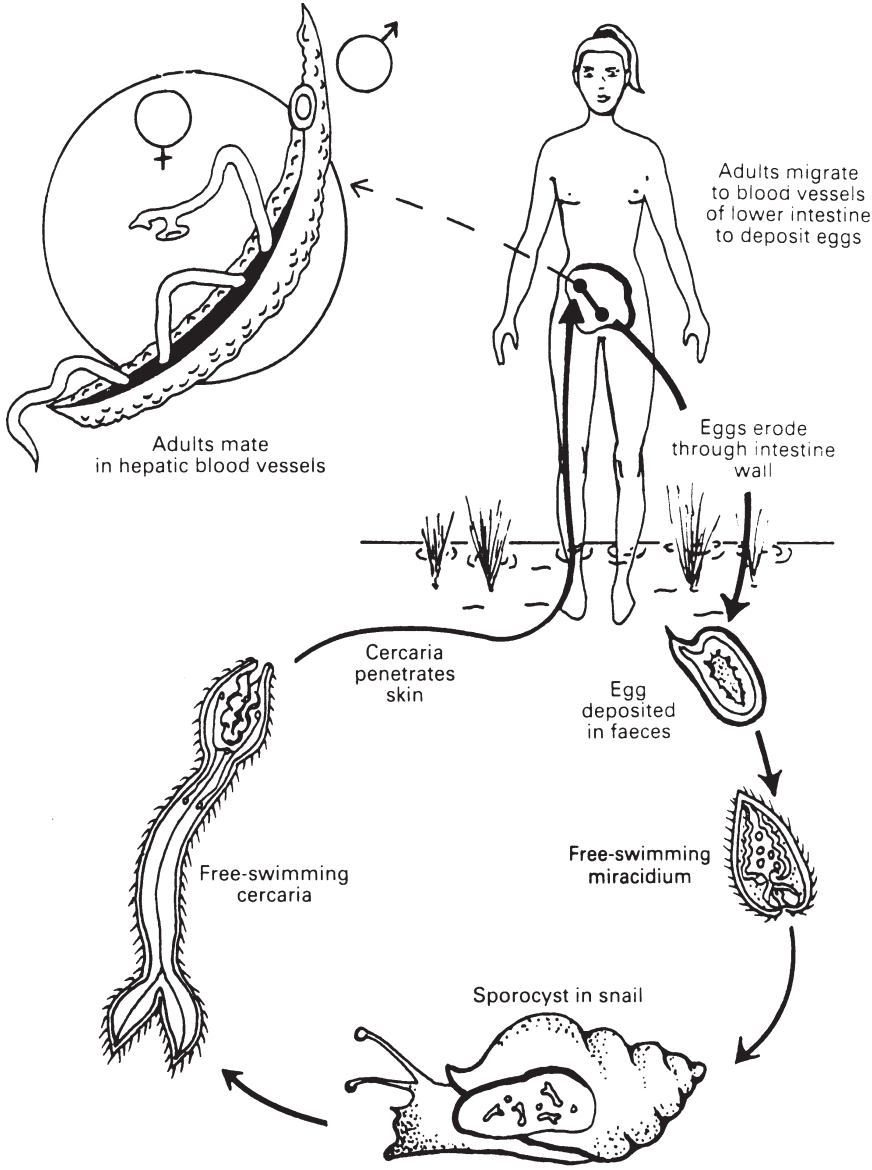


**Figure 5.2** Life cycle of a typical trematode parasite of humans, *Schistosoma mansoni* (not to scale)

Control of the spread of parasitic diseases is an enormous public health problem in many parts of the world. UNEP (1991) estimates that currently 600 million people in the world are at risk from Schistosome infection, and 200 million are actually infected. Detailed consideration of the life cycles of parasites is required

in order to understand their modes of transmission, the control measures which may be effective against them and the relationship between water pollution and the spread of parasitic infection. Where the life cycle includes one or more intermediate hosts, control measures may be directed against the intermediate host in order to break the cycle of infection. Aquatic molluscs living in polluted waters can readily become established as a reservoir of human infection. It is often important, therefore, to eliminate aquatic molluscs by various means, including the use of selective poisons. In this case, an understanding of the toxicological principles and techniques discussed in Chapter 4 clearly becomes important. Alternative methods of eliminating aquatic molluscs include management of the flow regime of drainage and irrigation channels, where this is technically feasible, since many aquatic molluscs are restricted to static or slow-flowing waters.

Strict regulation of food processing and handling is also important, including effective systems for the inspection of meat and other foodstuffs to avoid infected material being used for human consumption. Education of the general public in personal hygiene, and in the appropriate techniques for the preparation and cooking of food can do much to limit the spread of disease. Deep freezing food (-20°C for 24 h) is usually sufficient to kill infective stages of parasitic worms. Adequate medical facilities for the diagnosis and treatment of infection are of obvious importance. Nevertheless a large proportion of the most serious parasitic diseases—whether caused by protozoa, nematodes, cestodes or trematodes—have in common that their transmission is facilitated by the contamination of watercourses or land with human faecal wastes. The monitoring and control of water pollution, and the development of adequate methods for the safe treatment and disposal of wastes, is therefore arguably the most powerful single measure that can be taken against many parasitic diseases.

### *5.1.4 Pathogens in the Aquatic Environment and in Waste Water Treatment Processes*

Deprived of the favourable growth conditions provided by their hosts, many pathogens die quite quickly. They may, however, remain viable sufficiently long to create a hazard to the health of bathers or other recreational users of the receiving water, and to render the water unsuitable for domestic or agricultural uses. It is therefore necessary to consider the survival rate of pathogens in the aquatic environment, and the effectiveness of waste treatment processes in removing them from effluents.

Some representative data on the survival of bacteria are summarised by James M.Montgomery Inc. (1985). For a range of enteric bacteria and pathogens in well water, times for 50% reduction in the initial population at 9.5°C to 12.5°C varied from 2.4 to 24 hours. For a 99.9% reduction, however, much longer periods of 20 days or more appear to be necessary for some pathogens. Factors which influence the survival

times of bacteria include the temperature, the light intensity and the quality of the water. It is therefore highly desirable that sewage treatment processes remove as many pathogenic bacteria as possible. Conventional treatment processes (see Chapter 6) are often reasonably effective in removing bacterial pathogens from the final effluent, although the pathogens may become concentrated in the sludge. Further, treatment plants cannot always be operated at maximum efficiency, particularly at times of high storm-water input. Under ideal conditions, primary and secondary treatment of sewage reduce the numbers of pathogens in the effluent by 90% or more, compared with the raw sewage. Much lower removal rates are obtained during the frequent periods when treatment plants do not operate under ideal conditions.

Viruses survive at least as long as bacteria. Sattar (1981) in his summary of a large number of reports refers to enteric viruses in natural fresh waters requiring from less than one day to over 21 days for 99% loss of infectivity. In sea water, there are several reports of periods over 100 days for 99% loss of infectivity. A wide range of physical, chemical and biological factors have been reported to influence virus survival (Block, 1983; Sattar, 1981). The behaviour of viruses in sewage treatment plants is almost impossible to predict (Gerba, 1981; Sorber, 1983). In some cases, almost complete removal of virus particles has been reported, whereas in others the viruses have been found to pass freely. It is possible that high removal rates can be achieved in pilot plants operating under continuously optimal conditions, but that under the varying and often sub-optimal conditions which prevail in operational treatment plants, virus removal is much less efficient. As with bacteria, it is likely that viruses removed from the liquid effluent are to a large extent retained in the sludge. While conventional primary and secondary treatment cannot be considered generally effective in virus removal, some forms of tertiary treatment have been found to be successful (Rao and Melnick, 1986). Tertiary treatment of sewage, however, is still carried out only exceptionally even in better-developed communities.

At least some parasitic organisms may be expected to survive in the environment for long periods. The life cycle of many parasites includes a stage which is specifically adapted for survival in the external environment, to facilitate passage from one host to another. Some intestinal nematode parasites of humans which do not require any intermediate host do require a period in the external environment in order to complete their life cycle. Being relatively large, they settle readily and are often efficiently removed from sewage effluent by conventional treatment processes, although again they may tend to be concentrated in the sludge.

## 5.2 Monitoring Pathogens in Water

Since practicable waste treatment processes, however efficient, do not remove all the pathogens from sewage it is necessary to monitor receiving waters for the presence of pathogens. This is particularly necessary in waters which are used for

bathing, fisheries or as a source of further water supply. It also provides information on the operational efficiency of the treatment plant. Because there are so many potential pathogens, in routine monitoring generally no attempt is made to identify them all. Most commonly, the presence or level of abundance of coliform bacteria is used as an overall indicator of contamination of the water with faecal material. Coliform bacteria are so-called because they resemble *Escherichia coli,* a normal inhabitant of the human intestine which is excreted in vast numbers in the faeces of healthy individuals. *E. coli* itself is not normally pathogenic, although some other coliforms are. Depending upon the environmental conditions, coliforms survive only a few hours or days outside their hosts; therefore their presence in water suggests that it has been recently contaminated, and that pathogens are likely to be present. Further, because the non-pathogenic *E. coli* is excreted by healthy individuals, it will nearly always be far more abundant in sewage than other coliforms or other enteric pathogens. Therefore by ensuring that the numbers of coliforms are kept at a low level, it is possible to be reasonably certain that the numbers of pathogens present are very small indeed. Coliform bacteria can be readily isolated, identified and counted from water samples (see Section 5.2.1).

Despite these advantages, coliforms are by no means the only organisms which can be used as indicators of faecal contamination. Not all coliforms are of human faecal origin, so simply counting the total number of coliforms present can be misleading. It also sometimes happens that pathogens may be present when coliforms are absent or rare. The different types of coliform can be distinguished from one another by biochemical tests, and it is often suggested that specifically human faecal coliforms, rather than total coliforms, are a better indicator of sewage contamination (Bonde, 1977). Other bacterial indicators are available, such as faecal streptococci; this group is also easy to identify and occurs in large numbers in the faeces of healthy individuals. Pathogenic viruses, and the infective life stages of parasites, behave in many respects differently from bacteria. Although the presence of viruses or parasites is not routinely used to indicate faecal contamination, there are circumstances where it is advisable, for reasons of public health, to monitor water bodies for their presence. Standard methods for monitoring waters for pathogens have been widely published (e.g. APHA, 1995), and a brief review of some modern developments is given by Singh and McFeters (1992).

### *5.2.1 Monitoring for Coliforms*

Coliforms are Gram-negative rod-shaped bacteria which are most readily distinguished from others by means of their biochemical properties. A common test for the presence of coliforms in water is to inoculate a suitable liquid growth medium with a quantity of the water and to observe the result. The medium used (lactose broth) contains lactose, a sugar which is fermented by coliforms to produce acid and gas. The medium may also contain substances which inhibit

the growth of non-coliform bacteria while promoting the growth of coliforms. The production of acid is detected by incorporating an indicator dye in the medium; gas production is detected by inserting into the culture vessel a small inverted test tube (Durham tube). After a period of incubation at 35°C, positive cultures are easily recognised by the fact that the medium has changed colour and a bubble of gas has collected at the top of the Durham tube. This simple presumptive test for coliforms does not distinguish between coliforms of faecal origin and those from other sources. Confirmation of the presence of faecal coliforms requires that positive cultures are tested on a solid medium, lactose-peptone agar containing sodium sulphite and basic fuchsine. On this medium, colonies of faecal coliforms acquire a greenish colour, non-faecal coliforms a red colour and non-coliform colonies are uncoloured. Alternatively, a definitive one-stage test for faecal coliforms is to test for acid and gas production in lactose broth at 44.5°C. However, this test gives misleading results unless the incubation temperature is very precisely controlled. The growth media required are readily available commercially in made-up form.

Estimation of the numbers of coliforms present in a sample of water is done in one of two ways, the plate-count method or the Most Probable Number (MPN) method. In the plate-count method, an aliquot of the test water, usually about 1 ml, is mixed with the growth medium before the agar has set. When the medium has solidified and the plate has been incubated, each viable bacterium in the original aliquot will have given rise to a colony on the plate which can be readily observed. If a number of replicate plates are set up, the mean number of coliforms per ml of water can be calculated, along with its confidence limits, giving a reasonably accurate estimate of the number of coliforms present in the water.

A variation of the plate count technique which has some advantages is to use commercially-available millipore filters with a pore size of 0.45 microns. A known volume of water is filtered through the sterile filter disc, which is subsequently placed on a suitable solid medium. The number of colonies which develop during incubation corresponds to the number of viable cells present in the original sample.

The MPN method is simpler but much less accurate. Suppose that the sample of water actually contains 100 bacteria per ml. A series of tenfold dilutions will each contain, on average, ten bacteria per ml. If each of these dilutions is further diluted by a factor of ten, and an aliquot of 1 ml taken from each, then on average each 1-ml aliquot will now contain one bacterium. However, some will contain more than one, and some will contain none. If these aliquots are incubated in liquid medium as described above, those aliquots which contain no bacteria will score negative, and those which contain one or more will score positive. If aliquots from the original tenfold dilution were incubated in a similar fashion, very few of them would be negative, since

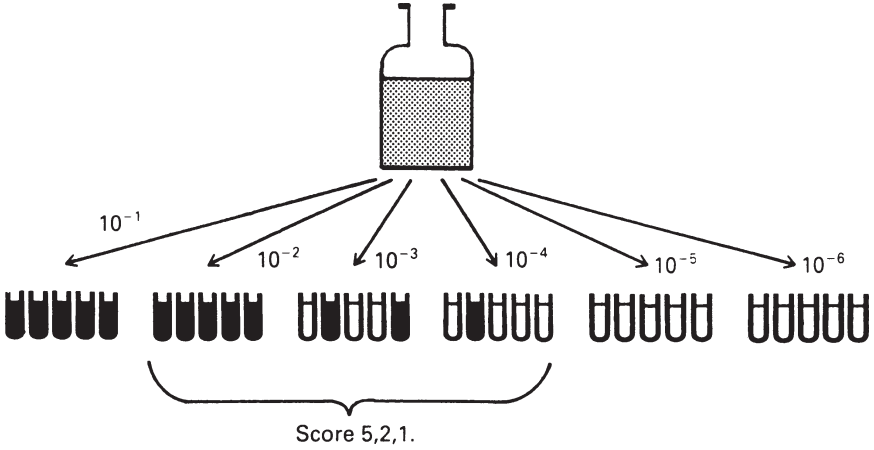


**Figure 5.3** Determination of the most probable number of coliforms in a sample of water. See text for explanation

the probability of a 1-ml aliquot containing no bacteria is small when the average content of bacteria is 10 per ml. However if a 1000-fold dilution of the original sample were made, only one aliquot out of ten would be expected to contain a bacterium and to score positive. This line of reasoning forms the basis of the MPN method, since for a given concentration of bacteria in the original sample, a given dilution factor and a given aliquot size, the ratio of positive to negative results that would be expected on average can be calculated statistically. In practice, there are a number of variants of the MPN procedure, but all are based on the same principle. One way to proceed is progressively to dilute the original sample by factors of 10. From each dilution, a number of culture tubes (usually three to five) are inoculated with an aliquot of the diluted sample and incubated. When the bacteria are present in high concentration, all five tubes will score positive. When the dilution is very great, all five tubes will be negative. At intermediate dilutions, some tubes will be positive and some will be negative.

In the example shown in Figure 5.3, aliquots of the original sample have been inoculated into liquid media in groups of five tubes, so that the original sample is diluted to $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$ and $10^{-6}$ of its original strength. As expected, at the highest dilutions ($10^{-5}$ and $10^{-6}$), all the tubes score negative—no coliforms were present in any of the inocula. At the lower dilutions ($10^{-1}$, $10^{-2}$), all five are positive, that is each tube was inoculated with at least one coliform. At the intermediate dilutions ($10^{-3}$ and $10^{-4}$), some tubes are positive and some are negative. The three sets of tubes indicated in the diagram are the ones used to determine the MPN, the number of positive scores in each set of tubes being

five, two and one respectively. Using a table of MPN values specifically calculated for this procedure, the MPN corresponding to a score of 5, 2, 1 can be determined. Note that, starting from the left of the diagram, scores of 5, 5, 2; 5, 2, 1; 2, 1, 0; or 1, 0, 0 could be generated. Every MPN protocol specifies a set of rules for deciding which sets of tubes are used to obtain the score. In this case, the rule is 'select the greatest dilution giving some positive reactions together with the two preceding sets of dilutions'. Numerous variants of the basic MPN procedure are employed. These variations include the number of tubes tested at each dilution, the range of dilutions required, the size of the inoculum and the method of scoring. They are necessary because the numbers of bacteria found in raw sewage, river water and treated drinking water are obviously greatly different. It is important to realise, therefore, that MPN tables can be used only in conjunction with the exact procedure for which they were calculated. Detailed descriptions of various MPN procedures for different purposes are given, for example, by APHA (1995) and by HMSO (1983b).

An important disadvantage of the MPN procedure is that it is intrinsically inaccurate and potentially misleading. Assume that an MPN value is obtained of 100 organisms per 100 ml of water. This figure may be the most probable number, but a value of 80, or 120, is only slightly less probable; and the probability that the true number is either 80 or 120 could be greater than the probability that the true number is actually 100. In fact, although 100 is the most probable single number, it is almost certain that the true number is some greater or lesser value. Most MPN tables show, in addition to the MPN itself, the 95% confidence limits of the calculated value. Strictly, the test is incapable of estimating the number of bacteria in the original sample; it can only estimate with 95% confidence that the number of bacteria lies within a certain range. If this range were sufficiently narrow, this would not matter very much. In practice, however, the range is wide. For example, most published MPN tables show that for an MPN value of around 100 organisms per 100 ml, the 95% confidence limits embrace values from about 25 to about 270. Generally, the method cannot reliably distinguish between bacterial counts an order of magnitude apart. The MPN procedure is widely and routinely used in water quality monitoring, and arguably its limitations are not as fully appreciated as they should be.

Monitoring for other bacteria, and indeed for other microorganisms such as yeasts, algae and protozoa, can often be carried out (whether qualitatively or quantitatively) using procedures similar in principle to those used for coliforms, but with the appropriate culture media. Many proprietary kits are available from various manufacturers to reduce the time and labour required by the conventional methods, and many of these have been officially approved for use by regulatory agencies such as the US Environmental Protection Agency.

### *5.2.2 Monitoring for Viruses*

Isolating, identifying and counting viruses from the aquatic environment is intrinsically more difficult than monitoring bacteria, and there is less agreement on standard methods (Dobberkau *et al.,* 1981; Gerba, 1983; Rao and Melnick, 1986). As they are generally far less numerous than bacteria, and because the efficiency of virus recovery is often low, large volumes of water (100 litres or more) sometimes have to be processed by special filtration procedures and other complex techniques for concentrating the virus particles. The viruses must then be inoculated into cultures of living cells and allowed to grow. They are then isolated from the cell cultures and identified. The identification of viruses itself requires specialised techniques, including the observations of their effects on the cells in culture, electron microscopy, immunological techniques and biotechnological methods (Reynolds and Rose, 1991). Many viruses of potential interest are notoriously difficult to grow in cell culture, or grow only very slowly. It takes much longer, often weeks rather than days, to obtain the eventual results than is the case with bacteria, and accurate quantitative results can be obtained only with great difficulty. For these reasons, and because of the specialist skills and facilities required for virological work, routine monitoring for viruses is not practised very widely, but it may become more common as new methods are developed. In view of the difficulties of isolating and identifying viruses from water, it would be advantageous for routine monitoring purposes to use a single, readily-identifiable virus species as an indicator of the possible presence of pathogenic viruses. One possibility is to use attenuated poliovirus for this purpose. In many countries, children are immunised routinely against polio by means of an oral vaccine containing live but attenuated (non-pathogenic) poliovirus. Thus in any large community, there will always be some people excreting the attenuated virus, and its presence in water might be taken to indicate that enteric viruses, including pathogens, are likely to be present in the water.

### *5.2.3 Monitoring for Parasites*

Monitoring for parasites requires skilled personnel rather than specialised equipment. The most common method of identifying parasites is direct visual examination, under the microscope, of the water sample by someone trained to recognise the various life stages of parasitic organisms. The identification may be aided by certain staining procedures, and quantitative results can be obtained by using standard microscopical techniques of counting. Sometimes it is necessary to concentrate the suspended matter in a water sample of known volume, for example by centrifugation. Some parasitic protozoa, including *Entamoeba*

*histolytica,* can be cultured in suitable growth media and treated rather like bacteria (Diamond, 1983), and similar techniques for *Giardia* and *Cryptosporidium* are under development (e.g. HMSO, 1990; Musial *et al.,* 1987; Singh and McFeters, 1992). In areas where amoebic dysentery is endemic, a group of non-pathogenic intestinal amoebae (of the genus *Entamoeba* but distinguishable from *E. histolytica*) are useful indicators of the possible presence of pathogenic protozoa, rather as coliforms can be used as indicators of pathogenic bacteria.

## 5.3 Water Pollution and Water Supply

An important consequence of water pollution is that polluted water is generally less acceptable than clean water for most of the purposes to which water may be put. To take an obvious example, water carrying a heavy burden of potentially pathogenic organisms may be unsuitable for use as a source of potable water supply, or may only be rendered suitable by means of elaborate and expensive purification processes. These difficulties are exacerbated by the increasing need for water to be reused as demands on water resources increase.

### *5.3.1 Potable Water Supply*

The requirement that potable water be free from pathogenic organisms is obviously of prime importance, and water treatment processes are designed to achieve this end. Conventional water treatment processes are less effective in removing chemical contaminants which may give rise to harmful effects. Nitrates are common pollutants of water (see Chapter 2), and may be found in high concentrations in water bodies used as sources for potable supply. Excessive nitrate in drinking water is dangerous, particularly to young children and to babies fed on dried or bottled milk. Nitrate ions are readily converted in the stomach (at the low pH of gastric juices) to nitrite ions which pass readily into the bloodstream. The nitrite ions react with the haemoglobin of the blood, forming methaemoglobin, a relatively stable compound which has a lower oxygen-carrying capacity than haemoglobin. Cyanosis (blueing of the skin) is the most obvious symptom of severe methaemoglobinaemia. Some thousands of fatalities have been recorded around the world, and in many countries nitrate in water supplies is routinely monitored to ensure that the concentration does not exceed 50–100 mg $l^{-1}$. In some areas of Britain, for example, it is necessary to distribute bottled water at times when the nitrate levels in the public water supply exceed the safe level. Nitrates (converted to nitrites in the stomach) can also react in the gut with amines to form potent carcinogens, although the epidemiological significance of this process is not yet clear.

Benes (1978) lists, in addition to nitrates, a large number of potentially toxic substances which can occur, in trace amounts, in drinking water derived from polluted sources. These include trace metals, pesticides, and a large number of

organic compounds, some of which are derived from industrial or agricultural pollution, some from leaching of chemicals from plastic and other materials used in the water distribution system, and even some derived from chemicals used in the processing of water and waste water. The significance of these 'micropollutants' is not well established, but their presence in drinking water is a potential cause for concern. In Europe, member states of the European Union are subject to two European Community Directives (see Chapter 6) governing the presence of trace pollutants and are obliged to ensure that levels of trace pollutants do not exceed certain limits. The two directives are the so-called 'Surface Water Abstraction' directive, which sets limits on the quality of water intended for human consumption *before* treatment, and the 'Drinking Water' directive which sets limits for water *after* treatment. These directives reflect public and official concern, though many argue that the limits set are unnecessarily strict, imposing high monitoring costs for no strongly-established scientific reason; and in some cases the limits set are so low that they may be technically impossible to achieve.

Nevertheless many national authorities attempt to follow guidelines laid down by international bodies such as the World Health Organisation, and may be subject also to mandatory standards laid down nationally or, as in the case of members of the European Union, through the relevant EU Directives. A useful guide to the problems of trace pollutants in potable water is given by Nicolson (1993).

### *5.3.2 Agricultural Water Supply*

Agricultural activities are a major source of serious water pollution, but at the same time agriculture imposes a heavy demand for supplies of clean water. Noy and Feinmesser (1977) briefly summarised some of the difficulties which can arise from the use of polluted water for agricultural purposes. The seriousness of such problems depends, to a large extent, on the varying conditions which prevail in different parts of the world, especially in relation to the climate, the availability of water and the extent to which waste treatment facilities and general public health provisions are established. The volume edited by Shuval (1977a) includes examples from several different countries. The disposal of waste waters, whether treated or not, and of sludges from treatment processes to agricultural land has many economic and agricultural benefits, but there are a number of hazards associated with these practices. Even where the deliberate reuse of water is not practised, it is increasingly likely that agricultural water supplies will be drawn from water bodies which have previously received waste matter.

The most obvious adverse effects of polluted water in agriculture relate to the presence of toxic matter, especially heavy metals, and of pathogenic organisms. Agricultural animals probably do not differ much from humans in their sensitivity

to toxic heavy metals, and heavily-contaminated water is no more acceptable to them than it would be as a potable supply for humans. The sensitivity of plant species to heavy metals in their environment is well known. Although there is some possibility that crops may accumulate sufficient heavy metal to be hazardous to consumers, in practice the effects of toxic metals are initially economic; some crops will give a reduced yield, or fail altogether, if the levels of toxic metals in the soil or irrigation water are too high. Thus water pollution can restrict the uses to which land can usefully be put, or impose extra costs relating to the supply of water of adequate quality. Boron, for example, is like many other elements an essential requirement in trace amounts for plants; in excess, however, it is toxic. The widespread use of perborates in detergent formulations has led to concern that domestic sewage effluent, without any contamination from industrial sources, could so elevate the boron content of receiving waters as to exert an adverse effect on crops. Consequently it has been necessary for national and international agricultural agencies to formulate detailed recommendations concerning the chemical quality of water used for various agricultural purposes.

Pathogenic organisms in water or sludges applied to agricultural land present obvious potential dangers. Again, the magnitude of the hazard depends greatly upon the climatic, agricultural and general public health situation of the geographical area concerned. Infective life stages of parasites can be ingested by animals from contaminated water and transmitted to human consumers; some parasites may be transmitted directly to human consumers, particularly from salad vegetables or other crops which are eaten uncooked. The hazards from pathogenic bacteria and viruses have not been properly evaluated in many parts of the world, but Shuval (1977b) and Vaughn and Landry (1983) have briefly reviewed some studies which suggest that microbial contamination of arable crops through irrigation with contaminated water can contribute to the spread of disease.

### *5.3.3 Industrial Water Supply*

Industrial usage accounts for a very large proportion of the total demand for water, and about two-thirds of the water used in industrial processes is used for cooling. The use in some industrial processes of sewage or other effluents, or of water which is too polluted for other water supply purposes, would appear to offer some advantages. In practice, the use of polluted water in industrial processes is subject to certain constraints. For example, the dairy and food-processing industries obviously require water of the highest quality, and it is not always economically feasible to construct and operate safely water distribution systems in which water of different quality is supplied to different factories within an area.

Many of the problems which can arise are not of a strictly biological nature; for example, the presence of ammonia and other chemicals can exacerbate corrosion problems in pipework. A common difficulty is the growth of bacterial slimes and the accumulation of organic detritus in cooling systems; this can be controlled by chlorination, provided that the presence of free chlorine at relatively high levels does not interfere with the normal operation of the factory. Shuval (1977b) drew attention to the possibility that the use of polluted water in cooling towers may spread pathogenic microorganisms over a wide area. Eden *et al.* (1977) discuss water reuse practices in Britain, and describe some examples of the use of polluted water for industrial water supply. A new and recent pressure is the demand from some very modern industries, such as the manufacture of electronic components, for water of a very high standard of purity. This could lead to the siting of manufacturing plants, often dealing with highly toxic chemicals, in the vicinity of the least-polluted water bodies of greatest utility and conservation value. In many countries planning considerations prevent this, and the alternative approach is to require potable water of the highest quality, and then to subject it to even further advanced treatment at the point of use. Some biologists may take the view that such developments should be vigorously opposed. Others may argue that the application of known principles and practices and of others yet to be discovered will allow an optimum water-use strategy to be devised which would not necessarily prohibit proposals which are of considerable social and economic value. The socio-economic dimension is here not directly relevant; but we may be certain that as one biological problem is solved, another will surely arise.

# 6

# Water Pollution Control

Biologists have an important function in the control of water pollution. First, many waste treatment processes depend upon the controlled application of biological phenomena. Secondly, the assessment of the effects of pollution is ultimately a judgement which can only be made by biologists. Appropriate strategies for the treatment and disposal of wastes, and the assessment of the efficacy of such strategies, are therefore impossible without sound biological advice. Additionally, however, effective pollution control requires the expenditure of money, the professional advice of other scientific specialists such as chemists, engineers and hydrologists, and the formulation and enforcement of laws, regulations and administrative practices. The extent to which pollution is successfully controlled therefore depends upon constructive interactions between specialists from a diverse spectrum of disciplines.

The amount of money spent on the design, construction and operation of a sewage treatment works can be fairly precisely estimated. Any competent chemist can accurately determine the concentration of zinc, phenol or ammonia in a sample of water. Biologists, however, do have difficulty in estimating the number of fish or mayfly nymphs in a lake; and non-biologists may be forgiven if they do not easily understand why a million mayfly nymphs are preferable to an equal number of beetle larvae, particularly if they have been informed that in other places and under other circumstances the beetle larvae must be preserved! A legislative assembly may understand that while it can pass a law stating that all rivers must contain salmon, it is futile so to do; but the legislators may wonder why a concentration of ammonia which is harmless in one river may be devastating in another. The role of legislation is, however, clear. Since pollution control is expensive, technical advances do not necessarily lead to more effective pollution control in the absence of legislative, administrative and economic pressures which

can be brought to bear on the individuals, industries and communities which are responsible for pollution. Equally, laws or international agreements on the control of pollution cannot be effective in the absence of the necessary technical and economic resources, including the availability of suitably skilled personnel.

Effective pollution control therefore depends upon the availability of adequate technical means, of an appropriate legislative and administrative framework, and of trained personnel drawn from a variety of disciplines who are able to communicate effectively with one another. In this chapter, some technical and administrative aspects of water pollution control are considered together.

## 6.1 Biological Treatment of Waste Water

Sewage, and wastes consisting largely of putrescible organic matter, can be very effectively treated by biologically-based processes. In practice, these processes are often effective in treating wastes containing toxic substances, provided that the concentration of toxic matter is not too high. If untreated wastes are discharged to water they are broken down and assimilated into the receiving water ecosystem by a variety of physical, chemical and biological mechanisms. This may, however, cause disturbances in the receiving water ecosystem such as were described in Chapter 2. The objective of waste water treatment is therefore to minimise these disturbances by ensuring that the processes responsible for the breakdown of organic matter, and for the removal of suspended solids, pathogens and toxic substances take place, as far as possible, within the treatment plant itself rather than in the receiving water. In this way the effluent will have a relatively small effect on the receiving water when it is discharged.

Biological waste treatment processes have evolved over a period of more than one hundred years, partly through trial and error but increasingly by design, as understanding of the underlying biological and engineering principles has improved. The biological aspects of waste treatment processes have been described and discussed in the volumes edited by Curds and Hawkes (1975, 1983a, 1983b) and Gray (1992). Texts which also cover the engineering aspects of waste treatment processes include those of Mudrack and Kunst (1986), Hanel (1988) and Horan (1990). In tropical and developing countries, the design, operational and public health aspects of waste water treatment are significantly different, and are treated by Cairncross and Feachem (1993). A useful introductory text to water and waste water treatment technology for biologists is that of the Open University (1993).

Numerous variations of the basic methods are available, but it is convenient to describe conventional processes of biological waste treatment

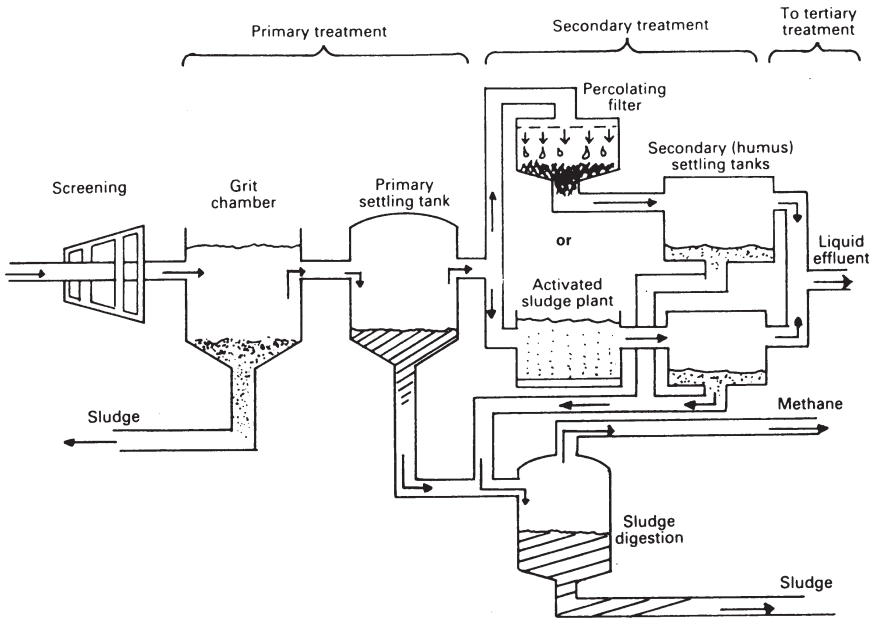


**Figure 6.1** The different stages of a typical sewage treatment process

in terms of a division into preliminary/primary, secondary and tertiary stages. A typical treatment process can be summarised as in Figure 6.1. The efficiency of the various stages of sewage treatment varies widely, according to the quality of the waste water, the design of the plant and the operating conditions, which themselves vary widely, but some typical data are summarised in Table 6.1. Some other relevant data were given in Chapter 2 (Tables 2.1 and 2.2).

### *6.1.1 Primary Treatment*

The initial treatment of the waste water is purely physical, consisting typically of screening and settling. The water is passed through series of screens formed from metal bars, to remove large objects such as bottles or pieces of wood. These large objects may be incinerated or otherwise disposed of, or may be comminuted into small particles and returned into the system. This stage, and a period of settlement to remove inorganic particles (grit), is often referred to as preliminary treatment. The water is then passed through one or more sedimentation tanks which are designed to allow a high proportion of the suspended organic particulate matter to settle at the bottom of the tank. This stage, together with the preliminary treatment stage, is often referred to as primary treatment. The settled organic particulate matter

**Table 6.1** Effect of various sewage treatment processes on the 5-day biological oxygen demand (BOD), suspended solids content and bacterial numbers in sewage. The values given are those typically obtained under good operating conditions. Data from Klein (1966)

| | Approximate percentage reduction (compared to values in raw sewage) | | |
|---|---|---|---|
| Process | 5-day BOD | Suspended solids | Bacteria |
| Sedimentation | 30–40 | 40–75 | 25–75 |
| Sedimentation + percolating filters | 80–90 | 80–90 | 90–95 |
| Sedimentation + activated sludge | 85–95 | 85–95 | 90–98 |
| Oxidation ponds | 75–95 | [a] | >99 |

[a]Suspended solids content of oxidation pond effluent can be very high, owing to the production of algae in the pond.

(primary sludge) may be disposed of in various ways (see Section 6.1.4), and the remaining liquid may then pass to the secondary treatment stage.

### *6.1.2 Secondary Treatment*

Secondary treatment, if applied, is most commonly undertaken by one of three methods; percolating filters, the activated sludge process, or oxidation (stabilisation) ponds. These are all essentially biologically-based processes in which organic matter is broken down aerobically by the metabolic activity of microorganisms. It is important that the breakdown of organic matter occurs aerobically; under these conditions, organic compounds (mainly composed of the elements carbon, hydrogen, oxygen, phosphorus, nitrogen and sulphur) are rendered into relatively innocuous inorganic compounds such as carbon dioxide, water, carbonates, nitrates, sulphates and phosphates. Under anaerobic conditions, organic matter is broken down by different metabolic pathways (usually by predominantly different microorganisms) to produce methane, ammonia, amines, organic compounds of phosphorus and hydrogen sulphide. These compounds are, in general, toxic to most forms of life as well as being aesthetically unacceptable. (Some of the reactions involved in aerobic and anaerobic decomposition processes were summarised in Section 2.3.) Anaerobic conditions in waste treatment plants are therefore generally avoided, as they impair the efficiency of the treatment process and produce an effluent with undesirable and potentially

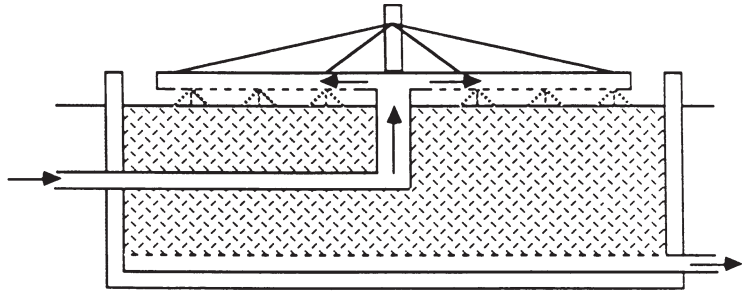

**Figure 6.2** Cross-section through a percolating filter

harmful characteristics. There are, however, some circumstances—such as sludge treatment (Section 6.1.4)—where the controlled use of anaerobic decomposition is beneficial. Also, anaerobic decomposition plays an important part in the functioning of oxidation ponds (see below).

Percolating (trickling) filters consist of circular or rectangular beds, usually about 2 m deep and from a few square metres up to about 2000 square metres in surface area, of stones, slag or synthetic material through which the settled sewage is allowed to percolate slowly (Figure 6.2). The preferred material for constructing the filter bed is fairly coarse (25–50 mm) and of more or less uniform size to ensure that air-filled void spaces are plentiful. Materials with rough surfaces (high surface area/volume ratio) are advantageous. Traditionally stones or slag were used in filter beds, but these are increasingly replaced by lightweight plastic materials specially designed for the purpose which are more efficient. When sewage is allowed to trickle through the filter, the surfaces of the filter medium become colonised with a thin film of bacteria and other microorganisms. This biological film is responsible for the breakdown of organic matter in the sewage. It typically contains heterotrophic bacteria which utilise complex organic compounds, and autotrophic bacteria which oxidise simple compounds, for example converting ammonia to nitrate. In addition, however, percolating filters support a variety of other organisms, including fungi, algae, protozoa, and many invertebrates, especially annelids, insect larvae, nematodes and rotifers.

The relationships between these various organisms in the percolating filter, and their role in the treatment process, have been the subject of much study (Curds and Hawkes, 1975; Hawkes, 1963). Some appear simply to colonise, opportunistically, the favourable environment which the filter provides. Some of the filter biota are undoubtedly a potential source of nuisance; flies, for example, can become very numerous in the vicinity of trickling filters, and excessive growth of fungal mycelium within the filter can fill the void spaces, reducing the filter's efficiency and in severe cases rendering it inoperative. However, many of the organisms typically found in percolating filters make a positive contribution to its function. Protozoa feed on bacteria, and/or particulate organic matter, thereby contributing to the biological processing of the matter present in the sewage. The invertebrate

species feed upon the meiofauna, on bacteria, and on particulate organic matter. They also browse upon the microbial film which is being continuously generated upon the surfaces of the filter medium. Their activities have three beneficial effects. First, they prevent the microbial film becoming too thick; excessive growth of the film would eventually tend to reduce the efficiency of the filter. Second, they process organic matter—whether newly-synthesised by microbial activity, or that originally present in the sewage—consolidating it into faecal pellets which settle out more readily at the secondary sedimentation stage. Finally, through their respiration they are responsible for the conversion of a significant proportion of the organic matter to carbon dioxide and water.

In effect, the trickling filter is an intensely-productive semi-controlled ecosystem whose energy source is the organic material present in the sewage. The precise characteristics of the community which develops in the filter depend upon the quality of the sewage and the operating conditions of the filter. The qualitative and quantitative characteristics of the filter bed community can sometimes be used to assess the performance of the treatment process in much the same way that indicator organisms can be used to assess the biological quality of receiving waters (Chapter 3). Overall, the filter ensures that the organic matter present in the sewage is biologically utilised under aerobic conditions. Were this matter to be discharged untreated into the receiving water, its utilisation would result in deoxygenation of the water, with the undesirable consequences described in Chapter 2. The effluent from the percolating filter is very different from the settled sewage which entered it a few hours earlier (Table 6.1), although it still contains a lot of organic matter. A large proportion of this, however, is recently-elaborated biomass. The biological film and its associated grazing organisms gradually break off from the underlying filter medium, and the liquid effluent from the percolating filter therefore requires further settling in tanks similar to those used in primary treatment. This gives rise to a secondarily-treated liquid sewage effluent (which may be discharged, or subjected to tertiary treatment) and to secondary sludge, or humus. The secondary sludge has a high organic content, and may be disposed of by various means (Section 6.1.4).

An alternative to percolating filters as a means of secondary treatment of sewage is the activated sludge process. In theory, secondary treatment by biological oxidation could be achieved simply by holding settled sewage in a suitable container and subjecting it to aeration. In practice, early experiments showed that the period of time required for acceptable treatment to be achieved by this simple approach was far too long. In addition, the exigencies of sewage treatment dictate that the process should be a continuous-flow rather than a batch-type operation. Various solutions to these problems have been devised, and the development and principles of the activated sludge process are described by Hawkes (1983a). The first operational plants which worked on a continuous flow basis appear to be those at Davyhulme, Manchester and in Worcester, both in England, in 1914

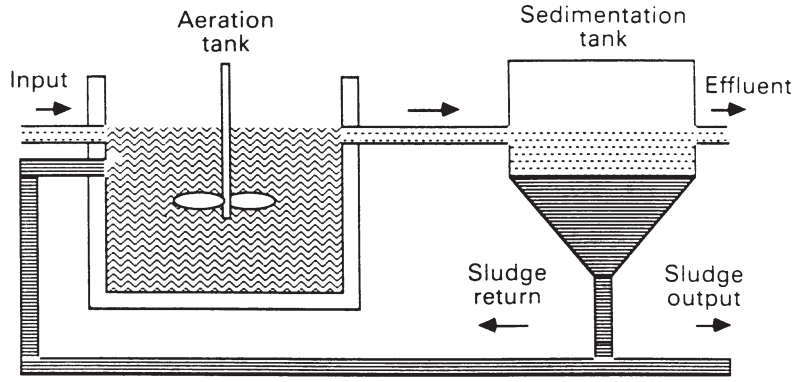


**Figure 6.3** The activated sludge process

and 1916 respectively. The method is about equally as effective as percolating filters, but in some circumstances has economic and engineering advantages. It is cheaper to install and requires less land, and nuisances caused by odours and insects associated with percolating filters are less troublesome. However, the process is more expensive to operate, and requires much stricter supervision of its operating conditions, so its suitability for a given location depends on local circumstances and the availability of qualified staff to operate the system.

There are many variants of the activated sludge process, but its basic principles are illustrated in Figure 6.3. Settled sewage is introduced to a tank at a rate which is designed to allow complete replacement of the tank's contents within a period of a few hours. During this time, the sewage in the tank is aerated vigorously. Microbial metabolism brings about breakdown of the organic material present in the sewage. As in the percolating filter, the end-products of this breakdown are carbon dioxide, water, simple inorganic compounds and a considerable quantity of newly-synthesised organic matter consisting largely of microbial biomass. The effluent from the tank is passed to settling tanks in which the organic solids are separated from the liquid component of the effluent. However, an essential feature of the activated sludge process is that a substantial proportion of the organic solids is returned directly to the reaction tank (Figure 6.3), while only the liquid effluent is discharged.

The biological processes which occur within the reaction tank have been the subject of much study (Geraldi, 1990; Hawkes, 1983a). Growth of bacteria, fungi, protozoa and microinvertebrates (particularly rotifers) results in the production of an organic floc. The physical and biological characteristics of this floc appear to be extremely important. The characteristics of the floc are greatly influenced by the operating conditions of the process, and in turn the efficiency of the process is clearly influenced by the characteristics of the floc. A plant which is performing badly is frequently found to have a floc whose physical appearance and biological composition is very different from that

found in a plant which is operating well. In fact, regular inspection of the floc characteristics is widely used in monitoring the correct operation of the activated sludge process. A rather narrower range of organisms is typically found in activated sludge tanks than in percolating filters. Again, the question arises of whether these organisms are fortuitously colonising a favourable environment, or whether they have a specific and desirable role in the process. Experimental studies (Curds, 1975) have shown clearly that protozoa are responsible for a marked reduction in the turbidity of the effluent, and for the reduction in the numbers of bacteria present in the effluent. Effluents from plants containing protozoa also have lower values of biological oxygen demand (BOD), chemical oxygen demand (COD), organic nitrogen and suspended solids compared to those from plants operating without protozoa. Rotifers also appear to be important. Doohan (1975) suggests that they are responsible for removing non-flocculated bacteria. They also promote floc formation, by producing faecal pellets and by breaking up large floc particles, both processes providing nuclei for the formation of further floc.

Oxidation (stabilisation) ponds are also widely used as a means of sewage treatment. Strictly speaking, oxidation ponds are not a means of secondary treatment, but rather an alternative means of complete sewage treatment to what have come to be regarded as conventional methods. Where oxidation ponds are employed, there is frequently no division of the treatment process into primary, secondary and tertiary. Some forms of tertiary treatment, as conventionally practised, in fact involve the treatment of secondary sewage effluent in what amounts to an oxidation pond (see Section 6.1.3). As Hawkes (1983b) points out, treatment of used waters in biological oxidation processes involves the speeding-up and intensification of the natural processes of purification which occur in natural ecosystems. Oxidation ponds represent the lowest practicable level of this intensification, while percolating filters and activated sludge processes represent respectively higher levels. Thus to treat the sewage from 1000 people typically requires an area of 35 square metres in an activated sludge plant; but 210 square metres of percolating filter and between 2000 and 50000 square metres of oxidation pond, depending upon climatic conditions, are required to treat the same amount of sewage to a similar standard. Oxidation ponds are therefore particularly associated with warmer climates and regions which are technologically less well developed, although they are utilised in all parts of the world where local conditions are appropriate.

As with other sewage treatment processes, many variations of the basic procedure have been developed (Mara, 1988). A typical process is that described by Hawkes (1983b) as the facultative oxidation pond. A typical pond (Figure 6.4) is about 1 m in depth. Waste is introduced near the bottom of the pond, which has a retention time of a few hours or days, depending upon local conditions. Frequently, ponds are arranged in series so that the

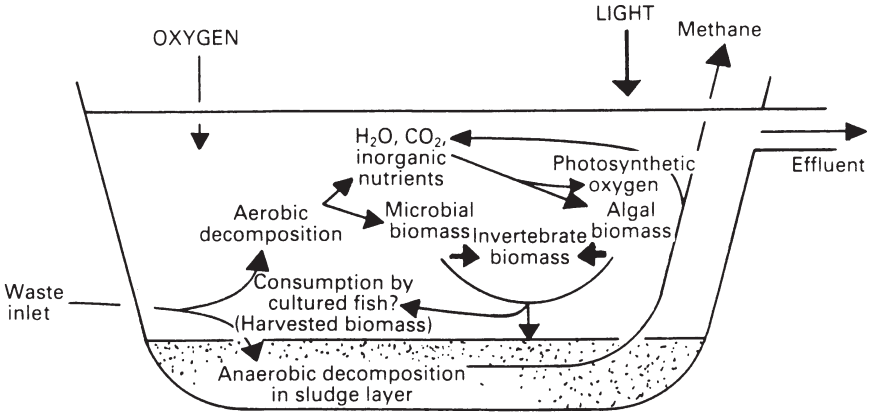


**Figure 6.4** Summary of the processes which occur in a facultative oxidation pond

effluent from one pond is passed to another for further treatment. The pond acts as a sedimentation tank, so that a layer of organically-rich sludge accumulates on the bottom. Within this sludge layer, anaerobic conditions quickly become established and decomposition of organic material takes place, resulting in the production of methane, carbon dioxide, ammonia and other metabolites typical of decomposition by anaerobic bacteria. These products themselves undergo further transformations in the upper, aerobic layers of the pond. Algae are particularly important in the functioning of oxidation ponds. If light is available, algal photosynthesis removes carbon dioxide and plant nutrients from the water, while providing photosynthetic oxygen. This allows the development of an aerobic bacterial flora which brings about further degradation of organic matter in the sewage. The waste material is thus broken down and resynthesised into new biomass. In part, this biomass eventually settles down into the sludge layer and is recycled within the system. However, the newly-synthesised material, through respiration, is responsible for converting a significant proportion of the waste matter to carbon dioxide and water. It also provides a rich source of energy which is capable of supporting a complex ecosystem. In some cases, waste treatment can be combined with aquaculture so that useful material (e.g. plant biomass, fish), can actually be harvested from the system. As a means of sewage treatment, stabilisation ponds compare well, under favourable conditions, with the standards achieved by other processes (Hawkes, 1983b; Mara, 1988).

### *6.1.3 Tertiary Treatment*

After secondary treatment and sedimentation, the effluent is often discharged without further treatment. Tertiary treatment, if necessary, is applied to the effluent to bring about specific improvements to the secondary effluent before it is

discharged. The precise nature of the tertiary treatment required depends upon the efficiency of the earlier stages of treatment, the characteristics of the receiving water and the uses to which the receiving water may subsequently be put. It is not therefore possible to describe a typical procedure for tertiary treatment, but the following examples illustrate the range of treatments which may be employed. The principal reasons for employing tertiary treatment are to bring about a further reduction in the BOD, suspended solids, ammonia, nitrate and phosphate in the effluent. Frequently, the most convenient method of achieving this is to pass the effluent through a further stage of biological filtration, perhaps using a modified form of percolating filter known as a 'high rate' filter. Nitrogen and phosphorus can be removed by a modified activated sludge process (Horan, 1990), and ion-exchange processes are available for the removal of phosphate and other inorganic ions, although they are expensive. Sand filters, similar to those used in water supply treatment plants, are effective in improving the quality of the final effluent. Sand filters, of course, are not simply physical filters; as in the percolating filter, the sand particles develop a microbial flora whose metabolism contributes to the effectiveness of the process.

An additional stage of simple sedimentation in a suitable tank has frequently been found to produce a useful reduction of BOD and suspended solids in the final effluent. Lagoons, similar to oxidation ponds, are a useful form of tertiary treatment where sufficient land is available, and effectively reduce BOD, suspended solids and nutrients. Traditionally, a widely-employed alternative is simply to allow the effluent to pass slowly over an area of slightly-sloping grassland. Organic solids are deposited and utilised in the soil, reducing suspended solids and BOD, and plant nutrients can be effectively absorbed by the growth of grass, which can be periodically mowed and removed; this method is however now largely obsolete. The optimum solution for any particular tertiary treatment problem depends primarily upon local conditions. Finally, tertiary treatment is sometimes required to remove pathogens from the effluent. Any form of tertiary treatment is likely to assist in removing pathogens, by sedimentation and mortality due to the passage of time outside the normal host. However, it is sometimes necessary to employ special techniques such as ultra-violet irradiation of the final effluent. A concise description of the major types of tertiary treatment in current use, and their applications, is given by IWEM (1994).

### *6.1.4 Sludge Disposal*

Sewage treatment generates substantial volumes of organically-rich sludge. In stabilisation ponds the sludge itself plays an important part in the treatment process, but in other processes the sludge which is generated following primary and secondary treatment must be disposed of. Various means are employed to treat and dispose of sewage sludge, the method chosen depending mainly upon local

conditions and the particular characteristics of the sludge. In general, sewage sludge is composed approximately of 98% water and 2% organic and inorganic particulate matter. Secondary sludge tends to have a higher organic content than primary sludge, and all sludges contain some quantity of potentially toxic material, and various pathogenic organisms.

One approach to the problem of sludge disposal is to regard it simply as waste material. In coastal areas, particularly in Britain and the USA, sewage sludge is frequently dumped at sea from specially-constructed vessels. The extent to which this practice represents a serious threat of marine pollution is a matter of controversy. Traditionally the United Kingdom, having a relatively long coastline, has regarded sludge disposal at sea under controlled conditions as a legitimate option, but under pressure from other European countries which have short coastlines, has agreed to eliminate this practice by 1997. At inland sites, disposal at sea is not practicable and other means of disposal must be sought. A major difficulty is to reduce the bulk of the sludge, and one of the simplest means of achieving this is to spread the liquid sludge onto drying beds, allowing the water to evaporate and consolidating the sludge into a more compact form, so that it can be accumulated on-site or perhaps transported elsewhere for disposal as landfill, as fertiliser or for other useful purposes. In some cases, liquid sludge is transported from the treatment site and spread onto agricultural land, where it may perform a useful function both as fertiliser and as a means of irrigation. However, the presence of toxic material, pathogens and parasites in sewage sludge presents a severe limitation on the disposal of sludge to agricultural land, and the commonly-held belief that this is a feasible means of solving the problem of sludge disposal is naive and largely false. At the very least, sludge requires dewatering and heat treatment before use in agriculture, and in addition high transport costs usually render this option unacceptable. Because sewage sludge is, potentially, a valuable resource rather than simply a waste material, many methods have been devised specifically for the treatment of sludge, and in particular for reducing its bulk (for ease of transport), and for eliminating pathogenic organisms. Descriptions and evaluations of sludge treatment processes are given by White (1978) and Frost *et al.* (1990); some important biological aspects of sludge treatment are discussed by Mosey (1983), Green (1983) and Frost *et al.* (1990). The methods of sludge treatment applied in particular circumstances depend largely upon local conditions and upon economic considerations.

Traditional sludge-drying beds are formed by spreading a layer of sludge, approximately 200 mm thick, over a drainage layer of ash or similar material. Liquid effluent from the drying beds can be collected by drainage channels and is ideally recirculated for further secondary treatment, as it has a high BOD and organic content. The remaining water is lost by evaporation. Sludge-drying beds require extensive land areas, especially in temperate or cool climates, and can create local nuisances due to odours and insects. They have largely been superseded by other methods of treatment. There are many advantages in reducing the water

content of sludge by other means, and the addition of chemical coagulating agents (such as compounds of iron and aluminium) to sludge has been found useful. Heat treatment of sludge (at about 200°C for 0.5 h) also appears to aid coagulation, and is useful in reducing the load of pathogenic organisms. Various processes of sludge dewatering which rely on filtration under pressure are also widely used.

Sludge treatment processes which are essentially biological in nature include anaerobic fermentation and composting. Anaerobic fermentation of sludge (sludge digestion) takes place in closed vessels, and results in a marked reduction in the organic content of the sludge as a result of anaerobic bacterial action. The operating conditions of sludge digesters vary (some, for example, are heated to promote the growth of particular bacteria, or to speed up the digestion process). In many cases, the end product of bacterial metabolism is methane, which can be collected and burned to provide energy for heating the digesters, or for other stages of the sewage treatment process. Composting of sludge is an aerobic process similar to the traditional garden compost heap; typically, sewage sludge is mixed with solid domestic waste in a ventilated enclosure. The end product of the process is an organic humus of value as an agricultural fertiliser. In many conurbations, the disposal of domestic solid wastes and of sewage sludge each pose a problem. Domestic waste has a high organic content but is too dry and frequently too deficient in nitrogen to allow composting to occur. Sewage sludge has too high a water content to allow aerobic decomposition to occur readily. Mixing the two forms of waste together under appropriate conditions can offer an economic solution to both problems. Finally, if no other method is appropriate to local conditions, sewage sludge can, like solid domestic waste, be incinerated.

### 6.1.5 Biological Treatment of Industrial Waste Water

Although conventional biological treatment processes were devised primarily for the treatment of sewage, they often work well with industrial wastes, though frequently the treatment processes have to be modified. Many industrial waste waters are similar in composition to raw sewage, particularly those from the dairy and foodprocessing industries, and from industries involved in the processing of natural materials. However, a common problem is that industrial waste waters are much more concentrated than typical sewage. Dairy wastes, for example, are difficult to treat by conventional biological filtration because the filters readily clog with fatty material and fungal masses. The concentrated nature of dairy effluents means that they rapidly begin to decompose anaerobically, so clogging of the filters rapidly leads to the breakdown of the whole treatment process. A typical means of overcoming this difficulty is to modify the conventional treatment process. First, the raw effluent is diluted; this can be achieved by mixing the raw effluent with a quantity of the final, treated effluent. Secondary treatment is carried out not with a conventional percolating filter, but by a modified process known as

alternating double filtration. In this system, two percolating filters are arranged in series. The effluent from the first is passed, after settling, through the second. The biological film in the first filter builds up rapidly; however, before the filter becomes clogged the flow of effluent is diverted so that the second filter receives the raw effluent first, while the first filter now receives partially-treated effluent. This has the effect of stripping out from the first filter the excessive quantity of film, which settles out in the sedimentation tank. When the second filter approaches the stage at which it begins to clog, the filters are alternated again. The regular alternation of the filters brings about a very effective treatment of the highly-concentrated waste; indeed, many conventional sewage treatment works operate on the alternating double filtration system simply to increase the speed of treatment.

A wide variety of industrial waste waters can be successfully treated by modified biological processes. A common practice is continuously to seed the percolating filter or activated sludge plant with a range of microorganisms which have been specifically isolated and cultured for their ability to metabolise the waste in question. Frequently, industrial wastes need special pretreatment before they are amenable to biological treatment. Common forms of pretreatment include: neutralisation of extreme pH; the removal of toxic substances by ion-exchange, coagulation or volatilisation; and the physical separation of oils, greases or other undesirable matter which may interfere with the normal treatment processes. A common difficulty is that many wastes, including some which are principally organic in origin, are deficient in one or more of the nutrients which are essential for the rapid development of the fauna and flora of the treatment process. For most biological treatment processes, the elements carbon, nitrogen and phosphorus should be present roughly in the ratio 100:5:1. In many industrial waste waters the nutrient elements are deficient, and frequently nitrogen, phosphorus or other nutrients in a biologically-available form are added to the waste water to enhance the efficiency of the treatment process. As the physical, chemical and biological processes underlying the treatment of different kinds of waste material become better understood, it becomes increasingly possible to develop specific processes by design rather than through the traditional, largely empirical approach (Rittman, 1992).

## 6.2 The Legislative and Administrative Framework of Water Pollution Control

Important as it is, the control of water pollution is only one aspect of the overall management and utilisation of water resources. Generally, the policies and practices associated with water pollution control are broadly compatible with those of water supply, fisheries, conservation and recreation. They may conflict, however, with political or economic priorities associated with waste disposal, industrial or agricultural development, transportation or other legitimate demands upon water resources. Pollution control policies must also be formulated in accordance with

the available technical, human and economic resources. Therefore the legislative and administrative measures which are employed in water pollution control vary widely from one country to another, and indeed change over time within a single country as circumstances change. This discussion introduces some examples of legislative and administrative approaches to water pollution control. It is based largely upon the procedures adopted in Britain, Europe and the United States. The systems adopted in these countries are not necessarily perfect, but they represent the kinds of system which have evolved in countries which are relatively wealthy, industrialised and, at least in some regions, very densely populated.

Most developed countries have some form of legally-constituted regulatory agency which is responsible for the monitoring and control of water pollution. The legislative framework within which the regulatory agencies operate may be broadly similar in different countries, but in practice the important consideration is the basis upon which consent conditions, i.e. permission to discharge waste material to water, are formulated. It is upon this issue that practices differ widely between different countries, depending upon local circumstances; and it is upon this issue that purely scientific or technical judgements need to be balanced against socio-economic criteria. As pollution is increasingly recognised as an international rather than a purely local problem, such differences in approach become correspondingly more important. To take a simple example, many European rivers pass through several different countries and differences in pollution control strategies can give rise to disagreements between sovereign states. In Europe, members of the European Union are increasingly subject to European laws and regulations which may dictate departures from their normal pollution control practices; differences which may arise between politicians from the different countries concerned are no more vigorously debated than those which occur between the scientists! Similar situations arise in the USA and in other federal countries, where state regulatory agencies may be subject to federal laws which are different in approach or emphasis from state laws or practices. It is therefore important to consider the various approaches which may be taken to the problem of what constitutes an 'acceptable' level of pollution.

For the purposes of this discussion, four general principles can be distinguished as forming the basic elements of an effective strategy of pollution control:

1 the 'polluter pays' principle;
2 receiving water quality standards;
3 emission standards; and
4 water quality objectives.

It is necessary to examine these basic elements before comparing how they are employed within different legal traditions to achieve the common objective of a satisfactory level of pollution control.

### *6.2.1 The 'Polluter Pays' Principle*

It is widely accepted that those who are responsible for pollution should contribute towards the costs of monitoring, regulating and controlling the adverse effects of pollution. However, the 'polluter pays' principle is clearly insufficient in itself as an effective and equitable mechanism of environmental protection. Consider, for example, a factory which produces a substantial amount of contaminated waste water. The factory owners may be unwilling to meet the costs of treating the wastes, or may lack the technical ability to do so. The factory therefore discharges untreated waste, thereby obtaining an economic benefit at the expense of the community at large. Clearly the factory should be made to compensate the community for this, perhaps passing on the economic charge to the eventual consumers of its products. The calculation of the charge to be made, however, is difficult. It should be possible to calculate the economic cost of treating the waste, and the extra costs incurred at water treatment works owing to the fact that the raw water is of lesser quality than it would otherwise have been. The additional costs to the regulatory authorities of monitoring the effects of the pollution, and the economic losses associated with any deterioration of fisheries, are in principle calculable, though not easily so. However, it is difficult to estimate in purely economic terms the amenity or aesthetic value of unpolluted water in comparison with polluted water, or to put a cost in money terms on the loss of species diversity in the aquatic community. It must also be recognised that not all polluters can be identified and charged. Further, there are several objections to the idea that permission to pollute should be given in exchange for a cash contribution to the community at large. In the absence of some additional form of regulation, the 'polluter pays' principle leads almost inevitably to the idea that pollution is a right which may be exercised by anyone who is able and willing to pay the appropriate charge. There are moral and philosophical arguments against such a proposition, but in any case the idea is impracticable. For example, some rivers may already be so polluted that any additional burden would create ecological or public health consequences which are unacceptable at any price. Some forms of pollution, such as radioactivity or certain toxic chemicals, are so potentially hazardous that their disposal demands special procedures; to allow their discharge simply in exchange for an economic charge would be extremely dangerous. Finally, some habitats are of particular scientific, conservation or amenity value and their protection from pollution would be undermined by acceptance of the simple proposition that pollution can be compensated for by a cash payment. Thus while considerations of logic and equity dictate that elements of the 'polluter pays' principle should be preserved, the principle must operate within a regulatory framework which prevents the inherent tendency of the principle to lead to environmentally undesirable consequences.

Some examples from British regulatory practice illustrate one way in which the 'polluter pays' principle can be incorporated into an overall pollution control

strategy. For example, every household pays a charge for the water it consumes which is distinct from general taxation, and includes also a charge for 'environmental services' (i.e. sewage treatment). Activities such as pollution monitoring, fisheries, river management, and flood prevention are the responsibility of the Environment Agency, and are financed partly from general taxation but also from charges levied on polluters directly, through levies on local communities collected by the local authorities and from the sale of, for example, fishing licences. The discharge of wastes to water is expressly forbidden except with the permission of the regulatory agency (Environment Agency—formed in April 1996 through the amalgamation of the National Rivers Authority with other statutory agencies concerned with environmental protection, such as Her Majesty's Inspectorate of Pollution). Permission can be refused altogether, to prevent discharges of especially hazardous waste or to protect specific habitats. The Agency may charge the polluter for the discharge consent, to cover the cost of any investigations which may be required. Where permission to discharge is granted, qualitative and quantitative limits are set which may not be exceeded. If the limits are exceeded, fines and penalties are imposed by the courts upon the offender. These economic penalties are often in themselves relatively insignificant, although recently they have been considerably increased (NRA, 1993), partly because of extra powers conferred upon the courts by the Water Resources Act of 1991. However, offenders can often be compelled to pay for the restoration of any damage they cause (such as restocking rivers with fish) and this cost is often the most significant penalty.

Sewerage charges to industrial premises are charged for at a rate which may reflect the costs of treating the waste water, of monitoring the impact of the discharge on the receiving water, of increased costs of managing and maintaining the receiving water and of increased water treatment costs of downstream water supply works. Large undertakings frequently undertake their own waste treatment, either in exchange for reduced sewage treatment charges or because the consent conditions for their discharges make this an absolute requirement. Smaller undertakings are permitted, within limitations, to discharge wastes to the public sewerage system in exchange for a payment. The level of these payments can, at least in principle, be set at a value which acts as an economic incentive to the factory operators to improve the quality or reduce the quantity of the effluent. Thus the 'polluter pays' principle operates only within regulatory constraints which are intended to ensure that the principle has beneficial rather than adverse environmental effects.

### *6.2.2 Receiving Water Quality Standards*

In order to decide whether or not a polluting discharge should be permitted, and if so under what conditions, it is obviously necessary to have some idea of the maximum quantity of pollutant which a water body can assimilate without undergoing adverse consequences. Where the receiving water is to be used as

a source of water supply, or for recreational or agricultural purposes, clearly special considerations may apply. However, for the purposes of this discussion it will be assumed that the dominant consideration is the preservation of aquatic life. The degree of pollution which is deemed 'acceptable' in any particular circumstances is only partly a biological question, but a reasonable objective which may be adopted is that the existing biological characteristics of the receiving water should not be significantly altered by the discharge of wastes to the receiving water. To implement this water quality objective (see Section 6.2.4) by means of specific decisions on the quantity and quality of effluent which may be discharged requires a reasonably reliable estimation of the likely effects of pollutant concentrations on individual species within the aquatic community, on the community as a whole and on the physico-chemical environment of the receiving water. If it can be established, for example, that a certain concentration of a toxic substance is unlikely to exert any significant effect in the receiving environment or upon its biota, then discharges can be regulated by various means to ensure that the concentration of the poison in the receiving environment is kept below that level.

Water quality standards for different forms of pollution are devised in different ways, because the means by which pollutants bring about their effects vary. Putrescible organic matter, for example, brings about a variety of changes in the receiving water environment (see Chapter 2) but these can largely be avoided if the dissolved oxygen concentration is maintained above a certain level. Knowing the BOD of the effluent, the degree of dilution afforded by the receiving water and certain physical characteristics of the receiving environment, it is possible to predict fairly accurately the oxygen sag curve which will occur. The dissolved oxygen requirements of many important aquatic species, especially fishes, are reasonably well known (see, for example, Alabaster and Lloyd, 1980). Therefore it is relatively easy to determine a quality standard for dissolved oxygen which can be translated into an emission standard (see Section 6.2.3) for organic matter. Toxic pollutants present greater difficulties, mainly because relatively little information exists on the effects of long-term, low-level exposure of animals to many poisons. Additionally, the toxicity of many poisons is greatly influenced by environmental conditions; many poisons are selective in their effects; and they normally occur in water in mixtures and at fluctuating concentrations (see Chapter 4). Water quality standards for toxic pollutants are derived on the basis of evidence from experimental studies and, where they exist, observations made during chemical and biological studies of polluted waters. For all but the most common pollutants, however, relatively little data are available from field studies and water quality standards have to be based on toxicological studies, especially toxicity test data of various kinds (see Chapter 4). This is obviously also true in the case of novel pollutants.

The bulk of the available toxicological data relates to lethal levels of pollutants; much less information is available on sublethal toxicity and the behaviour of pollutants at realistic levels in, for example, controlled or semi-controlled ecosystems. Data of the latter kind are accumulating slowly, as techniques are developed, but the main problem of formulating water quality standards from toxicological data remains that of using median lethal concentrations, or other data from relatively short-term experiments, as the basis for estimating concentrations which will not only fail to kill any organisms, but which will allow them to survive, grow and reproduce normally. One widely-used technique for deriving water quality standards from toxicological data is the 'application factor'. It is relatively quick and simple to determine lethal toxicity. The application factor is based upon the assumption that for every poison there must be a concentration which is so low that it has a negligible effect; and that this concentration should bear some approximate relation to the lethal threshold concentration, or other measure of acute toxic effect. Thus, multiplying the lethal threshold concentration by a factor (e.g. 0.1) should give a rough indication of the 'acceptable' level of the poison.

Methods of deriving application factors vary, and were briefly reviewed by Sprague (1971). Some methods are little more than simple extrapolation of the data, others are based upon statistical or other considerations, but all are subject to a degree of arbitrariness. There is little scientific validity in their use, but in practice they have been found useful and many regulatory agencies in Europe and North America have published recommended application factors for a wide range of poisons. Values vary from about 0.01 for persistent or cumulative poisons, to about 0.3 for rapidly-degradable poisons of fairly low toxicity. As Sprague (1971) emphasised, 'The value of the application factor is assigned on the basis of the judgement of scientists'. Although the basic concept of the application factor is, in practice, still widely used, the more recent literature contains few references to the idea. In part, this may be a reflection of the development of sublethal toxicity test methods and, particularly in the USA, of the idea of the NOEC (no observable effect concentration). Attempts were made, in the late 1960s and early 1970s, to determine application factors experimentally by calculating the ratio of the NOEC to the lethal concentration. However, the determination of NOEC is itself a somewhat arbitrary process (see Chapter 4), and the whole approach seems to have been superseded by what has come to be known as sequential hazard assessment (Duthie, 1977).

Sequential hazard assessment is not in fact a particularly novel approach to assessing the potential hazards of water pollutants. Rather, it is simply a codification of the sequence of investigative processes which has long been recognised as

necessary to derive water quality standards from toxicological data. The development of modern techniques, and very importantly the gradual accumulation of a reliable database of previous experience, render sequential hazard assessment possible; but the underlying process of scientific judgement remains the same as it always has been. The approach has been described, with examples, very clearly by Tooby (1978), and can be expressed in the form of a simple flow chart (Figure 6.5).

Essentially, a sequential hazard assessment scheme consists of a number of steps, each providing progressively more complex information. Each succeeding stage in the investigation is generally more expensive and time-consuming than the previous one. The advantage of such a scheme is that, at least in some cases, the later and more difficult steps can be dispensed with. For example, Figure 6.5 shows that the preliminary assessment is designed to establish the 'margin of safety'. Here, the results of a few simple screening tests can be compared with the projected environmental concentrations and patterns of use of the chemical. If, say, the 96-h LC50 of the chemical to a carefully-chosen range of test species is more than 10000 times greater than the expected environmental concentration, experience suggests that the chemical is not particularly hazardous. Taking into account its pattern of use (e.g. whether it will be permanently present in the water, or only for a few hours or a few days occasionally), the decision may be made that no further tests are required and that permission to discharge may be given, at least provisionally. If, on the other hand, the 96-h LC50 values are within an order of magnitude of the projected environmental concentration, the likely decision will be that the chemical is unacceptable, again without the need for further tests. Margins of safety lying between these two extremes would tend to indicate the need for further investigations, that is, to proceed to the next step of the scheme.

At any stage, the investigation can be terminated when sufficient information has been gained to make, in the light of previous experience, a decision. Both industry and regulatory authorities can follow a similar procedure, and the advantages in terms of speed and economy are obvious. The procedure not only allows the simple decision 'accept or reject the chemical' to be made. It clearly also permits decisions to be made upon, for example, the concentrations which are acceptable and any restrictions or conditions which should be put on the discharge of the pollutant (e.g. to protect early life stages at certain times of the year). Obviously, however, any water quality standard determined on this basis is tentative, and should ideally be evaluated in the light of ecological monitoring of the receiving waters subsequent to the discharge of the pollutant.

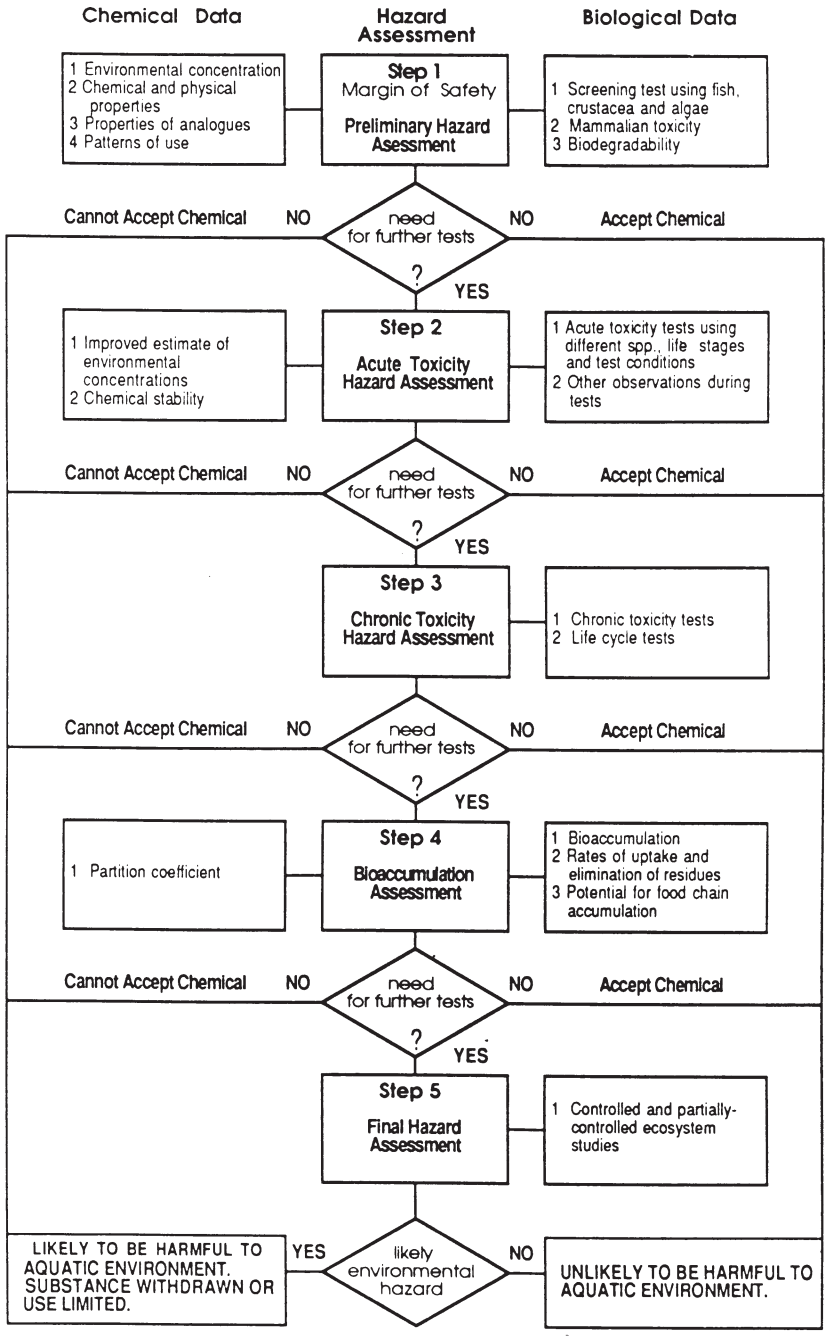


**Figure 6.5** Flow chart summarising the steps in the sequential hazard assessment procedure. After Tooby (1978)

In the case of common industrial pollutants of long standing, where for example it is desired to improve the status of a polluted receiving water, the approach to determining water quality standards is often significantly different. A large amount of toxicological information may already exist, and data from field observations are likely to assume a greater significance. A very good illustration of the considerations which may be taken into account in formulating water quality standards is the series of reviews edited by Alabaster and Lloyd (1980). These reviews recommend water quality standards for the protection of European freshwater fisheries against several common industrial pollutants. It is also useful to recall the sophisticated approach to formulating water quality standards for toxic substances described in Section 1.2. The common industrial pollutants copper, zinc, phenol, ammonia and cyanide frequently occur together, and their toxicity is greatly influenced by environmental conditions. The technique described in Section 1.2 offers the means to identify specific threats to, or potential improvements in, the biological status of a receiving water which can be fairly easily implemented by specific regulatory action against polluting discharges or by alternative strategies of river management. The successful application of this method requires, however, an extensive database of chemical, biological and toxicological information.

Water quality standards such as those recommended by EIFAC (Alabaster and Lloyd, 1980) are advisory rather than mandatory, but have been widely adopted and incorporated into national practice in many countries. They have been updated from time to time (see, for example, EIFAC Technical Paper series published by UN FAO, Rome). However, in many parts of the world they have been superseded by mandatory standards, either national or supranational, such as European Union directives which govern standards in member states of that Union; a situation rather similar to that which pertains to drinking water quality standards (see Chapter 5), where advisory WHO standards are increasingly superseded by similar mandatory standards.

What is not yet clear is the extent to which more recent developments in toxicological techniques—particularly those based upon cellular, biochemical and genotoxicological approaches such as described in Chapter 4—may play a role in the future in determining water quality standards. Computer modelling is also becoming widely used to predict the likely environmental impact of novel pollutants. These are likely to be areas of development over the next few years; some examples of applications in the rather different circumstances of the marine environment are discussed in Section 7.2.3.

### *6.2.3 Emission Standards*

Emission standards are the limit values set upon the quantity and/or quality of the effluent which may be discharged; in other words, the consent conditions which form the basis of permission to discharge wastes to water. In some jurisdictions,

such as the UK, emission standards are usually set having regard to considerations of the overall receiving water quality standards which are desirable; these in turn are based upon water quality objectives (see Section 6.2.4). In other jurisdictions, emission standards (often referred to as 'end-of-pipe' controls) are accorded much greater importance and form the major, if not the only, element of the water pollution control strategy.

An early example of an emission standard in Britain was the recommendation of the Royal Commission on Sewage Disposal in 1915 that sewage effluent should contain no more than 30 mg $l^{-1}$ suspended solids and should have a BOD value not exceeding 20 mg $l^{-1}$. This recommendation (the so-called 30/20 standard) was based on the assumption that the effluent would normally be diluted at least eight times by the volume of the receiving water. In practice, consent conditions for the discharge of effluents may set limits on the strength and quantity of effluent which may be discharged; individual components of the effluent may be subject to specific restrictions; and consent conditions may vary according to the time of year, or the physical conditions of the receiving water such as temperature or the volume of dilution water available.

It is comparatively easy to monitor compliance with emission standards, and they have often been found useful even when set at more or less arbitrary levels. For example, the 30/20 standard resulted in a substantial improvement in the quality of many British rivers, even if the standard was not universally achieved, because at the time it was implemented most sewage was discharged either untreated, or treated only to a poor standard. Emission standards for oil and toxic substances can usefully be applied to effluents in which they are only rarely to be expected (for example as a result of plant failure), since the existence of the standard will require the effluent to be monitored and will thus tend to prevent pollution caused by unusual occurrences which may otherwise be undetected. Emission standards are also administratively convenient. Consider, for example, a river which is already polluted to which it is proposed to discharge a new effluent. The regulatory agency may decide that the river cannot accommodate a substantial increment of pollution, and may wish to set consent conditions for the new factory which are more restrictive than those which apply to the established discharges. This can lead to litigation or political lobbying from the owners of the new factory, who may feel that they are being treated inequitably in comparison with their competitors. An equitable and administratively convenient solution would be to revise the consent conditions of all the factories concerned so that each factory was subject to the same consent conditions—in other words, uniform emission standards would be imposed on all.

The administrative convenience of uniform emission standards tends to lead to their assuming a degree of importance in pollution control strategies which can be counter-productive. This problem is becoming more acute as pollution control

strategies become internationalised. Within the European Union, for example, member states have agreed in principle to adopt policies designed to reduce environmental pollution. Different states within the Community may each feel that their particular pollution control strategy is adequate in relation to local conditions. Inevitably, however, emission standards will vary from one place to another, and some states will be able to argue that the stricter constraints under which they operate impose upon them an unfair economic disadvantage. It is therefore difficult, politically and administratively, to avoid moving towards a policy of uniform emission standards regardless of local conditions. A similar situation can arise where several states enter into agreements to limit pollution in rivers, lakes or seas to which all discharge effluents.

An alternative approach to emission standards is to specify not a range of limit values relating to the quality or quantity of the effluent itself, but to specify a process or method by which the effluent must be treated before it is discharged. This approach is sometimes advantageous with particular effluents or with particular types of material, or where there is disagreement about the environmental quality standard which needs to be achieved. In the USA, the Clean Water Acts of 1972 and 1987 give the Environmental Protection Agency the powers to insist that an effluent is treated by the 'best available technology' (BAT). Of course, any effluent can be treated completely if cost is no object, so in practice a less rigorous code is used for all but the most dangerous substances, which specifies treatment by the 'best practicable technology' (BPT). This implies that some form of cost-benefit analysis needs to be undertaken before the decision is made concerning what constitutes the BPT, which tends to give rise to extensive litigation. In Europe, a similar idea has gained currency, and is known as 'best available technology not entailing excessive cost' (BATNEEC).

The principal disadvantages of uniform emission standards are that they are not always appropriate for the protection of the receiving environment, and that they can lead to the inefficient use of resources. For example, a sewage works discharging to a river which is already heavily polluted could operate to a comparatively low standard of effluent quality and still actually improve the quality of the receiving water, perhaps leaving resources to spare which could be better utilised elsewhere. Conversely, a new discharge which meets a uniform emission standard could have a seriously adverse effect if sited on a river which was previously unpolluted. For this reason, in Britain and several other countries, a fourth element of pollution control strategy has been developed, the concept of water quality objectives.

### *6.2.4 Water Quality Objectives*

The concept of pollution regulation by water quality objectives (WQOs) is based on the argument that the most effective use of resources and the greatest degree of protection and improvement of receiving waters is in practice achieved by the

formulation of emission standards which are designed to be appropriate to the particular receiving water under consideration. In other words, uniform emission standards are specifically rejected. Instead, each receiving water is considered individually, and a technically and economically feasible management objective is determined. An example of such an objective may be that the receiving water should sustain a healthy population of cyprinid fish. The appropriate receiving water quality standards are then determined, and can be translated into emission standards designed to achieve the initial objective.

The principal difficulty of the WQO approach is that it can only be sensibly and effectively applied on the basis of extensive and reliable information about the current status of receiving waters and of the pollution loads which they are receiving. Without this information, feasible water quality objectives cannot be set, and if the objective is not realistic the resulting emission standards will be as arbitrary and inefficient as uniform emission standards. For example, it may be highly desirable that all rivers sustain populations of salmonid fishes and a diverse invertebrate fauna. Such an objective ignores the fact that many rivers are, for various reasons, naturally unable to do so. Also, regulatory agencies are more likely to enjoy the co-operation of industry and the support of the general public if the economic resources devoted to pollution control result fairly quickly in a perceptible improvement in environmental quality. It is therefore important that water quality objectives are realistic in relation to the current status of the receiving waters.

In Britain, the application of WQOs to pollution control rests upon extensive national surveys of all substantial rivers. These surveys began in 1958, and now take place every five years. The survey work is undertaken by the National Rivers Authority (now part of the Environment Agency, see Section 6.3). An important aspect of the national river surveys is the development of a national scheme for the collection and analysis of data. This was a major factor in the development of the BMWP Score, a biotic index of water quality devised to be appropriate in all parts of the country and in all types of river (see Section 3.4.3). On the basis of the survey information, a national system of river classification was developed over a period of time (NRA, 1991) which can be used to formulate sensible water quality objectives. The classification scheme currently in use is shown in Table 6.2, and a summary of the national survey results since 1958 is shown in Table 6.4.

The classification system immediately suggests several objectives. For a Class 1A or 1B river, an obvious objective is to prevent any deterioration at all. For rivers of intermediate classification, a reasonable objective is at least to preserve them in their present condition, and where feasible to improve them so that they attain the next-highest classification. In a typical scheme devised by the Severn-Trent Water Authority (Young, 1980) it was proposed to leave Class X rivers as they are; to eliminate Class 4 rivers by improving them to Class 3 quality; to improve selected rivers of Classes 3 and 2 to 2 and 1B respectively; and to preserve Class 1A rivers

in their present condition (Table 6.3). The 1990 National Survey (NRA, 1991) in fact showed that the projected improvements did not take place, and that the 1990 situation was almost the same as that of 1980. Results of the 1995 survey are not yet published.

Note that an important aspect of the classification system is the current or potential uses of the water, which both determine the present classification of the water and help to identify those rivers for which improvements would be most cost-effective. Prior to 1980, the classification scheme was based largely upon physical and chemical criteria and are often referred to as RPS (River Pollution Survey) classes. The new classification scheme differs essentially in that the basis of the classification gives rather more weight to the actual and potential uses of the water, and to biological characteristics; the old Class 1 (unpolluted) has been split into Classes 1A and 1B; while the old Class 4 (grossly polluted) has been replaced by Classes 4 and X. Classes 1A and 1B are both generally of good quality, and a 1B river cannot be promoted to a Class 1A. They are different for natural reasons, for example 1A rivers tend to be upland, and 1B lowland stretches. Class X rivers are defined as insignificant ditches or watercourses; they often act as surface drains, and the principal management objective is to prevent them becoming a health hazard or public nuisance.

Table 6.4 summarises the classifications of non-tidal rivers and canals in England and Wales in national surveys between 1958 and 1990, demonstrating a steady overall improvement in water quality during most of that period. The effect of the change in classification system in 1985 is shown by the 1980 data, which in Table 6.4 are presented both in the old and new formats. The report of the 1985 survey showed that the rate of improvement had slowed, and by 1990 that some rivers had clearly deteriorated (NRA, 1991). This is less obvious from the summary data shown in Table 6.4 than from the more detailed region-by-region breakdown shown in the original report. Significant deteriorations which occurred in some regions are masked, in the summary table, by improvements elsewhere. The report argues that this is at least partly due to changes in survey methodology, though there is little doubt that much of the deterioration is significant and real. However, the reasons for the deterioration, which were the subject of much ill-informed speculation, actually emerge from the data in the report itself and from other data in the public domain (e.g. NRA, 1992, 1994a). For example, the South-West region of the country, where considerable deterioration took place between 1985 and 1990, is not an industrial area but is dominated by intensive dairy farming, much of it subsidised by the European Common Agricultural Policy. The area suffers from chronic water shortage and during the period concerned suffered from a four-year drought. Therefore the common assumption that the problems were related to industry or to any laxity on the part of the newly-privatised water utility companies is largely unsus

**Table 6.2** The National Water Quality Classification Scheme used in Britain

| River class | Quality criteria | Remarks | Current potential uses |
|---|---|---|---|
| | Class limiting criteria (95 percentile) | | |
| 1A Good quality | (i) Dissolved oxygen saturation greater than 80%<br>(ii) Biochemical oxygen demand not greater than 3 mg/l<br>(iii) Ammonia not greater than 0.4 mg/l<br>(iv) Where the water is abstracted for drinking water, it complies with requirements for A2[a] water<br>(v) Non-toxic to fish in EIFAC terms (or best estimates if EIFAC figures not available) | (i) Average BOD probably not greater than 1.5 mg/l<br>(ii) Visible evidence of pollution should be absent | (i) Water of high quality suitable for potable supply abstractions and for all other abstractions<br>(ii) Game or other high class fisheries<br>(iii) High amenity value |
| 1B Good quality | (i) DO greater than 60% saturation<br>(ii) BOD not greater than 5 mg/l<br>(iii) Ammonia not greater than 0.9 mg/l<br>(iv) Where water is abstracted for drinking water, it complies with the requirements for A2[a] water<br>(v) Non-toxic to fish in EIFAC terms (or best estimates if EIFAC figures not available) | (i) Average BOD probably not greater than 2 mg/l<br>(ii) Average ammonia probably not greater than 0.5 mg/l<br>(iii) Visible evidence of pollution should be absent<br>(iv) Waters of high quality which cannot be placed in Class 1A because of the high proportion of high quality effluent present or because of the effect of physical factors such as canalisation, low gradient or eutrophication<br>(v) Class 1A and Class 1B together are essentially the Class 1 of the River Pollution Survey (RPS) | Water of less high quality than Class 1A but usable for substantially the same purposes |

| | | | |
|---|---|---|---|
| 2 Fair quality | (i) DO greater than 60% saturation<br>(ii) BOD not greater than 9 mg/l<br>(iii) Where water is abstracted for drinking water it complies with the requirements for A3[a] water<br>(iv) Non-toxic to fish in EIFAC terms (or best estimates if EIFAC figures not available) | (i) Average BOD probably not greater than 5 mg/l<br>(ii) Similar to Class 2 of RPS<br>(iii) Water not showing physical signs of pollution other than humic colouration and a little foaming below weirs | (i) Waters suitable for potable supply after advanced treatment<br>(ii) Supporting reasonably good coarse fisheries<br>(iii) Moderate amenity value |
| 3 Poor quality | (i) DO greater than 10% saturation<br>(ii) Not likely to be anaerobic<br>(iii) BOD not greater than 17 mg/l. This may not apply if there is a high degree of re-aeration | Similar to Class 3 of RPS | Waters which are polluted to an extent that fish are absent or only sporadically present. May be used for low grade industrial abstraction purposes. Considerable potential for further use if cleaned up |
| 4 Bad quality | Waters which are inferior to Class 3 in terms of dissolved oxygen and likely to be anaerobic at times | Similar to Class 4 of RPS | Waters which are grossly polluted and are likely to cause nuisance |
| X | DO greater than 10% saturation | | Insignificant watercourses and ditches not usable, where the objective is simply to prevent nuisance developing |

*Notes*: (a) Under extreme weather conditions (e.g. flood, drought, freeze-up), or when dominated by plant growth, or by aquatic plant decay, rivers usually in Class 1, 2 and 3 may have BODs and dissolved oxygen levels, or ammonia content outside the stated levels for those classes. When this occurs the cause should be stated along with analytical results.

(b) The BOD determinations refer to 5-day carbonaceous BOD (ATU). Ammonia figures are expressed as $NH_4$.

(c) In most instances the chemical classification given above will be suitable. However, the basis of the classification is restricted to a finite number of chemical determinands and there may be few cases where the presence of a chemical substance other than those used in the classification markedly reduces the quality of the water. In such cases, the quality classification of the water should be down-graded on the basis of biota actually present, and the reasons stated.

(d) EIFAC (European Inland Fisheries Advisory Commission) limits should be expressed as 95 percentile limits.

[a]EEC category A2 and A3 requirements are those specific in the EEC Council Directive of 16 June 1975 concerning the Quality of Surface Water Intended for Abstraction of Drinking Water in the Member State.

**Table 6.3** Proposed river quality objectives for the Severn–Trent Water Authority area in 1978 (data from Young, 1980)

| Classification | Existing quality km | Proposed quality km |
|---|---|---|
| 1A | 877 (14.3%) | 877 (14.3%) |
| 1B | 2417 (39.5%) | 2543 (41.5%) |
| 2 | 2085 (34.1%) | 2360 (38.6%) |
| 3 | 565 (9.2%) | 292 (4.8%) |
| 4 | 128 (2.1%) | 0 (0%) |
| X | 46 (0.8%) | 46 (0.8%) |

tainable in view of the facts. In 1995, however, a national drought occurred which caused much greater disruption to water supplies than had occurred during earlier droughts in 1976 and 1983. On this occasion, the National Rivers Authority supported the widespread public criticism of some water utilities for poor investment in, and management of, water resources. Many households faced emergency restrictions on water use; and greatly increased abstraction rates from rivers exacerbated pollution and ecological problems which were the NRA's responsibility to deal with.

Pollution control by quality objectives is arguably the most scientifically and economically sophisticated approach to the problem of devising an effective system for regulating waste discharges. However, its effectiveness and public acceptance depend crucially upon extensive and accurate information. This in turn depends upon the establishment of nationally-based or regionally-based systems of monitoring and co-ordination, and of an appropriate technical and administrative infrastructure; these are, unfortunately, well established in relatively few parts of the world. It is for this reason that regulatory strategies differ from one country to another, although these differences primarily consist of differences in the relative emphasis given to the 'polluter pays' principle, water quality standards, emission standards, and water quality objectives. Some of the issues involved are discussed in the volumes edited by Stiff (1980) and Lack (1984).

## 6.3 Water Pollution Law in Practice

Biologists are not lawyers, but they do need to interact with lawyers, particularly if they are working for a regulatory agency, or advising individuals or organisations concerned with water pollution. The following discussion is a brief comparative account of the law relating to water pollution mainly based upon Britain, Europe, and North America. McLoughlin and Bellinger (1993) provide a general discussion of the relevant principles, and a comparative survey of practice in a variety of different countries.

**Table 6.4** Summary of the results of the British National River Surveys, 1958–1990. Data from NRA (1991) (see text for details)

| | Former classifications 1958–1980 surveys | | | | | | | | | New classification 1980–1990 surveys | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Non-tidal rivers and canals | | | | | | | | | Freshwater rivers and canals | | | | | | |
| | 1958 | | 1970 | | 1975 | | 1980 | | | 1980* | | 1985 | | 1990 | | |
| Class | km | % | km | % | km | % | km | % | Class | km | % | km | % | km | % | |
| Unpolluted | 24 950 | 72 | 28 500 | 74 | 28 810 | 75 | 28 810 | 75 | Good 1A | 13 830 | 34 | 13 470 | 33 | 12 408 | 29 | |
| | | | | | | | | | Good 1B | 14 220 | 35 | 13 990 | 34 | 14 536 | 34 | |
| Doubtful | 5220 | 15 | 6270 | 17 | 6730 | 17 | 7110 | 18 | Fair 2 | 8670 | 21 | 9730 | 24 | 10 750 | 25 | |
| Poor | 2270 | 7 | 1940 | 5 | 1770 | 5 | 2000 | 5 | Poor 3 | 3260 | 8 | 3560 | 9 | 4022 | 9 | |
| Grossly polluted | 2250 | 6 | 1700 | 4 | 1270 | 3 | 810 | 2 | Bad 4 | 640 | 2 | 650 | 2 | 662 | 2 | |
| | | | | | | | | | X | – | – | – | – | 39 | – | |
| | | | | | | | | | Unclassified | – | – | – | – | 17 | – | |
| Total | 34 690 | | 38 400 | | 38 590 | | 38 740 | | Total | 40 630 | | 41 390 | | 42 434 | | |

*As revised in 1985

### *6.3.1 Water Pollution Law in Britain*

The United Kingdom consists of Great Britain (England, Wales and Scotland), and the province of Northern Ireland. For our purposes, the law and legal systems of England and Wales are identical. Scotland has its own legal system and its law is different in many ways from that of England and Wales, and there are special considerations relating to Northern Ireland, so for purposes of this discussion the term 'Britain' relates largely to England and Wales only. In Scotland, the overall thrust of the legal provision is the same as in England, but the methods of administration and enforcement are different. Reid (1993) gives a brief summary.

Until 1974, responsibility for the various aspects of water management was shared between a large number of different organisations. Broadly speaking, water supply was the function of a large number of separate bodies, of which some were municipal undertakings and others were private companies. Sewage disposal was the responsibility of municipal authorities around the country; and river management (drainage, flood prevention, fisheries, conservation and pollution monitoring) was undertaken by a number of River Authorities. Separate management of these three basic functions worked reasonably well (although many municipal authorities neglected their sewage treatment works), and is still practised in many countries. However, the potential advantages of a unified management system for all aspects of the hydrological cycle were widely recognised. The Water Act 1973 led to the establishment of ten Regional Water Authorities in England and Wales in 1974 (Scotland retained the old system), each Authority being responsible for all aspects of water management within its area. These areas were defined not by political boundaries, but by the major river catchment areas. These large, unified authorities with their considerable physical, human and financial resources were intended to be better able to cope with the increasingly complex management and technical problems associated with increasing demands for water. Arguably, it is easier to devise and implement policies on the interrelated problems of water supply, pollution control and monitoring, conservation, river management, recreation, fisheries and so on within a single organisation. However, one paradox of the unified management system was that the organisation responsible for monitoring and regulating waste discharges was itself the single most important polluter of water, since the water authorities were themselves responsible for sewage disposal. This difficulty was resolved by requiring that the water authorities, which were normally empowered to grant or refuse permission to discharge wastes to water, required the approval of the Secretary of State (Environment Minister) for their own discharges. In practice, the system did not work very well. Faced with continuous demands for public investment from the water authorities to meet environmental quality standards, successive governments could not resist the temptation to relax consent conditions as an alternative to

increasing public expenditure. However, by the late 1980s a further major change took place.

Under the Water Act 1989, the Government privatised the water utilities. This was a politically controversial move, but the reasons for it were only partly ideological. It was becoming obvious that in many parts of the country, substantial capital investment would be required to renew water supply, drainage and sewerage systems, many of which were reaching the end of their useful lives. Additionally, demand for water was increasing and environmental quality standards were becoming inexorably more demanding. Rather than face the burden of these enormous costs on the public purse and make substantial increases in general taxation, it appeared politically more comfortable to turn the Water Authorities into private utility companies able to raise capital through the commercial money markets and by the issue of shares in the companies. One problem remained, which arose through Britain's obligations under European Community law to designate a "competent authority" to fulfil the role of a regulatory agency, which could not be a private company. Bearing in mind the problems referred to earlier which had arisen from the 1973 Water Act, the opportunity was taken to create a new agency, the National Rivers Authority (NRA), to undertake most of the regulatory and river management functions, leaving water supply and sewage disposal as the responsibility of the private water utility companies. The 1989 Water Act conferred a range of powers and responsibilities on the NRA to enable it to carry out its functions. In April 1996, the NRA became part of the new Environment Agency.

It is not easy to give a summary of water pollution law in Britain which remains up-to-date, as it remains in a state of rapid flux. However, the following summary attempts to give an overall picture of the general pattern of legal provision. A useful and fairly brief account is given by NSCA (1992, and later editions; see also *Croner's Environmental Management,* 1995). The powers and responsibilities of the water utilities and the NRA in Britain are defined in a number of laws, including the Rivers (Prevention of Pollution) Acts of 1951 and 1961; The Water Acts of 1945, 1973, 1983 and 1989; the Salmon and Freshwater Fisheries Act 1975; the Control of Pollution Act 1974 and the Environmental Protection Act 1990. Many of the provisions of these Acts remain in force, but the key Acts which are directly relevant today are the Water Act 1989, subsequently amended by the Water Resources Act 1991 and the Water Industry Act 1991. Howarth (1990) provides an accessible guide to the 1989 Act. In fact the 1991 Water Resources Act substantially repealed the 1989 Act and replaced it with an amended version, but the general situation remains much the same.

The basic legal position is that the discharge of waste matter to water bodies is prohibited by any person, organisation or other legal entity unless permission has been granted by the NRA (or Environment Agency). Consent is normally given only if certain conditions are met, such conditions relating to both the quality and quantity of the effluent, any other conditions which the NRA deem necessary, and normally requiring in addition that the quality, quantity and effects of the effluent be systematically monitored and recorded. Failure to observe the consent conditions

can lead to prosecution of the offender. In addition, the NRA has a general duty to ensure that the waters for which it is responsible (i.e. all fresh waters and also estuaries and coastal waters) do not deteriorate, and indeed that under appropriate circumstances the quality of the waters is actually improved. For this reason, large-scale programmes of chemical and biological monitoring are undertaken. This activity not only ensures that their statutory responsibilities are being met, but also provides useful data for the formulation of water quality objectives (see Section 6.2.4).

It is possible to anticipate some future developments in British law and practice which are currently under discussion. For example, it is proposed to introduce *statutory* water quality objectives, that is WQOs which have legal force. At present, WQOs are primarily used as management, planning and administrative tools as described in Section 6.2.4. A statutory WQO would be designated for a particular body of water and it would become a legal obligation for that objective to be achieved and maintained. The purpose of this is to prevent the possibility that established WQOs could be arbitrarily altered by administrative decision as a result, say, of negotiations between the NRA, other planning or regulatory authorities and polluters themselves. Statutory WQOs, once designated, could only be reviewed or revised as a result of a legally-enforceable and defined process; this will place increasing responsibility on polluters and regulatory authorities alike.

From 1989 to 1996, the NRA was the principal regulatory agency of the aquatic environment in Britain; other agencies had responsibilities which sometimes overlapped or complemented the NRA's activities. These included Her Majesty's Inspectorate of Pollution, mainly concerned with air pollution and pollution associated with solid wastes; and the Drinking Water Inspectorate, specifically concerned with the quality of drinking water and of water bodies used as a source for drinking water supply. Local authorities also had some regulatory functions. These organisations and their functions have now been merged into a single Environmental Agency. It remains to be seen what influence this will have on policy and practice in the future.

However, undoubtedly the major source of rapid change at the present time in British pollution control practice stems from the UK's membership of the European Union.

### 6.3.2 European Water Pollution Law

Currently 15 countries have acceded to the European Union (formerly the European Economic Community), and further countries are expected to join in the near future. It is not clear at present how the Union may develop in the future, but its status is perhaps best described as a union of independent sovereign states which are bound by a series of treaties to co-operate and to harmonise national policies in areas such as trade, social and economic policy, and the environment. Thus there is no such thing as Federal law, as exists for example in the USA, and European environmental law impinges upon the national law of member states largely through

the adoption by the member states of legal instruments known as directives. Directives are developed and proposed by the European Commission, and adopted by the member states, sometimes with amendments or derogations to reflect particular national interests or circumstances. Directives essentially set targets or objectives to be achieved, usually within a time limit, but do not specify the means by which these objectives are to be achieved; that is left to each member state according to its own custom and practice. Obviously, in some circumstances a particular state may already have achieved the objective, so the directive may have no influence on existing custom and practice; in other cases, in order to comply with the directive a member state may have to amend its custom and practice, or even pass new legislation through its own Parliament, create new agencies, or take other appropriate steps. Therefore European legislation can have a significant influence on individual member states.

EC directives which are relevant to water have been published by the Commission of the European Communities (1992), which lists 17, though the number increases annually. Some of the more important ones are:

- 76/464/EEC—Directive on pollution caused by certain dangerous substances discharged into the aquatic environment of the community.
- 78/659/EEC—Directive on the quality of fresh waters needing protection or improvement in order to support fish life.
- 79/923/EEC—Directive on the quality required for shellfish waters.
- 91/676/EEC—Directive concerning the protection of waters against pollution caused by nitrates from agricultural sources.
- 91/271/EEC—Directive concerning urban waste water treatment.

At the time of writing, a draft directive concerning the ecological quality of water has been circulated for consultation. One of the major thrusts of this directive is to establish in all member states a system of biological monitoring of water quality and national surveying and reporting thereof, similar to that which has been in operation in the UK since 1958 (see Section 6.2.4)

The Commission of the European Communities (1992) publication simply prints the text of the relevant directives up to that date. Of more direct interest is the effect the directives have on national policy and practice, and indeed the origin and intent of the directives. Haigh (1990, 1995) explains the background and impact of each relevant directive on UK policy and practice; *Croner's Environmental Management* (1995) is also a useful and practical guide. A report on the implementation in the UK of the Freshwater Fish Directive (78/659/EEC) has been published by the NRA (1994b). The ostensible reason for the involvement of the European Union in environmental matters is that pollution is a genuinely transnational problem which demands transnational solutions. However, many directives betray the origin of the European Union as essentially a trading bloc or economic community, in that a major force behind many of them seems to be that one member state should not obtain undue economic

advantage over others by adopting lower environmental standards. (Similar arguments apply in relation to other matters such as social security policy or environmental safety in the workplace.) There seem to be several different categories of directive, in terms of their origin and purpose. Some, such as those listed above, are of fairly clear relevance. A number of directives are concerned with specific pollutants or specific industries, of which some have scientific validity but others really represent nothing more than the expression of national interest. A good example is the directive on waste from the titanium dioxide industry (78/176/EEC) (Haigh, 1995). In this case, a dispute between France and Italy over an Italian factory which was obsolete, badly sited and poorly regulated arose because its wastes were polluting the sea between Italy and Corsica. The obvious solution was to tackle the problem by national action to close, improve or regulate the offending factory. Since the Italian government was unable or unwilling to do this, France initiated against the entire industry a directive whose economic impact is very damaging but whose environmental relevance is extremely doubtful.

Many directives are of a very technical nature, though no less important for that. Several are concerned with methods of measurement or analysis, or frequency of sampling for chemical or microbiological purposes. The need for such directives appears to arise because, as we have seen earlier in this book, the methods of measurement chosen, whether for counting coliforms in water or estimating the toxicity of pollutants, can greatly influence the results. Further, the reliability and accuracy of measurements depend crucially on the sampling size, frequency, distribution in space, and so on. Some member states are technically unable or politically unwilling to comply fully with the requirements for environmental data, so a need is created for these 'methodological' directives as a means of quality control on the information forwarded to the European Commission. A further source of doubt about some aspects of European legislation concerns the inadequate scientific advice which some legislators appear to receive; one may suggest there are too many lawyers and not enough scientists involved! For example, careful study of some directives will reveal limit values for chemicals enacted as environmental quality standards, or specified detection limits, which are actually beyond the best available technology, and certainly beyond the capability of most laboratories involved in routine monitoring.

There will certainly therefore be still further changes in European law over the next few years, as the process of harmonisation of practices continues, as the less technically well-endowed members of the Union improve their standards, and as scientific reality intrudes upon legislative optimism. It will certainly be interesting to see whether, now that the European Union extends from the Arctic to the Mediterranean, the bureaucratic obsession with standardisation can be sustained in the face of climate and biogeography. In the meantime, we may expect continuing controversy because of the different traditions and stages of development of the various member states. The UK, and some other Northern European countries, for example, reject the European emphasis on emission

controls because they have already developed strategies based on water quality objectives which they consider superior. It is an inevitable consequence of this that *some* waters 'will not meet EC standards'; that is the whole point. Most waters, as the monitoring data show, are much better than EC standards. However, those states which have elaborate monitoring systems in place can manage through water quality objectives, whereas most member states do not have the necessary information, though they may do if and when they begin to comply with the draft ecological directive referred to above! However, it must be pointed out that the moves in Europe towards water quality objectives are completely at variance with moves which are also under consideration to move towards emission controls based on BATNEEC (see above). The two approaches are fundamentally different, and it may be of interest to study the experience of the United States, where after two decades of technology-driven emission controls which produced little improvement, increasing emphasis is being placed on the development of water quality standards and water quality objectives.

### *6.3.3 Water Pollution Law in the USA*

The United States has a different legal and constitutional tradition from Europe. The primacy of Federal law over state law, and the powers of Federal agencies over state or local agencies, are much more clearly defined than in Europe; and the Constitution guarantees certain rights and is frequently invoked. Despite the differences in legal mechanisms, however, there are certain parallels in the legal position regarding water pollution. A summary of US law relating to water pollution is given by Anderson *et al.* (1990) and Wilcher (1993).

Historically, the responsibility for the control of pollution has passed gradually from local to state authorities, and now resides ultimately with a Federal agency, the Environmental Protection Agency, which was established in 1970. The key items of legislation are the Federal Water Pollution Control Act 1972, amended in 1977; and the Clean Water Act 1987. Federal policy evolved through three stages. The first was to confine itself to *post hoc* action against polluters, usually through prosecution under Federal law. The second stage was to involve State governments by requiring them to identify waters which could be used to assimilate different levels of waste, and to plan water management strategies accordingly ('water use zoning'); federal action would only be taken as a last resort. Finally, a concerted national effort was put in place to control pollution by 'end-of-pipe' controls based on the best available technology, with the State governments acting as agents of Federal policy. Throughout this time, little or no attention was paid at Federal level to the question of receiving water quality standards; emission controls based on best available technology was, in effect, the only strategy employed.

Certain States, however, were beginning to realise the drawbacks of this approach and began to develop strategies based upon water quality standards and a rudimentary form of water quality objectives; however without Federal

recognition, their progress was limited. There was no incentive to improve, or even monitor, receiving water quality under this system; permission to discharge would be granted as long as the polluter had fitted the right technology to his outfall pipe. This failure to consider receiving water quality standards meant that while conventional point sources of pollution, such as sewage discharges and discharges of unexceptional industrial discharges were tolerably catered for, no policy was formulated or could be legally enforced relating to toxic substances, or to discharges from non-point sources. Eventually the Natural Resources Defense Council brought a lawsuit against the Environmental Protection Agency, alleging that the EPA had failed, through over-reliance on an inadequate policy, to carry out its responsibilities. As a result, the EPA was required to regulate the discharge of 65 categories of toxic pollutant, and began to develop a control strategy for toxic pollutants. The 1977 amendments to the Federal Water Pollution Control Act, and the 1987 Clean Water Act, were closely related to this sequence of events.

US commentators regard as highly significant the provision under the Clean Water Act 1987 for 'citizens' suits' (Wilcher, 1993). Under the Clean Water Act, any citizen of standing may bring a lawsuit against any person guilty of a violation of water pollution law. Citizens have access to relevant data under the Freedom of Information Act, and the term 'of standing' merely means any citizen who has been affected in any way by the violation. (Interestingly, until 1973 under English common law a similar provision existed, but it was abolished by the Water Act 1973.) Suits may be brought not only against polluters, but also against regulatory agencies who do not fulfil their statutory obligations.

Thus the legal situation in the USA is also in a rapidly-changing situation, though for different reasons from those which apply in Europe. In effect, the legislation and corresponding Federal policy since 1972 has largely failed. Parr (1994) briefly cites some data showing the extent of contamination of US watercourses. At present, the pollution control strategy is broadly as follows. The EPA remains the ultimate regulatory agency, and its main policy plank is the National Pollutant Discharge Elimination System (NPDES). Any person or organisation wishing to discharge waste to water must apply for an NPDES permit. The permit specifies discharge limits, but these are still heavily based upon best available technology. However, states are now encouraged by the EPA to make local determinations of receiving water quality standards, and if best available technology requirements do not meet receiving water quality standards, more stringent requirements may be incorporated into the permit. The EPA has now developed criteria for toxic pollutants, including 108 which are hazardous to human health and 27 which threaten aquatic life. The EPA is now requiring states to develop biological criteria for the determination of water quality standards; it always had this power under the Clean Water Act, but did not use it until recently. Thus the USA seems to be moving towards a system of biological monitoring leading to a water quality objective-based approach, and perhaps will end up with a system similar to that which is evolving in Europe, though by

a different route. The individual states may determine quality standards according to local conditions, meeting broad objectives set and enforced by the Federal agency. Already nearly half the states are using some kind of biological assessment, and some have begun to develop a water quality classification scheme (Parr, 1994).

So far, the discussion has applied only to identifiable, point-source discharges. Under the earlier policy of EPA, virtually nothing could be done about diffuse sources, mainly of agricultural origin. Currently, the EPA consults with state and local governments to develop local plans based upon good management practices. State schemes which are approved by the EPA are eligible for financial assistance from Federal sources.

The US experience perhaps offers a salutary lesson in the non-cost-effectiveness of emission standards alone as a pollution control strategy, particularly those based on technology-driven criteria. The Federal government recognised that best available technology controls were expensive, and undertook a scheme of financial assistance to publicly-operated treatment works (POTW). Between 1972 and 1988, despite all the evidence that this particular policy was having no effect, the total cost of the POTW programme from Federal and State funds was 58 billion dollars; this does not, of course, include costs to private industry. There can be no clearer illustration of the need for scientists to give accurate advice and information, and for legislators to resist the pressures to be seen to be taking action until they fully understand what action they need to take.

# 7

# Estuarine and Marine Pollution

The effects of water pollution, and their consequences for public health, became obvious in lakes and rivers long before any general threat to estuaries and seas was widely perceived. Consequently the methodology and literature relating to the study of estuarine and marine pollution are less well developed, and many of the basic approaches to the study of pollution in maritime habitats are derived from ideas developed for freshwater habitats. Certainly, the answers sought from the study of freshwater, marine and estuarine habitats are essentially the same; nevertheless there are significant physical, chemical and biological differences between freshwater and maritime environments. Because of these differences, the technical and conceptual approach to the study of pollution in maritime habitats must be different, at least in emphasis, to that which is appropriate to the study of fresh waters. This chapter introduces some of the special problems of pollution which occur in estuaries and in the sea, and considers the extent to which the concepts and methodology which are appropriate for fresh waters can be applied to the study of pollution in maritime habitats. A good general introduction to the problems of marine pollution is given by Clark (1992), and more detailed reviews of some important aspects may be found in Kinne (1984a, b) and Preston (1989).

During the present century social, economic and industrial trends have combined to increase the threat of pollution to estuaries and coastal seas. Most of the world's major cities are now situated on estuaries or close to the coast. Increasingly, large industrial sites are required to be placed away from centres of population, but close to sources of water for cooling and waste disposal, and with access to transportation facilities (e.g. by ships of steadily-increasing size) for the import and export of raw materials and of finished products. Thus major industrial development increasingly takes place in the lower reaches of estuaries

or at coastal sites, often on reclaimed land. In many parts of the world, the availability of relatively cheap international travel has led to the development of an economically-important tourist industry. This has increased the pressures of urbanisation upon coastal areas especially; but at the same time it has created strong economic, aesthetic and public health pressures in favour of uncontaminated beaches and coastal seas. Additionally, it has become necessary to consider whether the seas themselves, in spite of their vastness, can accommodate the wastes of which they are the ultimate recipient.

## 7.1 Pollution of Estuaries

Estuaries vary so widely in their characteristics that it is difficult to define them satisfactorily, but Barnes' (1984) definition is a very useful one: 'An estuary is a region containing a volume of water of mixed origin derived partly from a discharging river system and partly from the adjacent sea; the region usually being partially enclosed by a land mass'. In order to understand the effects of pollution in estuaries, it is first necessary to consider some of their special characteristics. Concise descriptions which emphasise those characteristics of estuaries which are particularly relevant to their response to pollution include those of Barnes and Green (1972), Perkins (1974), Arthur (1975) and Barnes (1984).

Barnes (1984) recognises four main types of estuary, based on their geomorphological characters:

1 Drowned river valleys (coastal plain estuaries), formed by the rise in sea level which occurred at the end of the last glaciation, about 10000 years ago.
2 Tectonically-produced estuaries, formed by the subsidence of land and consequent invasion by the sea.
3 Fjords, glacially-overdeepened valleys into which the sea penetrates. These frequently have a sill at their mouth which greatly restricts the interchange of water between the estuary and the open sea, so that the volume of water lying within the fjord but below the level of the sill is effectively isolated, and behaves rather like a lake.
4 Bar-built estuaries, formed by the deposition of sand or shingle in a line parallel to the shore which blocks or diverts a discharging river system, forming an estuary in a former area of sea between the land and the sand or shingle bar.

The precise physical characteristics of an estuary greatly influence the biological processes which occur within the estuary, to the extent that it is difficult to generalise about the biological properties of estuaries and the way in which pollution affects them. In some estuaries, for example, the volume

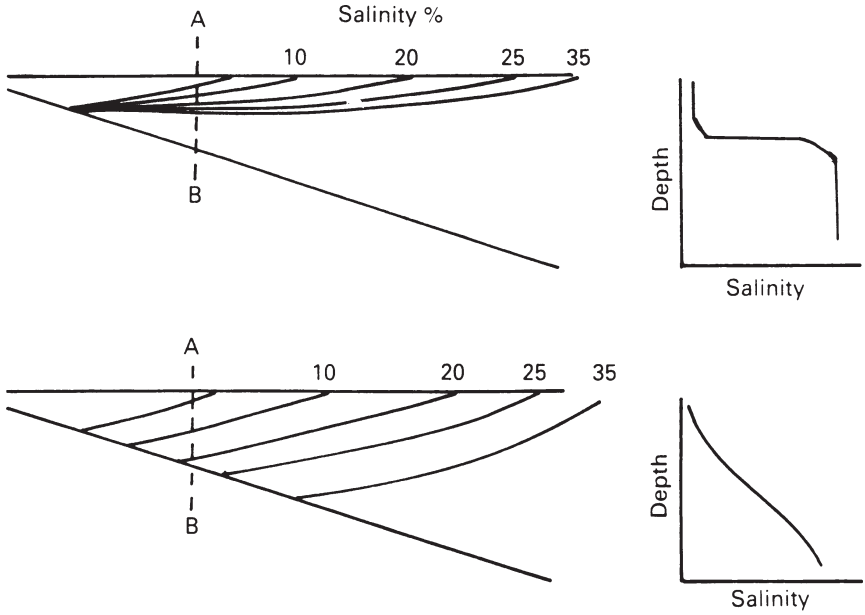


**Figure 7.1** Horizontal and vertical salinity profiles of two estuaries. In the upper diagram, the position of the isohalines indicates little vertical mixing, the fresh water tending to flow out above a salt-water intrusion (salt-wedge). There is a sharp discontinuity in the vertical salinity profile through the section A–B. The lower diagram represents an estuary where vertical mixing is more pronounced

of fresh water discharged may greatly exceed the amount of sea water intrusion; in others, the reverse is the case. The ratio of fresh water to sea water, their patterns of mixing and the consequent influences upon the biological characteristics of the estuary depend ultimately upon the shape and size of the estuary, the magnitude of the tidal influences, and the geomorphology and geochemistry of the area. This discussion attempts to describe some relevant aspects of representative coastal plain estuaries (the most common type).

The precise pattern of mixing of fresh water and sea water in estuaries varies greatly, depending upon geomorphological factors. However, all estuaries display both a longitudinal and a vertical gradient of salinity (Figure 7.1). Further, the salinity of the water at a given point in the estuary tends to vary greatly from hour to hour, according to the state of the tide. Since most living organisms do not tolerate rapid and wide fluctuations in the salinity of their environment, the number of species which can live under estuarine conditions is restricted by comparison with the biota of the open sea, or of fresh water. The majority of species found in estuaries are either freshwater organisms which penetrate the estuary downwards, and which become progressively less

numerous as they reach their limits of tolerance; or marine organisms which, in similar fashion, become numerically depleted as they penetrate further upstream. Physico-chemical processes within the estuary also produce conditions which limit the biota. By the time a river reaches the sea, most of its load of suspended matter has been deposited, and only the finest particulate matter remains in suspension. The mixing of fresh water with sea water involves a marked increase in the pH and in the level of dissolved salts; these promote the coagulation of fine particulate matter, and of organic and inorganic colloidal matter (Phillips, 1972). Since, during each tidal cycle, the water mass is sometimes moving in one direction and sometimes in the opposite direction, it follows that at every point in the estuary at some time the net water velocity is zero. These processes encourage the deposition of fine organic and inorganic matter on the bed of the estuary. Since most estuaries are also sheltered from wind and wave action, particulate matter brought into the estuary with the incoming sea water also tends to be deposited within the estuary. The bed of an estuary therefore typically consists of fine silt with a high organic content which many animals will not tolerate, particularly since estuarine muds will tend to be hypoxic owing to the high biological oxygen demand (BOD) (see Section 2.3) imposed by its organic content.

Plants, whether macrophytic or planktonic, will also be limited because the high turbidity characteristic of estuarine waters inhibits photosynthesis. In addition, it appears that few animals and plants have evolved specifically to estuarine conditions. One reason for this may be that, geologically speaking, estuaries are short-lived features; fluctuations in sea level and the corresponding changes in the position of the coastline mean that an estuary rarely occupies the same position for more than a few tens of thousands of years. The estuarine biota is derived from the freshwater and marine organisms which penetrate the estuary from either end. At the seaward end, the biota is similar to that of the adjacent sea, but species die out rapidly with increasing distance from the sea as they reach their limits of tolerance to decreasing salinity. A similar process occurs to limit the penetration of freshwater species from the landward end. These factors combine to create the typical situation whereby the estuary has a varied biota at its seaward and landward extremes, but species diversity is characteristically low in the middle reaches. Nevertheless, the handful of species which can survive in the estuary proper are often found in great abundance. The enormous numbers of sea birds which congregate in estuaries at low tide, to feed upon the abundance of invertebrate life, are readily observable. Similarly, estuaries are important sources of food for fish which visit at high tide. The export of biomass from estuaries is almost certainly very important in sustaining populations of ecologically and commercially important species which are not themselves actual residents of the estuary. In addition, many estuaries yield large quantities of shellfish of high commercial value, either from exploitation of natural populations or by means of aquaculture, as well as being crucial in the life cycle of migratory fish.

Given these natural characteristics, it is not difficult to recognise that the principal pollution threat in estuaries is likely to arise from deoxygenation of the water column and sediments due to the accumulation of organic matter, and the accumulation of toxic materials in the sediments. Many polluted estuaries have been studied in detail, and in general this has been found to be the case. In Britain, several of the largest and most industrialised estuaries have been under investigation for about 60 years (e.g. Department of Scientific and Industrial Research, 1935, 1964; Porter, 1973; Royal Commission on Environmental Pollution, 1972). In some of them pollution control measures applied in recent years have led to dramatic improvements, and although there remain some grossly-polluted estuaries, most have recovered to a good quality (NRA, 1991). While the precise situation varies in detail from one estuary to another (and these differences in detail are very significant in the design and implementation of appropriate pollution control strategies), some important general features emerge from these and similar studies.

First, it is clear that the long-held assumption that polluting matter is rapidly flushed from an estuary is wrong. Flushing times depend upon the topography of the estuary, the amount of fresh water input and the degree of mixing which occurs between the sea water and the fresh water. However, in most cases flushing times are relatively long, and in some cases essentially the same body of water moves up and down the estuary, according to the tidal cycle, for days or even weeks on end. In addition, indentures and embayments of the estuary shoreline can entrap polluting material more or less permanently. Partial deoxygenation of the water and sediments is a normal phenomenon of many unpolluted estuaries, but in polluted estuaries extensive sections become completely anoxic, either at regular intervals or more or less permanently (Figure 7.2), and are consequently devoid of higher forms of life. The accumulation of toxic matter in the sediments undoubtedly contributes to the elimination of species. Valuable sport and commercial fisheries are eliminated. The predominance of anaerobic microorganisms, producing hydrogen sulphide and methane as by-products of their respiration, is both inimical to the normal estuarine biota and aesthetically unpleasant. Combined with the obvious presence of tar balls, faecal matter, vegetable and domestic wastes and sewer scum, this effect destroys the recreational and commercial potential of the estuarine shores.

The estuary of the River Tees in northern England provides a salutary, but unfortunately by no means unique, example. In the early years of the present century the river supported migratory (salmonid) fish which were exploited commercially and for sport. Above the estuary even today the river is very little polluted, but industrial development of the estuary (Porter, 1973) including substantial petrochemical, agrochemical and steel-making complexes together with dramatic population increases, eliminated the fishery by 1937 (Perkins, 1974). A major survey of the estuary (Department of Scientific and Industrial Research, 1935) recorded the virtual absence of life

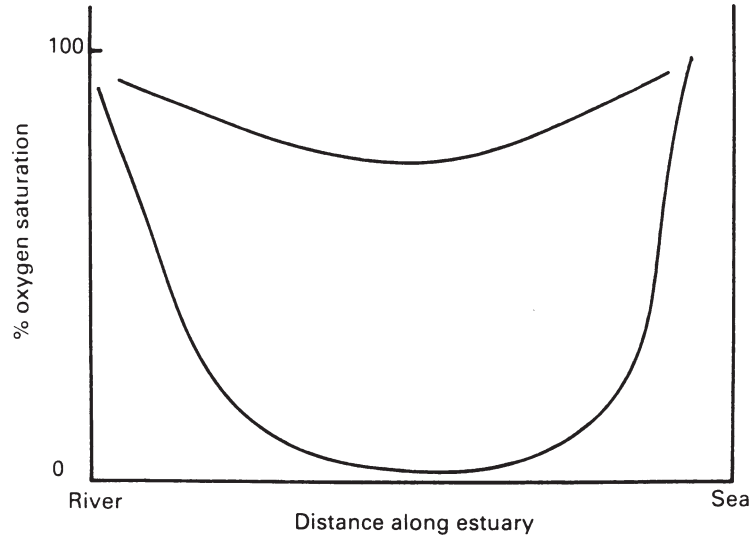


**Figure 7.2** Oxygen sag curves in (upper line) an unpolluted estuary and (lower line) a heavily-polluted estuary

from the most polluted reach, extending up to 6km in the central part of the estuary. Severe deoxygenation of the water was a more or less permanent feature, and on occasions the estuary water was rapidly lethal to fish. A more recent survey carried out in 1967 showed that in many respects the position had actually worsened (Porter, 1973). Fortunately here and in some other British estuaries, remedial action is now being implemented, with considerable success but at great cost. A good example of what can be achieved is the Thames. Wood (1982) described the decline of this major estuary from about 1800 to 1950, and the subsequent efforts to restore its water quality, a process which has continued to the present day.

## 7.2 Pollution of the Seas

Through the direct discharge of wastes to the sea, the discharge of polluted rivers into the sea, from land runoff and atmospheric fallout, the seas are the ultimate recipient of most pollution in most of its various forms. It is now very obvious that extensive areas of sea, particularly in coastal areas, are very seriously affected. What is not clear, however, is the extent to which these are purely local problems which are amenable to local solutions, such as improved waste treatment or the more effective dispersal of wastes over a wider area; or alternatively, the extent to which the severe and obvious local disturbances have more subtle adverse consequences further afield, possibly involving alterations to the entire marine ecosystem. It is virtually impossible to assess the ecological significance of the

substantial quantities of some potentially dangerous pollutants which enter the seas through atmospheric fallout. Except in the case of the most severe incidents of pollution, it is very difficult clearly to distinguish the effects of pollution from the natural fluctuations and variations which are known to occur in marine populations and communities. This is true even of communities and species which have been extensively studied on account of their ecological or commercial importance. Nevertheless the quantity and variety of polluting materials which now enter the seas are so great that the long-standing assumption that the seas are sufficiently vast to act as a waste sink of infinite capacity must be questioned. To determine the extent to which the sea's capacity to absorb waste matter is being approached or exceeded is the central question facing biologists in the study of marine pollution. A brief discussion of some of the commoner forms of marine pollution will illustrate the difficulties.

### *7.2.1 Oil Pollution*

The public perception of oil pollution as a major threat to the sea has been distorted by the occurrence of a relatively small number of major accidents (shipwrecks, oil-well blowouts), by the enormous tonnage of oil transported around the world, by the vigorous and expensive efforts made to clear oil spills (more often for aesthetic than ecological reasons) from beaches, and by the fact that sea birds, in substantial numbers, are often the most conspicuous casualties of major spills.

Detailed reviews of the scientific evidence, such as those given by Johnston (1984) and in the volume edited by Clark (1982a), afford a more realistic perspective. First, only about one-third of all the oil which enters the sea does so as a result of activities associated with oil transportation, and of that only about one-quarter is due to accidents and major spillages; the remainder is accounted for by normal operational losses. About 45% of the oil which reaches the sea does so from polluted rivers, urban runoff, municipal wastes and effluents from non-petroleum industries. Up to 20% of the total enters the sea from natural oil seeps, that is locations where oil naturally escapes from under the sea bed. Finally, oil is produced, ultimately, by living organisms, and hydrocarbon production by contemporary populations of marine phytoplankton almost certainly far outweighs anthropogenic inputs of hydrocarbons to the sea. On the basis of figures cited by Davenport (1982), hydrocarbon production by phytoplankton is at least 30 times greater than the anthropogenic input. Johnston (1984) estimates that five million tons of oil enter the sea annually from anthropogenic sources, compared to a total production of biogenic hydrocarbons of at least $10^{17}$ tons annually.

Experience of major oil spillages such as that caused by the wreck of the *Torrey Canyon* in 1967 (Smith, 1968; Southward and Southward, 1978) and the *Amoco Cadiz* in 1979 (Conan, 1982) has shown that the principal danger is damage to the littoral and sublittoral communities when the oil is washed ashore. Even large oil

slicks on the open seas readily disperse, a process which may be aided by the use of oil dispersants or other means. The more volatile fractions evaporate, and the heavier fractions eventually sink and are degraded by microbial action. Commonly, substantial amounts of heavier material form tar balls, usually a few millimetres in diameter, which are of more or less neutral buoyancy and float around in the water for long periods. There are few regions of the world where tar balls are not found, and in some areas, such as the Mediterranean, they are washed up on beaches where they form a considerable nuisance. Whether tar balls or sunken oil have any serious biological consequences is not known. Since oil is degraded by microorganisms, it has been suggested that oil could have a eutrophicating effect similar to that which may occur when other forms of organic material are discharged to water. Oil which is washed ashore after major spillages initially causes dramatic damage in the littoral and sublittoral zones, but in most of the major spills which have occurred in the last 20 years it has been found that recolonisation and restoration of the affected areas occur spontaneously and quite rapidly, within three or four years in high-energy environments (rocky, wave-washed areas) and over a rather longer period in more sheltered shores. Wave action has an important effect in physically breaking up and dispersing the oil; in sheltered areas, oil may initially be simply buried with sand and may be subsequently re-released during rough weather, so the time required for complete recovery may be ten or more years.

Although polluted shores recover fairly quickly and spontaneously, the community which is re-established may differ in some ways from that which was destroyed. More recent spills have shown that improved techniques of response to and management of the accident, particularly in the early hours after the accident, are most important in minimising environmental damage. The 1989 accident, in Alaska, to the *Exxon Valdes* gave rise to great concern, partly because it was the first major accident in Arctic waters, and partly because there was a degree of delay and confusion in the initial response which led to increased damage to the environment. However, in 1993 the spill in the Shetland Islands, UK, from the *Braer* was rapidly contained and the Government committee charged with investigating the incident was able to conclude within two years that 'the impact of the spill on the environment and ecology of South Shetland has been minimal' (ESGOSS, 1994).

The worst case to date of oil spillage into the marine environment occurred as a result of the Gulf War in 1991. Following the invasion of Kuwait by Iraqi forces in late 1990, an international force acting under United Nations authority forced the Iraqi troops to withdraw early in 1992. During their retreat, the invaders destroyed hundreds of oil wells and deliberately discharged massive quantities of oil into the Persian Gulf. Since that time, an enormous international effort has been made to study the effects of this spillage and to remediate the environmental damage. A preliminary account of these studies is given, for example, in the volume edited by

Price and Robinson (1993). Once again, it appears that initial estimates of the extent of environmental damage were probably over-pessimistic; however, the studies will undoubtedly continue for some time and may in future add a great deal to the understanding of the effects of major oil spills.

It is now generally agreed that a large part of the damage associated with oil spillages is in fact attributable to the toxicity of the oil dispersant materials which are often applied as an emergency measure. The newer oil dispersants are much less toxic than those in use formerly, but it is now widely accepted that they should be used cautiously, if at all, in coastal waters. They represent an additional toxic hazard and are usually applied for aesthetic rather than ecological reasons, for example to accelerate the removal of the oil from recreational beaches in tourist areas. Probably the best strategy is to sink the oil at sea before it reaches the shore. Surprisingly little research has been done in areas subject to natural oil seepage from under the sea bed, but the available data indicate that in these areas the effects of the oil are readily detectable in the immediate locality of the discharge but do not spread very far. In zones of intermediate oil concentration, the microbial breakdown of oil may cause an enriching effect leading to increased productivity, if not an actual increase in species diversity.

Chronic oil pollution of coastal areas, in harbours and around oil installations and refineries, is much more significant, in terms of the total area affected, than the more dramatic major accidents. The effects of chronic pollution are readily detectable in such locations, but experience has shown that while some effects may be long-lasting, rapid improvements take place following the installation or upgrading of appropriate pollution control facilities. The effects of offshore oil installations, if they are properly managed, are confined to the immediate vicinity of the installations. There is little evidence that oil pollution in the open seas associated with normal shipping operations has any effects other than purely local ones. Similarly, there is no evidence that oil pollution of any kind has far-reaching consequences for ecologically or commercially important species or communities (Clark, 1982a). Sea bird deaths in large numbers are conspicuously related to oil pollution, but while this may be distressing for some people the numbers involved are actually small in relation to natural mortality rates. In any case, adult mortality rates are not, for sea bird populations, generally the principal factor governing population density, and no sea bird population is known to be seriously threatened by oil. No evidence has been found to suggest that fish stocks, except in localised areas, are adversely influenced by oil pollution (or, indeed, pollution of any other kind in the sea). Planktonic organisms are, like polluting oil, mobile and patchily distributed; although they are sensitive to oil, damage again appears to be confined in time and space to the immediate vicinity of the discharge.

It is reasonable to conclude that some of the more extravagant fears about oil pollution of the sea are probably unfounded. Nevertheless oil is a serious pollution threat. Although the problems may be localised, there are many large areas detectably affected. Some habitats of particular ecological importance, such as mangrove swamps, mud flats, coastal bays and estuaries, are permanently at risk from accidents. The potential does exist for a local problem to have far-reaching consequences; for example, a major spill occurring in the nursery grounds of a fish population, if it occurred at an appropriate time, could have major ecological and economic implications. Finally, the view that oil pollution is a matter for local rather than global concern may simply reflect the fact that the techniques and knowledge currently available are not sufficient to allow the detection of potentially important but subtle effects. The threat of oil pollution therefore fully justifies both further research and the continuing efforts to control and abate it.

### *7.2.2 Sewage and Domestic Wastes*

Coastal towns and cities all over the world discharge sewage, often completely untreated, into the sea. In addition, substantial quantities of sewage enter the sea from grossly-polluted rivers. It is now widely recognised that this has created significant ecological damage and measurable public health risks, particularly in the many locations where sewage is discharged at the shoreline or through short outfalls perhaps only 100 or 200 metres in length. The health hazards arise mainly from the high numbers of pathogenic organisms which may be found in the water and on the beaches, presenting a danger to bathers and recreational users of the beach, and from the accumulation of pathogens in fish and shellfish which may be used for human consumption. Reish (1984) provides a detailed review of the effects of sewage discharges in marine environments.

The effects of sewage in the marine environment are, in principle, exactly the same as the effects in fresh water, and arise through the same mechanisms (see Section 2.3). The extent to which the adverse consequences manifest themselves seems to depend greatly on local conditions. In some cases, apparently large discharges appear to be accommodated without serious disturbance to the ecosystem, and the nutrient-enriching effect may even be considered beneficial. In other cases, severe disturbance of the benthic communities, even to complete elimination, has been recorded. In addition, problems similar to those associated with extreme eutrophication in lakes occur, including deoxygenation of the hypolimnion and sea bed, and the creation of algal blooms. Many of the algal blooms are associated with the production of algal toxins which are damaging to fish and other species of both ecological and commercial significance. As in fresh water, it is possible to rank species of benthic invertebrates in approximate order of susceptibility to organic pollution. The most severely polluted zones contain few species which are apparently tolerant of pollution, and species diversity tends

to increase in a fairly predictable fashion as the intensity of pollution decreases. Thus the effects of sewage pollution on the marine benthic community can be monitored in a similar way to the effects in fresh water, at least in some localities. Bellan (1970) described a zonation of benthic species in the vicinity of sewage discharges into the sea off Marseilles, a pattern which has been observed, with some variations, in other locations (Perkins, 1974, 1979). However, in a number of locations where such a pattern may be expected, by reason of known substantial sewage discharges, it is not apparent. This may be due to local conditions of tides and currents which affect sediment movements and the dispersion of the pollution, but until more data are available it is difficult to decide whether the absence of the expected zonation indicates that the pollution is exerting little effect, or whether the available techniques of monitoring are insufficiently sensitive to allow significant effects to be detected. For various reasons, techniques of biological monitoring are less well developed in the marine environment than in fresh water (see Section 7.2.3).

Whether or not sewage discharges exert serious effects on the receiving environment therefore appears to depend upon the extent to which satisfactory dispersion can be achieved through the design and location of the outfall in relation to the local conditions of tides, winds and currents. Determining the optimum strategy for marine sewage disposal requires a detailed knowledge of local conditions, and probably each case needs individual consideration. Frequently, improvements to the local environment can be achieved by constructing a sufficiently long outfall of suitable design and in a favourable location. In many circumstances, however, this is not technically or economically feasible and actual treatment of the sewage is required. In Britain, a common solution is to give sewage primary and sometimes secondary treatment, discharging the liquid effluent through a relatively short outfall (sufficiently long to avoid public nuisance, for example) and dumping the sludge from specially-constructed ships further out to sea. The extent to which this practice is acceptable is currently controversial; the British government, under pressure from neighbouring states, has agreed to end this practice by 1997. There is greater agreement, however, that sewage pollution of the sea is a very serious problem in many areas of the world, and that the waste disposal facilities of coastal towns and cities are in urgent need of upgrading. In Europe, for example, at least three substantial sea areas, totalling up to one million square kilometres in area, are thought to be in imminent danger. These are the northern Adriatic, the Baltic and parts of the eastern North Sea. In these seas, periodic deoxygenation of the sediments and water column have been recorded, often associated with heavy losses of marine life. In addition, excessive plant growth and toxic phytoplankton blooms are increasingly frequent occurrences, and the potential for serious ecological damage and economic loss affecting wide areas is now recognised.

Clark (1992) provides some brief case studies of seas in other parts of the world apparently under threat for similar reasons.

The Mediterranean sea provides a good example of a sea under serious threat although the extent of the threat is still not fully quantified. The sea is more or less enclosed, and its freshwater input is less than the rate of water evaporation, so its salinity is in places measurably higher than most seas. The loss of water by evaporation is replaced by an influx of cold water from the Atlantic ocean via the Straits of Gibraltar. This influx displaces a volume of warmer water which flows out at the surface in the opposite direction. There is no significant interchange with the waters of the Black Sea, and it is estimated that the volume of the Mediterranean is replaced only once every 80 years. The sea supports a substantial number of species—probably several hundred—which are not found elsewhere, and there is evidence of colonisation of non-indigenous species from the Indian Ocean via the Suez Canal, whose ecological impact is not clear. Most of the population of its 18 coastal states live in the narrow coastal strip, and depend heavily upon the sea, directly or indirectly, for their livelihoods. Some of these coastal states are advanced, industrialised countries, others are undergoing rapid development and some are among the poorest countries in the world. At least two states, Libya and Algeria, are substantial producers of oil and gas, and the search for exploitable minerals from the Mediterranean is well advanced. The indigenous population of about 100 million people is supplemented by about 30 million tourists per year who visit various parts, mostly in coastal areas. About 120 cities discharge sewage and industrial wastes into the sea, about 80% of which is untreated or treated to a poor standard. Identifiable ecological damage and/or health hazards affect about 25% of the coastline, and in many areas the coastline is over 90% developed or urbanised. Table 7.1 lists UNEP's estimate of the quantities of some common pollutants discharged annually to the Mediterranean. To this must be added up to 25% of the annual input of petroleum hydrocarbons into the world's seas, as the Mediterranean is one of the world's busiest shipping areas; this excludes inputs from major accidents (UNEP, 1985, 1989b).

In an attempt to evaluate and alleviate some of these actual and potential problems, UNEP established the Mediterranean Action Plan. This and other examples of international efforts to regulate marine pollution are discussed in Section 7.4.

### *7.2.3 Toxic Pollutants*

Of the many toxic pollutants which enter the sea, probably the greatest concern is due to the conservative pollutants, such as heavy metals and refractory organic substances like certain pesticides, polychlorinated biphenyl compounds and similar substances. In coastal areas all over the world, the discharge of toxic wastes causes readily-identifiable ecological damage in

**Table 7.1** Estimates of the quantities of pollutants entering the Mediterranean Sea annually from anthropogenic sources. Data from UNEP (1985)

| Pollutant | Quantity (tons) |
|---|---|
| Mineral oils | 120 000 |
| Phenols | 12 000 |
| Synthetic detergents | 60 000 |
| Mercury | 100 |
| Lead | 3 800 |
| Chromium | 2 400 |
| Zinc | 21 000 |
| Phosphorus | 320 000 |
| Nitrogen | 800 000 |

localised areas. In at least one well-documented case, Minamata Bay in Japan, widespread and serious human illness was caused by consumption of contaminated seafood (Tsubaki and Irukayama, 1977). There is an enormous literature on the effects of toxic pollutants such as metals (Bryan, 1984) and pesticides (Ernst, 1984) in the sea, but perhaps the most intractable problem is to discover whether their effects are confined to the localities in which they are released, or whether more widespread damage is occurring. Conservative pollutants have, at least in theory, the capacity to accumulate in sediments, and in the tissues of living organisms, until they reach concentrations which may be harmful; such effects may not become obvious until many years after the pollutants have been discharged at what may initially appear to be a 'safe' rate.

In the case of heavy metals such as lead, mercury and cadmium which have no known biological function, inputs to the sea from anthropogenic sources approach or exceed the natural rates of input due to weathering of rocks. Many refractory organic substances are completely synthetic and in the absence of anthropogenic inputs would not be found in the sea at all. This suggests that study of the biogeochemical cycles of these substances should be a useful approach. If, for example, it could be shown that the levels of mercury in sea water, sediments or living organisms were steadily rising, it could be argued that existing controls on mercury emissions are inadequate and that contamination is more than simply a local problem. However, in practice great uncertainty exists about some basic aspects of the biogeochemistry of mercury and other metals. Aston *et al.* (1986) point out that estimates of the mercury concentrations found in open sea water have actually been lowered by about one order of magnitude over the last two decades. This has happened because the levels being measured are at or close to the detection limits of the analytical procedures. As measurement techniques improved, the problems of contamination during the taking and processing of the samples became more apparent, and as increased precautions

against contamination were employed, the levels of mercury recorded have actually declined. This casts considerable doubt on the validity of most of the results available, even if obtained by the very best available techniques; in effect, there is no realistic baseline with which to compare the values currently being recorded.

An alternative approach is to measure the concentrations of toxic substances in sediments and living organisms, and a large volume of information is being accumulated. Collation of this information into a comprehensive bank of baseline data presents formidable problems of quality control, and difficulties of comparison caused by differences in methodology, but will undoubtedly be useful in the future. Such studies do frequently indicate, however, the existence of 'hot spots', or areas of apparently elevated concentrations of toxic matter, usually associated with urban or industrial pollution. The extent to which these elevated concentrations are harmful can only be assessed by toxicological studies. Bryan (1979) drew attention to some of the difficulties of interpreting data on the measured tissue levels of pollutants in marine organisms (see also Section 4.4.3). As with many other forms of toxicological investigation, the large differences between the best available experimental techniques and the reality of the field are particularly problematical when studying marine pollution. In laboratory experiments, uptake of pollutant occurs largely from solution. In the sea, animals almost certainly obtain most of their body burden of pollutants by ingestion of contaminated food or sediment. It is well known that the route of entry of a poison greatly influences the extent of its toxic effect. Further, some species appear to be able to immobilise and store pollutants within their bodies, and some appear to have developed a measurable degree of tolerance through genetic and/or acclimation mechanisms. Therefore the existence of a given level of a pollutant in the tissues does not necessarily indicate that it is intrinsically harmful.

In earlier chapters the principles of toxicological investigation and their applications to freshwater pollution were discussed. In fresh water, it frequently occurs that the concentrations of pollutants actually found in the environment are close to those which can be reliably maintained in controlled laboratory experiments. The results of toxicological studies can, cautiously, be applied successfully to many field situations. In the sea, except for the grossest and most localised forms of pollution, the difference between the concentrations found in the environment and those which can be maintained accurately in controlled experiments is much wider, frequently several orders of magnitude. This, together with the generally inadequate understanding of the effects of the marine environment on the chemical form and speciations of toxic substances (Burton, 1979), casts great doubt on the usefulness of conventional toxicological approaches when applied to marine organisms. Some alternative approaches are discussed in the volume edited by Cole (1979), and developed further by Abel and Axiak (1991).

Abel and Axiak (1991) argue that the whole question of assessing the impact of toxic pollutants in the marine environment requires a radically different toxicological approach. In fresh waters, it is both feasible and demonstrably successful to determine water quality standards (for example, in the form of maximum acceptable concentrations) and to devise emission standards based upon these. In the sea, it is not possible to determine a water quality standard, for the reasons outlined above. Even if it were, it would be impossible to regulate emissions accordingly. In practice, at least for the foreseeable future, the optimum pollution control strategy would appear to be one based upon some form of hazard assessment linked to strict emission controls, possibly technologically-based ones. In other words, the pollution control strategy best suited to the marine environment is very different from that most suited to fresh waters which was described in Chapter 6. This in turn means that the nature of the toxicological information we need for the protection of the marine environment is rather different from that which has historically been found useful in fresh waters. We do not wish to know the maximum 'safe' level of a pollutant in the sea; we wish to know whether a given level of contamination which we can detect is or is not exerting any adverse effect. Therefore, except in certain special circumstances, the kinds of toxicological investigation which have been found useful in fresh waters have little place when our concern is the assessment of toxic effect in marine environments. This probably explains why many of the more recent biochemical, physiological and genotoxic approaches have been largely developed by marine rather than freshwater scientists.

A promising application of biochemical techniques in assessing the response of the mussel *Mytilus edulis* to pollution was described by Bayne *et al.* (1979). Measurements of some biochemical parameters in animals from different natural populations were used to derive a model to allow prediction of growth rates. The predictions agreed well with the observed growth rates of animals in the field. Animals were transplanted from their natural environment to polluted locations, and subsequent measurements of the biochemical indicators showed that they had responded to the polluted conditions in a way which was detectable. These responses could be related to fecundity. Depending upon the degree of specificity and sensitivity which can be obtained, techniques of this sort probably represent a more directly useful approach to problems of measuring toxicity than those which have been found appropriate in fresh waters. Stebbing (1979) argued that instead of carrying out toxicity tests and seeking to compare the results with the environmental concentrations, it may be more useful in the marine situation to use suitably sensitive organisms to bioassay the sea water; subsequent treatment of the sea water to remove pollutants can, if the response of the test organism is abolished, then be used to indicate which pollutants are exerting a measurable biological effect.

Stebbing (1979) reported some success with this approach, using the growth and development of the colonial hydroid *Campanularia flexuosa*. This organism appears to be sufficiently sensitive to allow the bioassay of polluted sea water,

and has the advantage that variation due to genetic variability can be excluded by the use of a single clone. (American readers may be confused by the distinction between the terms 'toxicity test' and 'bioassay', since many American authors have in recent years begun to use the two terms synonymously. The difference between the terms is important as the present example indicates, and has been clearly explained by Brown (1973). It is unfortunate that the distinction in meaning between these terms which was universally accepted for decades has become blurred.) Further examples of toxicological techniques found useful in assessing the impact of pollutants in the sea are discussed in Section 4.3, also in the volume edited by Abel and Axiak (1991), and in a short review by Gray (1992).

To the extent that studying the toxicity of pollutants to individual marine organisms is of limited value, the study of toxic effects at the population or community level of organisation is of even greater importance in the sea than in fresh waters. Several attempts have been made using artificial or semi-natural ecosystems (Davies and Gamble, 1979; Steele, 1979), but these are difficult to maintain and control on sufficiently large scale and with a sufficient degree of replication to allow them to be widely used as predictive tools. In any particular set of circumstances, such experimental systems indicate the range of possible outcomes or mechanisms rather than what actually does happen in the field. They are probably best regarded as attempts to study natural processes on a larger scale, though not necessarily in a more realistic manner, than is possible in the laboratory. The most ambitious experimental ecosystems involve the enclosure of large volumes of water, together with the biomass they contain. However, the very existence of an artificial boundary is likely to interfere with the processes which occur within the enclosure, for example by preventing lateral water movement and by affording a surface for colonisation by algae and microorganisms which would normally not be present. Boundary effects can, of course, be reduced by increasing the size of the enclosure, but the expense of maintaining the systems soon becomes prohibitive and renders replication of experiments impossible.

In practice, it is sometimes found that variability between control replicates exceeds that due to the experimental manipulation. Further, in an enclosure which is sufficiently large to ignore boundary effects, the degree of control available to the experimenter is scarcely better than that available in the completely natural environment; and in any case, in very large enclosures the degree of variability found in different regions within the system is such that the procedures of sampling and analysis which must be employed, and the precision of the results obtained, show little improvement over what would be required if the enclosure were not there at all. Steele (1979) concluded that 'the experiments conducted with large scale ecosystems so far have probably taught us more about the general ecological interactions within such systems than about subtle long-term effects of pollutants'. Such studies have, however, drawn attention to the importance of specific processes which undoubtedly

require further study. These include interspecific and intraspecific interactions, and the role of sedimentary processes and of sediment-water interactions in the behaviour of pollutants in ecosystems.

## 7.3 Marine Biological Monitoring

Since laboratory experiments and other simulations of the marine environment are inevitably much further removed from reality than is the case with freshwater systems, the importance of studying the effects of pollution directly in the field is obvious. In Chapter 3, the concept and practice of biological monitoring of polluted waters were discussed. The same principles should, in theory, be applicable in the marine environment, but for various reasons this is not, in practice, always the case. One important general reason is that, apart from severely-polluted coastal areas and other special circumstances, the extent of the community response that may occur as a result of pollution in the sea is probably much smaller in relation to naturally-occurring spatial and temporal variations than is the case in fresh waters. Thus the difficulty of distinguishing pollution effects from natural phenomena is much greater. Perkins (1979) pointed out that even in fresh waters, biological monitoring has been much better developed and more successful in rivers than in lakes; to some extent lakes and the seas resemble one another more closely than they do rivers, particularly in certain physical features. It is, therefore, important to consider some of the special problems which may be encountered in seeking to apply conventional techniques of biological monitoring in the marine environment.

### *7.3.1 Indicator Organisms*

The concept of indicator organisms is central to biological monitoring (Chapter 3). Two requirements of indicator organisms are relatively limited mobility, and reasonably long life cycles. Thus the organisms will reflect, by their presence, absence or abundance, the environmental conditions of the place where they are situated over their entire life up to the time of sampling. However, many marine organisms have a planktonic phase in the life cycle, so the distribution and abundance of the adults may reflect events which took place at some previous time in a distant location, perhaps at a crucial early stage of the life cycle, or at metamorphosis. Most freshwater invertebrates have more or less annual life cycles, though some have two or more generations per year and some have overlapping generations in a life span of two years or so. Perhaps for this reason (and perhaps because depopulated stretches of river can be rapidly recolonised by downstream drift of organisms), in rivers even after catastrophic pollution incidents the ‘normal’ flora and fauna tend to become re-established within a few months.

In contrast, many marine species appear to have much longer life spans, and there is evidence that in marine communities a single event can have profound consequences which, because of the long life span of the organisms and the

nature of interspecific interactions, can persist for a very long time. Lewis (1972) followed population changes of four littoral species on an unpolluted English shore over four years. A combination of climatic events and biotic interactions resulted in repeated large fluctuations in population density, in some cases of more than tenfold. A major determinant of the marked community changes which occurred during the observation period was the success or failure of the planktonic stages in settling and metamorphosis to the sessile adult. Perkins (1979) refers to other examples where single events, not related to pollution, had consequences which persisted for several years. Thus it appears that in marine communities single events, often of natural origin, can cause profound changes which persist long after the disturbing influence has ceased. To this extent, the use of marine organisms as indicators of environmental quality may give a very misleading picture; certainly it is difficult to distinguish pollution-induced changes from natural phenomena.

Many marine species are also known to undergo long-term fluctuations in their levels of abundance. This is particularly well known in fishes, occurs independently of fishing pressure, and completely negates any possibility, on the basis of current knowledge, of reliably using fishery statistics as indicators of marine pollution, although the idea is remarkably common. Invertebrates almost certainly undergo similar fluctuations. A cautionary example is *Acanthaster planci,* the 'Crown of Thorns' starfish, which attracted much attention in the Pacific 15–20 years ago. Its increased abundance and damaging effect on coral reef ecosystems were widely attributed to pollution or other anthropogenic influence, but it is likely that in fact periodic increases in its population density are a natural feature of its ecology (Moore, 1978).

This does not mean that the concept of indicator organisms is invalid or inappropriate in marine systems. There are some species, such as the polychaete *Capitella capitata,* which have been widely associated with polluted conditions (Reish, 1973). However, it is clear that the concept cannot simply be transferred from fresh water without further fundamental research. The fact that the idea works successfully in rivers may be due to a fortuitous peculiarity of the river ecosystem—perhaps its simplicity—but it is certainly in part due also to the much greater knowledge that exists about rivers and their biota. In interpreting the data from biological surveys, we implicitly or explicitly compare what we have found with what we expected to find. Our expectations depend upon knowledge of the previous condition of the habitat under investigation, on contemporary knowledge of physically-similar and geographically-adjacent habitats, or at worst upon a broad but detailed knowledge of the ecophysiology of our indicator organisms. For marine organisms, this knowledge does not exist, and is urgently required. Perkins (1979) refers to an example which illustrates the need for more background knowledge of the ecology of marine indicator

organisms. Observations of the benthic community along a transect away from a marine waste outfall showed that the diversity and abundance of invertebrates increased with increasing distance from the pollution source. The obvious conclusion, that the outfall was exerting a significant polluting effect, was however erroneous. Subsequent studies in an adjacent, physically-similar area showed that the impoverished community near the pollution discharge was due to natural, physical disturbances caused by wave action, and that the pollution in fact had no detectable effect.

Clearly, then, the concept of indicator organisms in marine pollution studies is likely to remain of limited value until a great deal of additional information is obtained. Survey data will have to be evaluated in the light of the normal, natural variations in community structure and population density which occur in relation to temporal and spatial variations of the physical environment, and due to processes intrinsic to the populations and communities involved. Long-term studies, extending ideally over decades rather than years, of both polluted and unpolluted habitats, are ultimately required before indicator organisms can be used in the marine environment in the same way that they can be used in rivers. Again, however, there is a case to be made that the identification and evaluation of indicator organisms in the marine environment should not necessarily follow the same route which was pursued in freshwater studies. A very promising and potentially rapid method of identifying marine indicator organisms has been suggested (Gray and Pearson, 1982; Pearson *et al.,* 1983) based upon the study of the distribution of individuals between species in marine communities.

In many ecological communities, a few species are represented by a large number of individuals, while the majority of species are represented by a very small number of individuals. In practice, it is frequently found that in a reasonably representative sample of the community, most of the species are recorded as one or two individuals, one or two species contain nearly all the individuals, and some species are represented by intermediate numbers of individuals. This pattern is also commonly found in the benthic invertebrate communities of unpolluted marine habitats. The species present in the sample may be divided into abundance classes: Class I for species represented by just one individual; Class II for those with two or three individuals; Class III for four to seven individuals; Class IV for eight to 15; and so on. A graph or histogram of the percentage of species belonging to a particular abundance class, against the abundance class (Figure 7.3) approximates to one half of a normal curve. In such communities, the distribution of individuals between species is log-normal. In a community which is disturbed by pollution, some species will be rarer and some commoner than might be expected in an unpolluted area, and this log-normal

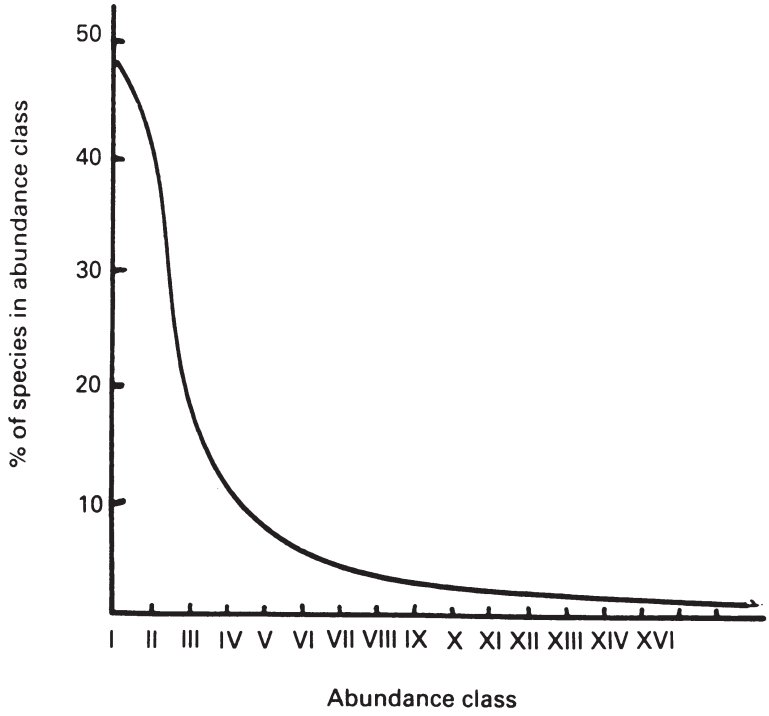


**Figure 7.3** Log-normal distribution of individuals between species in a typical benthic community. Deviations from the log-normal can be detected by simple statistical tests

distribution will be distorted. These distortions have been shown to be associated with polluted conditions (Gray, 1979).

Pearson *et al.* (1983) argue that species which may be useful indicators of community responses to pollution can be identified objectively, without any need for detailed knowledge of their general ecology or sensitivity to pollution. Species in abundance Classes I and II, the rare species, are rejected as indicators. Some of these may be rare because of pollution, but since the majority of species are rare and there are many possible reasons to account for rarity, rarity alone is an insufficient ground for recognising an indicator. The very few species which are found in the higher abundance classes, that is the most abundant species, are also rejected as indicators, for reasons explained below. Those species in abundance Classes V and VI are those which are tentatively identified as those which will be the most sensitive indicators in a community of externally-induced disturbance such as pollution. In practice, these species would typically be those which contain between 16 and 63 individuals per sampling unit; the sampling unit being typically five replicate 0.1-$m^2$ grab samples (Pearson *et al.,* 1983).

It is implicit in this method that the conventional approach to the recognition of indicator species is rejected. To take an example, the polychaete *Capitella capitata* is, conventionally, widely regarded as an indicator of organic pollution in marine habitats, because it is often found in abundance in such conditions. Gray (1979) and Pearson *et al.* (1983) argue that this view is mistaken. First, it appears that *C. capitata* is not, in fact, particularly tolerant of pollution. Several other polychaete species are measurably more resistant to various forms of pollution in experimental studies, but are not found abundantly in polluted conditions in the field. They argue that the association of *C. capitata* with polluted conditions is not due to its tolerance of pollution, but to the fact that it is an opportunist species with a high reproductive capacity and good powers of dispersal, which allow it readily to colonise disturbed areas, and quickly to make good any population losses due to adverse environmental conditions. Its population density is subject, in any particular location, to rapid and massive fluctuations. Second, the authors argue that *C. capitata* is a complex of at least six subspecies, which are difficult to distinguish by conventional taxonomic means. Similar considerations apply to many of the marine species which are regarded, on the conventional approach, as useful indicators. Therefore the use of the most abundant species as indicators is potentially misleading.

In contrast, the use of this approach offers several interesting possibilities. First, the deviation of community structure from the log-normal is amenable to statistical tests of significance, which pollution and diversity indices are not. Second, it allows the identification, by objective means, of a relatively small number of species which, in any particular location, are likely to respond to environmental change. This allows the expense and effort of monitoring to be concentrated on these species, rather than on more abundant, more taxonomically-difficult species whose significance as indicators is in any case in doubt. In their analysis of six extensive data sets from different parts of the world, Pearson *et al.* (1983) found that their method rapidly identified similar lists of species to those determined by more time-consuming methods. Further, the method allows the identification of indicator species specific to particular forms of pollution. Their analysis also suggests that in different parts of the world, different indicator species will be involved in community responses to pollution. Although, therefore, it may be necessary to identify different sets of indicator species for each form of pollution and each individual location, the method affords the means whereby this can be done relatively quickly.

### *7.3.2 Sampling Methodology*

The importance of adequate sampling techniques, and the relationship between sampling strategy, data analysis and the validity of the final results were discussed in Chapter 3. The same considerations of course apply also

**Table 7.2** Comparison of the number ($n$) of replicate 0.1 $m^2$ samples required to give standard error of counts per taxon equal to 20% of the mean. Data from survey of Forties oilfield (Hartley, 1979), given by Hartley (1982). The number of samples required ($n$) is generally <5 for gross taxonomic groupings (polychaetes, molluscs, crustaceans). However, for individual species the number of replicate samples required is much larger. Note that only the more abundant species have been considered. For rare species, the number of samples required may be much larger

| | | | | | Range and mean of values of ($n$) for numerically dominant species[a] | |
|---|---|---|---|---|---|---|
| | $X$ | $\bar{X}$ | $s^2$ | $n$ | Range | Mean |
| Station 15 | | | | | | |
| Polychaetes | 420 | 84.0 | 278.5 | 0.99 | | |
| Crustaceans | 58 | 11.6 | 17.3 | 3.21 | 0.6–18.6 | 5.51 |
| Molluscs | 132 | 26.4 | 11.3 | 0.41 | | |
| Station 16 | | | | | | |
| Polychaetes | 407 | 81.4 | 419.3 | 1.58 | | |
| Crustaceans | 59 | 11.8 | 11.2 | 2.01 | | |
| Molluscs | 157 | 31.4 | 31.3 | 0.79 | | |
| Station 17 | | | | | | |
| Polychaetes | 431 | 86.2 | 237.7 | 0.92 | | |
| Crustaceans | 66 | 13.2 | 16.2 | 2.32 | 2.7–96.1 | 20.68 |
| Station 18 | | | | | | |
| Polychaetes | 341 | 68.2 | 17.2 | 0.09 | | |
| Crustaceans | 62 | 12.4 | 32.3 | 5.25 | | |
| Molluscs | 127 | 25.4 | 43.3 | 1.68 | 1.5–14.9 | 6.00 |

[a]Regarded as those top ranked species contributing to the first 50% of the number of individuals at each station (10–12 taxa for these stations).

to the marine environment. Unfortunately, sampling in the marine environment is notoriously difficult and expensive, especially if the use of offshore vessels is involved. There is therefore an inevitable tendency to attempt to minimise the sampling effort undertaken in marine survey work. There is a danger that in some cases the effort undertaken is, in the end, wasted, or worse, that inaccurate information is obtained which serves only to confuse or mislead. This may appear, to some, an extreme statement; but as recently as 1982 the editor of a well-known journal in the field publicly drew attention to the poor quality of much research, and cited inadequate sampling as a major reason (Clark, 1982b). Hartley (1982) discussed some common failings of many investigations, and made some specific recommendations. In particular, he showed that the number of samples required to

estimate accurately the population density of benthic invertebrates is, as in fresh water, often inconveniently large (Table 7.2). Other sources of error, and of difficulty in comparing results from different sources, are the different efficiencies of the various designs of grab (or other sampling device) which are used, and variations in the procedure employed to extract the animals, usually by some form of sieving. Sources of guidance on the subject of sampling in marine ecological research are available (e.g. Dybern *et al.,* 1976; Holme and McIntyre, 1984; Stirn, 1981), but an urgent need is for wider general agreement on standardised methods for pollution surveys.

### *7.3.3 Data Analysis*

The interpretation of biological survey data is essentially a series of comparisons—temporal, spatial or both—and some of the methods available were described in Chapter 3. One difference between marine survey data and that from fresh water is immediately apparent. For practical purposes, a river is usually considered as a one-dimensional system, that is having length, but negligible width or depth. At least, width and depth effects are, in practice, usually ignored in biological surveys; and the comparison of communities above and below a source of pollution, and at various distances from the pollution source, usually allows any effects of pollution on the receiving water fauna to be readily detected. The relatively strong and unidirectional flow of rivers, and the accretion of unpolluted water along the length of the river, tend to accentuate differences between communities at different points. Marine habitats, in contrast, are at least two-dimensional, and may be three-dimensional if the pelagic biota are to be considered. Further, since observations at a single point in time are clearly of very limited value, the additional dimension of time is likely to be involved in many surveys. Thus the analysis of marine survey data is intrinsically more complex than that of river data. It follows that the data required from a marine survey, for the purposes of meaningful spatial and temporal comparisons, the design of the sampling programme which produces the data, and the methods of data analysis and interpretation employed, must also be more complex.

Index methods (Section 3.4) have often been found useful in analysing survey data in freshwater surveys, and have also been applied to marine work. Diversity indices have been widely used, but are subject to the limitations discussed in Section 3.4 (see also Gray and Pearson, 1982; Reish, 1984). The use of deviation from the log-normal distribution of individuals among species to identify disturbed communities (Gray and Mirza, 1979; see also Section 7.3.1) seems to offer some advantages. Multivariate methods of analysis such as cluster analysis (Section 3.4) appear promising. Analysis based on inspection of a matrix of similarity coefficients, but stopping short of constructing the dendrogram, was recommended under the

name 'trellis method' by Stirn (1981). Some examples of cluster analysis applied to the interpretation of marine survey data are discussed by Reish (1984). Biotic and pollution indices have been rarely used, for the obvious reason that this form of index relies on some form of ranking of known species in order of susceptibility to pollution, and for most marine species it is as yet impossible to do this. Perhaps the nearest approach to the idea is the nematode: copepod ratio. It has frequently been observed that in samples of the benthic meiofauna, the ratio of nematodes to copepods varies with the intensity of organic pollution in the area from which the samples were taken. The use of this ratio as an index of organic pollution was suggested by Rafaelli and Mason (1981). The idea has not been fully tested. Amjad and Gray (1983) found that it produced satisfactory results in Oslo Fjord, but in the Firth of Forth (Shiells and Anderson, 1985), the ratio varied along apparent pollution gradients in an inconsistent manner.

This latter example illustrates an important difference between maritime habitats and rivers which is often overlooked. Whereas, in a river, it is reasonable to expect that a pollution gradient exists along the length of the river, with pollution decreasing with increasing distance downstream from the source of the discharge, in estuarine and marine habitats this is not the case. In these environments there are complex vertical and horizontal patterns of water movement, which are not constant or unidirectional and which are influenced greatly by tides and winds. Therefore it cannot be assumed that pollution decreases unidirectionally with increasing distance from the point of discharge. In the above case, it is possible either that the pollution index used is not valid, or that the pollution gradient is not as it is assumed to be, or indeed that the pollution is not exerting a sufficiently large effect to be detectable.

## 7.4 International Co-operation on Marine Pollution

It was argued in Chapter 6 that effective pollution control requires adequate scientific and technical means, combined with a suitable legislative framework. Since the jurisdiction of any individual state does not extend beyond its own boundaries, it follows that any attempt to control or regulate marine pollution will require some degree of international co-operation. Added to this are a number of other difficulties. For example, in many situations there is inadequate background information available; many states do not possess the physical resources, or adequately trained personnel, which are required to tackle pollution problems. Many of these countries, especially if they are economically disadvantaged and facing numerous other social problems as a consequence, are inclined to regard industrial and agricultural development as a potential solution to their difficulties rather than as a problem requiring regulation. Finally, in many parts of the world long-standing antagonisms tend to inhibit co-operation even on non-political matters of mutual interest; in the Mediterranean, for example, between Turkey and Greece, or between Israel and

the Arab states, or in Africa until recently between the Republic of South Africa and its immediate neighbours. Nevertheless it is widely recognised that some form of international co-operation is necessary and some progress has been made, though many difficulties remain.

International law relating to marine pollution is both vague and weak (Brubacker, 1993). General principles exist which are based on ideas of abuse of rights, custodianship of the environment and good neighbourliness between states, but very few cases have been pursued in international courts and the principles of liability and rights to compensation have not been clearly established by case law. Nor are there any enforceable provisions relating to the type and quantity of pollution, its effects on the environment and how these may be determined. Thus in practice, international co-operation on the control of pollution depends mainly upon a series of treaties or conventions. These may be bilateral, regional or global, and provisions for dealing with violations of the treaty tend to vary. Frequently such provisions consist of some form of arbitration panel, established by mutual consent, with ultimate appeal to the United Nations, or some other international legal forum. Brubacker (1993) lists 89 different international legal instruments which are relevant to the control of marine pollution; to these should be added many provisions in the national laws of individual states, and obligations which arise out of broader forms of international agreement, such as the treaties binding many European states into the European Union, many of which are relevant. This section can therefore only cite a small number of examples.

One of the first treaties to cover marine pollution was the London Dumping Convention, arising from an intergovernmental conference on the Convention on the Dumping of Wastes at Sea, in London in 1972. This is a global convention, binding states which ratify it to prevent vessels under their jurisdiction from dumping certain specified waste materials and toxic substances (the 'Black List') anywhere at sea; a second, so-called 'grey' list of materials was subjected to restrictions, basically requiring parties to the Convention to regulate closely the disposal at sea of these substances. At about the same time a similar convention, the Oslo Convention, was negotiated on a regional basis by states bordering the North Sea and North-East Atlantic. The Paris Convention, in 1974, applies to the coastal states of the North and Barents Seas, and adopts similar black and grey lists. However, this Convention extended controls to pollution from land-based sources and from offshore installations.

The London, Oslo and Paris Conventions formed the model for the Barcelona Convention (United Nations, 1978, 1984), and its daughter conventions and protocols, which are the basis of the Mediterranean Action Plan. The United Nations Environment Programme, formed in 1972, recognised the threat to the Mediterranean and convened a conference of Mediterranean coastal states in 1975.

The Barcelona Convention, adopted in 1976 by all the coastal Mediterranean states (except Albania) and the European Economic Community (now the European Union), binds the participants to co-operate in four major areas: integrated planning, pollution monitoring and research, legal matters, and institutional and financial arrangements. UNEP recognised that given the very different levels of technical and economic development of the Mediterranean states, some form of assistance to the less well-endowed parties to the convention would have to be given. UNEP was therefore appointed to carry out co-ordinating and secretariat functions. The costs of the plan are mainly met by contributions in kind (manpower, facilities) from institutions of the participating states; only the European Union and the wealthier parties make substantial cash contributions. UNEP itself has funds allocated to it by the United Nations, from a Mediterranean Trust Fund established for the purpose. The money pays for the co-ordinating and secretariat functions, scientific conferences, research grants and the supply of capital equipment to the poorer laboratories.

All MAP activities are derived from the Barcelona Convention and its protocols. The 1976 Protocol on the Prevention of Pollution by Dumping from Ships and Aircraft is similar to the London, Oslo and Paris Conventions. The 1980 Protocol for the Prevention of Pollution from Land-based Sources binds the states to take measures to control sewage, industrial wastes and agricultural chemicals at source. The 1976 Protocol concerning Co-operation in Combating Pollution by Oil and Other Harmful Substances in Cases of Emergency commits the governments to co-operation in the case of serious accidents. A Regional Marine Pollution Emergency Response Centre was established in Malta, to provide training and technical advice, formulate contingency plans and co-ordinate government responses to accidents. The 1982 Protocol concerning Specially Protected Areas agrees policies for the designation, protection and management of the habitats of endangered species and other areas of biological importance. About 40 such areas have been designated so far. The Blue Plan, Priority Actions Programme and the Programme for Pollution Monitoring and Research in the Mediterranean (MEDPOL) derive from the Barcelona Convention itself. The Blue Plan is concerned with long-term planning. Pressure on the sea mounts through the development of industry and tourism, and population movements towards the limited coastal strip. The Blue Plan reports on subjects such as water resources, population trends, tourism, food requirements and transport. It offers advice to governments on the local, national and regional consequences of particular development policies. Its aim is to reduce long-term pressures on the sea by providing a rational basis for environmentally-acceptable development, and to advise on policies to prevent or reverse harmful development. The Priority Actions Programme offers technical advice and financial support for specific projects which are environmentally

advantageous and can be implemented fairly swiftly. Examples include projects on aquaculture, solar energy, water resource development, waste disposal and land use planning in earthquake zones. MEDPOL carries out biological and chemical monitoring, and research. Harmonised monitoring programmes for pollutants in water, sediments and living organisms have been established, along with reference methods tested and evaluated by intercalibration exercises. Laboratories were equipped and trained in the appropriate techniques. Provision was made for the servicing and maintenance of equipment, which is often installed in relatively remote locations. Studies are undertaken on, for example, the biogeochemical cycles of selected pollutants; input of pollutants from the atmosphere; microbiological quality of coastal waters, ecotoxicological and eutrophication problems.

The Mediterranean Action Plan in turn became the model for other areas of the world, under UNEP's Regional Seas Programme. Conventions exist for the Kuwait Action Plan Region, West and Central Africa, East Africa, East Asian Seas, the Caribbean, Southeast Pacific, Southwest Pacific, Red Sea and Gulf of Aden, and the Southwest Atlantic; all are basically the same as the Barcelona Convention and its protocols. This comprehensive framework has had mixed success, though this is hardly surprising. Some participating states, as happens with all international agreements, pursue their treaty obligations more assiduously than others. A major problem remains the shortage of suitably qualified and experienced personnel, aggravated by the limitations of the educational system, and its shortage of resources, in many countries. This has a number of consequences. First, a great deal of the monitoring and research data is in practice of limited value. Second, it is arguable that some substantial part of the total research effort is wasted on relatively unimportant problems of purely local relevance, or is based on technical or conceptual approaches which are outdated. This occurs, in part, because within the MAP and its sister plans the overall direction of the activities is strongly influenced by the scientists from the region who are participating in it. Even senior staff from many countries have, until recently, had limited access to the international literature and have had little opportunity to experience the pace and rigours of modern research. Also, precisely because staff are scarce, they are frequently under pressure to concentrate their efforts on local problems which need urgent attention. In many such cases, there is no real need for research as such, rather the application of existing knowledge is what is required; but in many parts of the world, particularly where the legislative framework of pollution control is weak, scientists have little experience of the regulatory aspects of pollution science. They are therefore inclined to tackle fairly simple problems by initiating relatively trivial research when the obvious solution is to adapt general principles which have been well tried elsewhere. Nevertheless, the MAP does show that progress can be made. Perhaps one of its major achievements will be the emergence of a corps of scientists, suitably

experienced by virtue of the opportunity they have had to participate more fully than before in the international scientific community. To this can be added progress towards more accurately quantifying the pollution threat to some of the world's seas, and measures in hand to combat the threat at the technical and legislative levels. Cheap and unbiased technical advice is now available, and many cities and industries have begun to construct pollution control facilities for the first time.

In Europe, directives of the European Union relating to the control of water pollution influence fresh waters, estuaries and coastal seas. Since a large part of the pollution discharged to the seas originates from polluted rivers, these directives will probably in due course have some ameliorative effect on the North Sea and the Mediterranean. Although compliance with EU directives is far from complete in many member states, historically the southern states have had a less rigorous approach to pollution control than some of the northern ones, so some improvement is to be expected. Also, the EU has effective means of enforcing compliance; such powers are not widely used, if they exist at all, under many other treaties. It is expected that some countries of Eastern Europe, where there remain many serious pollution problems, will in the foreseeable future join the EU and be subject to more strict environmental controls than at present, so some benefit may accrue in time to the Baltic and North Seas by this route.

One final example of international co-operation in preventing pollution of the seas relates to agreements regulating shipping operations, usually under the auspices of the International Maritime Organisation (IMO). Shipping operations can cause a great deal of pollution, for example by the discharge of oily ballast water, through accidents, and through negligent operation of routine procedures. The IMO therefore has enacted many regulations which are designed to reduce marine pollution. For example, the discharge of oily ballast water is illegal, and various modifications to the design, construction and operation of ships should be observed in order to avoid this. Ships are supposed to have a certificate of seaworthiness, to avoid major accidents; similarly, the crew are supposed to be properly trained and experienced so that they follow the often complex regulations, and avoid endangering their ships and cargoes. This has resulted in a considerable reduction in pollution from shipping operations, but some problems remain. For example, under international law the responsibility for certificating ships and their crews, for checking the design and construction of ships and for enforcing the regulations usually lies with the state with which the vessel is registered. A number of countries, not entirely confined to the Third World, generate considerable national income from ship registration, and many shipowners find it advantageous for various reasons to register their vessels under 'flags of convenience'. Some of these so-called Flag states are notorious for the laxity with which they enforce regulations. Because of this, many Port states (that is, states which a ship happens to be visiting) have begun to exercise

their rights to inspect visiting ships for seaworthiness, structural soundness, safety and pollution control equipment, and certification of crew qualifications. A typical outcome is that reported from Australia in 1995 (MER, 1996); approximately 10% of 2542 ships inspected were detained for remedy of serious deficiencies, and 56% of the ships detained were registered in one of only four countries. Similarly, records of ships lost at sea or otherwise causing pollution incidents show that certain countries of registration appear with astounding frequency.

# References


ABEL, P.D. (1974) Toxicity of synthetic detergents to fish and aquatic invertebrates. *J. Fish. Biol.* **6** 279–298.

(1976) Toxic action of several lethal concentrations of an anionic detergent on the gills of the brown trout, *Salmo trutta* L. *J. Fish. Biol.* **9** 441–446.

(1978) Mortality patterns in trout exposed to an anionic detergent in relation to concentrations and mechanisms of toxic action. *Freshwater Biol.* **8** 497–503.

(1980a) Toxicity of *?*-hexachlorocyclohexane (Lindane) to *Gammarus pulex:* mortality in relation to concentration and duration of exposure. *Freshwater Biol.* **10** 251–259.

(1980b) A new method for assessing the lethal impact of short-term, high-level discharges of pollutants on aquatic animals. *Progr. Water Tech.* **13** 347–352.

(1988) Pollutant toxicity to aquatic animals—methods of study and their applications. *Rev. Environ. Health* **8** 119–155.

(1991) Lethal toxicity tests—theory and methodology. In: Abel, P.D. and Axiak, V. (Eds) *Ecotoxicology and the Marine Environment.* Ellis Horwood, Chichester.

ABEL, P.D. and AXIAK, V. (Eds) (1991) *Ecotoxicology and the Marine Environment.* Ellis Horwood, Chichester.

ABEL, P.D. and GARNER, S.M. (1986) Further comparisons of median survival times and median lethal exposure times for *Gammarus pulex* exposed to cadmium, Permethrin and cyanide. *Water Res.* **20** 579–582.

ABEL, P.D. and GREEN, D.W.J. (1981) Ecological and toxicological studies on the invertebrate fauna of two rivers in the Northern Pennine orefield. In: Say, P.J. and Whitton, B.A. (Eds) *Heavy Metals in Northern England— Environmental and Biological Aspects.* University of Durham, Department of Botany.

ABEL, P.D. and PAPOUTSOGLOU, S.E. (1986) Lethal toxicity of cadmium to *Cyprinus carpio* and *Tilapia aurea. Bull. Environ. Contam. Toxicol.* **37** 382–386.

ABEL, P.D. and SKIDMORE, J.F. (1975) Toxic effects of an anionic detergent on the gills of rainbow trout. *Water Res.* **9** 759–765.

ADDISON, R.F. (1984) Hepatic mixed function oxidase (MFO) induction in fish as apossible biological monitoring system . In: Cairns, V.W., Hodson, R.V. and Nriagu, J.O. (Eds) *Contaminant Effects on Fisheries*. John Wiley, New York.

ADELMAN, I.R. and SMITH, L.L. (1972) Toxicity of hydrogen sulphide to goldfish *(Carassius auratus)* as influenced by temperature, oxygen and bioassay techniques. *J. Fish. Res. Bd Can.* **29** 1309–1317.

ADELMAN, I.R., SMITH, L.L. and SIESENOPP, G.D. (1976) Effect of size or age of goldfish and fathead minnows on use of pentachlorophenol as a reference toxicant. *Water Res.* **10** 685–687.

ALABASTER, J.S. (1972) Suspended solids and fisheries. *Proc. R. Soc. Lond.* **B180** 395–406.

ALABASTER, J.S., GARLAND, J.H.N., HART, I.C. and SOLBÉ, J.F. DE L.G. (1972) An approach to the problem of pollution and fisheries. *Symp. Zool. Soc. Lond.* No. 29, pp. 87–114.

ALABASTER, J.S. and LLOYD, R. (Eds) (1980) *Water Quality Criteria for Freshwater Fish.* Butterworths, London, for the Food and Agriculture Organisation.

ALEXANDER, R.MCN. (1970) *Functional Design in Fishes.* 2nd ed. Hutchinson, London.

ALLAN, J.D. (1995) *Stream Ecology—Structure and Function of Running Waters.* Chapman & Hall, London.

AMES, B.N., MCCANN, J. and YAMASAKI, E. (1975) Methods for detecting carcinogens and mutagens with the *Salmonella/mammalian* microsome mutagenicity test. *Mutat. Res.* **31,** 347–364.

AMJAD, S. and GRAY, J.S. (1983) Use of the nematode-copepod ratio as an index of organic pollution. *Mar. Poll. Bull.* **14** 178–181.

ANDERSON, F.R., MANDELKER, D.R. and TARALOCK, T.D. (1990) *Environmental Protection: Law and Policy.* Little, Brown and Co., Boston.

APHA (1995) *Standard Methods for the Examination of Waters and Wastewaters,* 19th ed. American Public Health Association, American Waterworks Association, Water Environment Federation. American Public Health Association, Washington.

ARMITAGE, P.D. (1980) The effects of mine drainage and organic enrichment on benthos in the river Nent system, Northern Pennines. *Hydrobiologia* **74** 119–128.

ARMITAGE, P.D., Moss, D., WRIGHT, J.F. and FURSE, M.T. (1983) The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Water Res.* **17** 333–347.

ARTHUR, D.R. (1975) Constraints on the fauna in estuaries. In: Whitton, B.A. (Ed.) *River Ecology.* Blackwell Scientific Publications, Oxford.

ARTHUR, J.W., ZISCHE, J.A. and ERICKSON, G.L. (1982) Effect of elevated water temperature on macroinvertebrate communities in outdoor experimental channels. *Water Res.* **16** 1465–1477.

ASH, I., MCKENDRICK, G.D.W., ROBERTSON, M.H. and HUGHES, H.L. (1964) Outbreak of typhoid fever connected with corned beef. *Br. Med. J.* **1** 1474–1478.

ASTM (1973) *Biological Methods for the Assessment of Water Quality.* American Society for Testing and Materials, Special Technical Publication No. 528. Philadelphia.

ASTON, S.R., FOWLER, S.W. and WHITEHEAD, N. (1986) Mercury biogeochemistry in the Mediterranean marine environment: an assessment of contamination. FAO/UNEP/WHO/IOC/IAEA Meeting on the biogeochemical cycle of mercury in the Mediterranean. Sienna, August 1984. *FAO Fish. Rep.* **325** (Supplement) 8–19.

AUSTIN, I. (1992) Acute sublethal effect of zinc on the feeding rate and behaviour of the freshwater shrimp, *Gammarus pulex.* Unpublished MSc thesis, Universityof Sunderland .

AXIAK, V. (1991) Sublethal toxicity tests: physiological responses. In: Abel, P.D. and Axiak, V. (Eds) *Ecotoxicology and the Marine Environment.* Ellis Horwood, Chichester.

AXIAK, V. and ABEL, P.D. (1991) Special cases of toxicity tests. In: Abel, P.D. and Axiak, V. (Eds) *Ecotoxicology and the Marine Environment.* Ellis Horwood, Chichester.

BADENOCH, J. (1990) *Report of the Group of Experts on* Cryptosporidium *in Water Supplies*. Her Majesty's Stationery Office, London.

BAKER, J.P. and SCHOFIELD, C.L. (1985) Acidification impacts on fish populations: a review. In: Adams, D.D. and Page, W.P. (Eds) *Acid Deposition: Environmental, Economic and Policy Issues.* Plenum Press, New York.

BALL, I.R. (1967a) The relative susceptibilities of some species of freshwater fish to poisons. I. Ammonia. *Water Res.* **1** 767–775.

(1967b) The toxicity of cadmium to rainbow trout (*Salmo gairdneri* Richardson). *Water Res.* **1** 805–806.

(1967c) The relative susceptibilities of some species of freshwater fish to poisons. II. Zinc. *Water Res.* **1** 777–783.

BALLOCH, D., DAVIES, C.E. and JONES, F.H. (1976) Biological assessment of water quality in three British rivers: the North Esk (Scotland), the Ivel (England) and the Taf (Wales). *Water Poll. Control* **75** 92–110.

BARNES, R.S.K. (1984) *Estuarine Biology.* 2nd ed. Edward Arnold, London.

BARNES, R.S.K. and GREEN, J. (Eds) (1972) *The Estuarine Environment.* Applied Science Publishers, London.

BAYNE, B.L., MOORE, M.N., WIDDOWS, J., LIVINGSTONE, D.R. and SALKELD, P. (1979) Measurement of the responses of individuals to environmental stress and pollution: studies with bivalve molluscs. *Phil. Trans. R. Soc. Lond.* **B286** 563–581.

BELL, H.L. (1971) Effect of low pH on the survival and emergence of aquatic insects. *Water Res.* **5** 313–319.

BELLAN, G. (1970) Pollution by sewage in Marseilles. *Mar. Pollut. Bull.* **1** 59–60.

BENENSON, A.S. (1982) Cholera. In: Evans, A.S. and Feldman, H.A. (Eds) *Bacterial Infections of Humans.* Plenum Publishing Corporation, New York.

BENES, V. (1978) Toxicological aspects of the water we drink. In: Plaa, G.L. and Duncan, W.A.M. (Eds) *Proceedings of the First International Congress on Toxicology.* Academic Press, New York.

BENSON, W.H. and BIRGE, W.J. (1985) Heavy metal tolerance and metallothionein induction in fathead minnows: results from field and laboratory investigations. *Environ. Toxicol. Chem.* **4** 209–217.

BLISS, C.I. (1935) The calculation of the dosage-mortality curve. *Ann. Appl. Biol.* **22** 134–167.

(1937) The calculation of the time-mortality curve. *Ann. Appl. Biol.* **24** 815–852.

BLOCK, J. -C. (1983) Viruses in environmental waters. In: Berg, G. (Ed.) *Viral Pollution of the Environment.* CRC Press, Boca Raton.

BOND, R.G. and STRAUB, C.P. (Eds) (1974) *Handbook of Environmental Control Vol. IV. Wastewater Treatment and Disposal.* CRC Press, Cleveland.

BONDE, D.J. (1977) Bacterial indication of water pollution. *Adv. Aquat. Microbiol.* **1** 273–364.

BRILLOUIN, L. (1951) Maxwell's demon cannot operate: information and entropy I and II. *J. Appl. Phys.* **22** 57–64.

BRINKHURST, R.O. (1993) Future directions in freshwater biomonitoring using benthic macroinvertebrates. In: Rosenberg, D.M. and Resh, V.H. (Eds) *Freshwater Biomonitoring and Benthic Macroinvertebrates.* Chapman & Hall, New York.

BROOKER, M.P. and MORRIS, D.L. (1980) A survey of the macroinvertebrate riffle fauna of the rivers Ystwyth and Rheidol, Wales. *Freshwater Biol.* **10** 459–474.

BROWN, A.W.A. (1978) *Ecology of Pesticides.* John Wiley & Sons, New York.

BROWN, B.E. (1976) Observations on the tolerance of the isopod *Asellus meridianus* Rac. to copper and lead. *Water Res.* **10** 555–559.

(1977) Effects of mine drainage on the river Hayle, Cornwall. A. Factors affecting concentrations of copper, zinc and iron in water, sediments and dominant invertebrate fauna. *Hydrobiologia* **52** 221–232.

BROWN, V.M. (1969) The calculation of the acute toxicity of mixtures of poisons to rainbow trout. *Water Res.* **2** 723–733.

(1973) Concepts and outlook in testing the toxicity of substances to fish. In: Glass, G.E. (Ed.) *Bioassay Techniques and Environmental Chemistry.* Science Publishers, Ann Arbor.

BROWN, V.M. and DALTON, R.A. (1970) The acute lethal toxicity to rainbow trout of mixtures of copper, phenol, zinc and nickel. *J. Fish. Biol.* **2** 211–216.

BROWN, V.M., JORDAN, D.H.M. and TILLER, B.A. (1967a) The effect of temperature on the acute toxicity of phenol to rainbow trout in hard water. *Water Res.* **1** 587–594.

(1969) The acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol and zinc. *J. Fish. Biol.* **1** 1–9.

BROWN, V.M., MITROVIK, V.V. and STARK, G.T.C. (1968) Effects of chronic exposure to zinc on toxicity of a mixture of detergent and zinc. *Water Res.* **2** 255–263.

BROWN, V.M., SHAW, T.L. and SHURBEN, D.G. (1974) Aspects of water quality and the toxicity of copper to rainbow trout. *Water Res.* **8** 797–803.

BROWN, V.M., SHURBEN, D.G. and FAWELL, S.K. (1967b) The acute toxicity of phenol to rainbow trout in saline waters. *Water Res.* **1** 683–685.

BRUBACKER, D. (1993) *Marine Pollution and International Law—Principles and Practice.* Belhaven Press, London.

BRYAN, G.W. (1979) Bioaccumulation of marine pollutants. *Phil. Trans. R. Soc. Lond.* **B286** 483–505.

(1984) Pollution due to heavy metals and their compounds. In: Kinne, O. (Ed.) *Marine Ecology Vol. V: Ocean Management Part 3: Pollution and Protection of the Seas.* John Wiley & Sons, Chichester.

BUHAGIAR, A. and ABEL, P.D. (1991) Development of a computer program for analysing toxicity test results—the 'Toxicologist' system. Ch. 4 in: Abel, P.D. and Axiak, V. (Eds) *Ecotoxicology and the Marine Environment.* Ellis Horwood, Chichester.

BUIKEMA, A.L., MCGINNIS, M.J. and CAIRNS, J. (1979) Phenolics in aquatic ecosystems: a selected review of recent literature. *Mar. Environ. Res.* **2** 87–181.

BURROWS, W.D. (1977) Aquatic aluminium: chemistry, toxicology and environmental prevalence. *CRC Crit. Rev. Environ. Control* **7** 167–216.

BURTON, D.T., MORGAN, E.L. and CAIRNS, J. (1972) Mortality curves of bluegills *(Lepomis macrochirus)* simultaneously exposed to temperature and zinc stress. *Trans. Amer. Fish. Soc.* **101** 435–441.

BURTON, J.D. (1979) Physico-chemical limitations in experimental investigations. *Phil Trans. R. Soc. Lond.* **B286** 443–456.

BYUS, C.V., COSTA, M., SPIES, I.G., BRODIE, B.B. and RUSSELL, D.H. (1976) Activation of 3':5'-cyclic AMP-dependent protein kinase and induction of ornithine decarboxylase as early events in induction of mixed function oxidases. *Proc. Nat. Acad. Sci. USA* **73**, 1241–1244.

CAIRNCROSS, S. and FEACHEM, R. (1993) *Environmental Health Engineering in the Tropics.* 2nd ed. John Wiley & Sons, Chichester.

CAIRNS, J. (1974) Protozoans (Protozoa). In: Hart, C.W. and Fuller, S.H. (Eds) *Pollution Ecology of Freshwater Invertebrates.* Academic Press, New York, pp. 1–25.

(1979) A strategy for the use of protozoans in the evaluation of hazardous substances. In: James, A. and Evison, L. (Eds) *Biological Indicators of Water Quality.* John Wiley & Sons, Chichester.

CAIRNS, J., DOUGLAS, W.A., BUSEY, F. and CHANEY, M.D. (1968) The sequential comparison index—a simplified method for non-biologists to estimate relative differences in biological diversity in stream pollution studies. *J. Water Poll. Contr. Fed.* **40** 1607–1613.

CAIRNS, J., HEATH, A.G. and PARKER, B.C. (1975) The effects of temperature upon the toxicity of chemicals to aquatic organisms. *Hydrobiologia* **47** 135–171.

CAIRNS, J. and PRATT, J.R. (1993) A history of biological monitoring using benthic macroinvertebrates. In: Rosenberg, D.M. and Resh, V.H. (Eds) *Freshwater Biomonitoring and Benthic Macroinvertebrates.* Chapman & Hall, New York.

CALAMARI, D. and MARCHETTI, R. (1973) The toxicity of mixtures of metals and surfactants to rainbow trout *Salmo gairdneri* (Rich.) *Water Res.* **7** 1453–1464.

CALAMARI , D., MARCHETTI , R. and VAILATI , G. (1980) Influence of water hardness on cadmium toxicity to *Salmo gairdneri. Water Res.* **14** 1421–1426.

CALOW, P. and PETTS, G.E. (Eds) (1992) *The Rivers Handbook, Vol. I.* Blackwell Scientific Publications, Oxford.

(1994) *The Rivers Handbook, Vol. II.* Blackwell Scientific Publications, Oxford.

CARPENTER, K.E. (1922) The fauna of the Clarach stream (Cardiganshire) and its tributaries. A preliminary study of the problem of lead pollution. *Aberystwyth Studies* **4** 251–258.

(1924) A study of rivers polluted by lead mining in the Aberystwyth district of Cardiganshire. *Ann. Appl. Biol.* **11** 1–23.

(1925) On the biological factors involved in the destruction of river fisheries by pollution due to lead mining. *Ann. Appl. Biol.* **12** 1–13.

(1926) The lead mine as an active agent in river pollution. *Ann. Appl. Biol.* **13** 395–401.

CARTER, F.W. and TURNOCK, D. (1993) *Environmental Problems in Eastern Europe.* Routledge, London.

CASTENHOLZ, R.W. and WICKSTROM, C.E. (1975) Thermal streams. In: Whitton, B.A. (Ed.) *River Ecology.* Blackwell Scientific Publications, Oxford, pp. 264–285.

CHAN, K.M., DAVIDSON, W.S. and FLETCHER, G.L. (1989) Metallothionein messenger RNA: potential indicator of metal exposure. Ch. 7 In: Nriagu, J.O. and Lakshmiraryana, J.S.S. (Eds) *Aquatic Toxicology and Water Quality Management*. Advances in Environmental Science and Technology Series, Vol. 22. John Wiley & Sons, New York.

CHANDLER, J.R. (1970) A biological approach to water quality management. *Water Pollut. Control,* **69** 415–422.

CHAPMAN, G.A. (1978) Toxicities of cadmium, copper and zinc to four juvenile stages of chinook salmon and steelhead. *Trans. Am. Fish. Soc.* **107** 841–847.

CHAPMAN, P.M., FARRELL, M.A. and BRINKHURST, R.D. (1982) Relative tolerances of selected aquatic oligochaetes to combinations of pollutants and environmental factors. *Aquatic Toxico.* **2** 69–78.

CHAPPELL, K.R. and GOULDER, R. (1994) Epilithic extracellular enzyme activity in a zinc-contaminated stream. *Bull. Environ. Contam. Toxicol.* **52** 305–310.

CLARK, R.B. (Ed.) (1982a) *The Long-term Effects of Oil Pollution on Marine Populations, Communities and Ecosystems.* The Royal Society, London. (Also published as *Phil. Trans. R. Soc. Lond.* **B297** 183–443, 1982.)

(1982b) Environmental science and all that. *Mar. Pollut. Bull.* **13** 335–336.

(1992) *Marine Pollution.* 3rd ed. Clarendon Press, Oxford.

COLE, H.A. (Ed.) (1979) *The Assessment of Sublethal Effects of Pollutants in the Sea.* The Royal Society, London. (Also published as *Phil. Trans. R. Soc. Lond.* **B286** 399–633, 1979.)

COLEMAN, M.J. and HYNES, H.B.N. (1970) The vertical distribution of the invertebrates in the bed of a stream. *Limnol. Oceanogr.* **15** 31–40.

COLWELL, R.R. (Ed.) (1984) *Vibrios in the Environment.* John Wiley & Sons, New York.

COMMISSION OF THE EUROPEAN COMMUNITIES (1992) *European Community Environment Legislation. Vol. 7—Water.* Office for Official Publications of the European Communities, Luxembourg.

CONAN, G. (1982) The long-term effects of the *Amoco Cadiz* oil spill. *Phil. Trans. R. Soc. Lond.* **B297** 323–333.

COOK, S.E. (1976) Quest for an index of community structure sensitive to water pollution. *Environ. Pollut.* **11** 269–288.

CRANE, M. (1995) Effect of zinc on four populations and two generations of *G. pulex* (L.) *Freshwater Biology* **33** 119–126.

*Croner's Environmental Management* (1995). Croner Publications Ltd, Kingston-upon-Thames.

CURDS, C.R. (1975) Protozoa. In: Curds, C.R. and Hawkes, H.A. (Eds) *Ecological Aspects of Used-water Treatment, Vol. I. The Organisms and their Ecology.* Academic Press, London, pp. 203–268.

CURDS, C.R. and HAWKES, H.A. (Eds) (1975), *Ecological Aspects of Used-water Treatment, Vol. I. The Organisms and their Ecology.* Academic Press, London.

(1983a) *Ecological Aspects of Used-water Treatment, Vol. II. Biological Activities and Treatment Processes.* Academic Press, London.

(1983b) *Ecological Aspects of Used-water Treatment, Vol. III. The Processes and their Ecology.* Academic Press, London.

CURTIS, E.J.C. and CURDS, C.R. (1971) Sewage fungus in rivers in the United Kingdom: studies of *Sphaerotilus* slimes using laboratory recirculating channels. *Water Res.* **5** 267–279.

DAVENPORT, J. (1982) Oil and planktonic ecosystems. *Phil. Trans. R. Soc. Lond.* **B297** 369–384.

DAVIES, J.M. and GAMBLE, J.C. (1979) Experiments with large enclosed ecosystems. *Phil. Trans. R. Soc. Lond.* **B286** 523–544.

DAVIES, P.H., GOETTL, S.P. and SINLEY, J.R. (1970) Toxicity of silver to rainbow trout *(Salmo gairdneri) Water Res.* **12** 113–117.

DAVIES, R.P. and DOBBS, A.J. (1984) The prediction of bioconcentration in fish. *Water Res.* **18** 1253–1262.

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH (1935) *Survey of the River Tees: Part II. The Estuary, Chemical and Biological.* Water Pollution Research Board Technical Paper No. 5. Her Majesty's Stationery Office, London.

(1964) *Effects of Polluting Discharges on the Thames Estuary.* Water Pollution Research Technical Paper No. 11. Her Majesty's Stationery Office, London.

DEPIERRE, J.W. and ERNSTER, L. (1978) The metabolism of polycyclic hydrocarbons and its relationship to cancer. *Biochem. Biophys. Acta* **47**, 149–155.

DEUTSCH, J., LENTZ, J.C., YANG, S.K., GELBOIN, H.V., CHANG, Y.L., VATSIS, K.P. and COON, M.J. (1978) Regio- and stereoselectivity of various forms of purified cytochrome P-450 in the metabolism of benzo (a) pyrene and (-) trans-7, 8-dihydroxy-7, 8-dihydro-benzo(a) pyrene as shown by product formation and binding of DNA. *Proc. Nat. Acad. Sci. USA* **75**, 3123–3127.

DIAMOND, L.S. (1983) Lumen-dwelling protozoa. In: Jensen, J.B. (Ed.) In vitro *Cultivation of Protozoan Parasites.* CRC Press, Boca Raton.

DIN (1983a) DIN 38412 T.13 *German Standard Methods for Examination of Water, Wastewater & Sludge. L13 Bestimmung von Sauerstoffproducktion und Sauerstoffverbranch im Gewasser mit der Hell-Dunkelflaschen-Methode SPG, SVG.* Berlin.

(1983b) DIN 38142 T.14 *German Standard Methods for Examination of Water, Wastewater and Sludge. L.14 Bestimmung von Sauerstoffproducktion mit der Hell-Dunkelflaschen-Methode unter Laborbedingungen, SPL.* Berlin.

(1987) DIN 38410 T.1 *German Standard Methods for Examination of Water and Wastewater. Biologisch-okologisch Gewasseruntersuchung Allgemeine Hinweise, Planung und Durchfuhrung.* Berlin.

(1990) DIN 38410 T.2 *German Standard Methods for Examination of Water and Wastewater. Biologisch-okologisch Gewasseruntersuchung Bestimmung des Saproflenindex.* Berlin.

DOBBERKAU, H. -J., WALTER, R. and LOGAN, K. (1981) The recovery of viruses from water: methods and applications. In: Goddard, M. and Butler, M. (Eds) *Viruses and Wastewater Treatment.* Pergamon Press, Oxford.

DOOHAN, M. (1975) Rotifera. In: Curds, C.R. and Hawkes, H.A. (Eds) *EcologicalAspects of Used-water Treatment, Vol. 1. The Organisms and their Ecology* . Academic Press, London, pp. 289–304.

DUGAN, P.R. (1972) *Biochemical Ecology of Water Pollution.* Plenum Press, New York.

DUTHIE, J.R. (1977) The importance of sequential assessment in test programmes for estimating hazards to aquatic life. In: Mayer, F.L. and Hamelink, J.L. (Eds) *Aquatic Toxicology and Hazard Evaluation.* Special Technical Publication No. 634, American Society for Testing and Materials, Washington.

DYBERN, B.I., ACKEFORS, H. and ELMGREN, R. (1976) Recommendations on methods for marine biological studies in the Baltic sea. *Balt. Mar. Biol. Publ.* **1** 1–98.

EDEN, G.E., BAILEY, D.A. and JONES, K. (1977) Water re-use in the United Kingdom. In: Shuval, H.E. (Ed.) *Water Renovation and Re-use.* Academic Press, New York.

EDWARDS, R.W., OSBORNE, A.C., BROOKER, M.P. and SAMBROOK, H.T. (1978) The behaviour and budgets of selected ions in the Wye catchment. *Verh. Int. Ver. Theor. Angew. Limn.* **20** 1418–1422.

EISLER, R. (1965) Some effects of a synthetic detergent on estuarine fishes. *Trans. Am. Fish. Soc.* **94** 26–31.

(1970) *Acute Toxicities of Organochlorine and Organophosphorus Insecticides toEstuarine Fishes* . US Dept. of the Interior: Bureau of Sport, Fisheries and Wildlife Technical Report No. 46.

(1971) Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. *J. Fish. Res. Bd Can.* **28** 1225–1234.

ELLIOT, J.M. (1977) S*ome Methods for the Statistical Analysis of Samples of Benthic Invertebrates.* Freshwater Biological Association, Scientific Publication No. 25.

ELLIOT, J.M. and DRAKE, C.M. (1981a) A comparative study of seven grabs used for sampling benthic macroinvertebrates. *Freshwater Biology* **11,** 99–120.

(1981b) A comparative study of four dredges used for sampling benthic macroinvertebrates in rivers. *Freshwater Biology* **11** 245–262.

ERNST, W. (1984) Pesticides and technical organic chemicals. In: Kinne, O. (Ed.) *Marine Ecology Vol. V: Ocean Management, Part 4: Pollution and Protection of the Seas.* John Wiley & Sons, Chichester.

ESGOSS (Ecological Steering Group on the Oil Spill in Shetland) (1994) Wreck of the tanker Braer: The environmental impact of the oil spill. *Spill Science & Technology Bulletin* **1** 101–107.

EVANS, G.P. and JOHNSON, D. (1984) Detection of pollution at drinking water intakes. In: Lack, T.J. (Ed.) E*nvironmental Protection: Standards, Compliance and Costs.* Ellis Horwood, Chichester, for Water Research Centre.

FINNEY, D.J. (1971) *Probit Analysis,* 3rd ed. Cambridge University Press, Cambridge.

FISHER, R.A., CORBET, A.S. and WILLIAMS, C.B. (1943) The relation between the number of species and the number of individuals in a random sample of an animal population. *J. Anim. Ecol.* **12** 42–58.

FLOWER, R.J. and BATTARBEE, R.N. (1983) Diatom evidence for recent acidification of two Scottish lochs. *Nature* **305** 130–132.

FOWLER, S.W., LAROSE, Y., UNLU, B., OREGIONI, J.P., VILLENEUVE, D.L., FUKAI, R., VALLEN, D. and BRISSON, M. (1979) Heavy metals and chlorinated hydrocarbons in pelagic organisms from the open Mediterranean sea. *J. Etud. Pollut. CIESM* **4** 155–158.

FRASER, P. (1984) Epidemiology and water quality. In: Lack, T.J. (Ed.) *Environmental Protection: Standards, Compliance and Costs.* Ellis Horwood, Chichester, for Water Research Centre.

FROST, R., POWLESLAND, C., HALL, J.E., NIXON, S.C. and YOUNG, C.P. (1990) *Review of Sludge Treatment and Disposal Techniques.* Report No. PRD 2306-M/1, Water Research Centre, Medmenham.

FURSE, M.T., WRIGHT, J.F., ARMITAGE, P.D. and Moss, D. (1981) An appraisal of pond-net samples for biological monitoring of lotic macro-invertebrates. *Water Res.* **15** 679–690.

GADDUM, J.H. (1948) P*harmacology. 3*rd ed. Oxford University Press, Oxford.

GALE, M.L., WIXON, B.G., HARDIE, M.G. and JENNET, J.C. (1973) Aquatic organisms and heavy metals in Missouri's new lead belt. *Water Res. Bull.* **9** 673–688.

GAMPER, H.B., TUNG, A.S.-C, STRAUB, K., BARTHOLOMEW, J.C. and CALVIN, M. (1977) DNA strand scission by benzo(a)pyrene diol epoxides. *Science* **197,** 671–674

GARLAND, J.H.N. and ROLLEY, H.L.J. (1977) Studies of river water quality in the Lancashire Tame. *Water Pollut. Contr.* **76** 301–326.

GAUCH, H.G. (1982) *Multivariate Analysis in Community Ecology.* Cambridge University Press, Cambridge.

GAUFIN, A.R., JENSEN, L.D., NEBEKEV, A.V., NELSON, T. and TEEL, R.W. (1965) The toxicity of ten inorganic insecticides to various aquatic invertebrates. *Water Sew. Wks. J.* July, 276–279.

GERALDI, M.H. (1990) *Wastewater Biology: the Microlife.* Water Pollution Control Federation, Washington.

GERBA, C.P. (1981) Virus survival in wastewater treatment. In: Goddard, M. and Butler, M. (Eds) *Viruses and Wastewater Treatment.* Pergamon Press, Oxford.

(1983) Methods for recovering viruses from the water environment. In: Berg, G. (Ed.) *Viral Pollution of the Environment.* CRC Press, Boca Raton.

GIDDINGS, J.M. (1983) Microcosms for the assessment of chemical effects on the properties of aquatic ecosystems. In: *Hazard Assessment of Chemicals: Current Developments.* Vol. 2, Academic Press, New York, pp. 46–89.

GILHOOLEY, E. (1988) Studies into the effects of dissolved zinc on the rate of downstream drift of *Gammarus pulex* in laboratory channels. Unpublished MSc thesis, Sunderland Polytechnic.

GILMOUR, C.G. (1992) Effects of acid deposition on microbial processes in natural waters. Ch. 2 In: Mitchell, R. (Ed.) *Environmental Microbiology*. John Wiley, New York.

GLEASON, H.A. (1922) On the relation between species and area. *Ecology* **3** 158.

GOODMAN, G.T. (1974) How do chemical substances affect the environment? *Proc. R. Soc. Lond.* **B185** 127–148.

GRANT, B.F. and MEHRLE, P.M. (1973) Endrin toxicosis in rainbow trout. *J. Fish. Res. Bd Can.* **30,** 31–40.

GRAY, J.S. (1979) Pollution-induced changes in populations. *Phil. Trans. R. Soc. Lond.* **B286** 545–561.

(1992) Biological and ecological effects of marine pollutants and their detection. *Mar. Poll. Bull.* **25** 48–50.

GRAY, J.S. and MIRZA, F.B. (1979) A possible method for the detection of pollution-induced disturbance on marine benthic communities. *Mar. Poll. Bull.* 142–146.

GRAY, J.S. and PEARSON, T.H. (1982) Objective selection of sensitive species indicative of pollution-induced change in benthic communities. 1. Comparative methodology. *Mar. Ecol. Prog. Ser.* **9** 111–119.

GRAY, N.F. (1992) *Biology of Wastewater Treatment.* Oxford University Press, Oxford.

GREEN, D.W.J. (1984) Ecological and toxicological studies on the invertebrate fauna of metalliferous streams. Unpublished PhD thesis, Sunderland Polytechnic.

GREEN, J. and TRETT, M.W. (1989) *The Effects and Fate of Oil in Freshwater.* Elsevier Applied Science, London.

GREEN, M.B. (1983) The Macrofauna of Sludge-drying Beds. In: Curds, C.R. and Hawkes, H.A. (Eds) *Ecological Aspects of Used-water Treatment, Vol. II. Biological Activities and Treatment Processes.* Academic Press, London pp. 261–300.

GREEN, R.H. (1979) *Sampling Design and Statistical Methods for Environmental Biologists.* John Wiley, Chichester.

HAIGH, N. (1990) EEC Environmental Policy and Britain. 2nd ed. Longmans, London.

(1995) Manual of Environmental Policy: the EC and Britain. 7th Release. Longmans, London.

HAINES, T.A. (1981) Acidic precipitation and its consequences for aquatic ecosystems: a review. *Trans. Am. Fish. Soc.* **110** 669–707.

HALL, D.J., LIKENS, G.E., FIANCE, S.B. and HENDRY,G.R. (1980) Experimental acidification of a stream in the Hubbard Brook Experimental Forest, New Hampshire. *Ecology* **61** 976–989.

HAMELINK, J.L. (1977) Current bioconcentration methods and theory. In: *Aquatic Toxicology and Hazard Evaluation. Proceedings of 1st Annual Symposium on Aquatic Toxicology.* American Society for Testing and Materials, Philadelphia, STP No. 634.

HANAWALT, P.C., FRIEDBERG, E.C. and Fox, C.F. (Eds) (1978) DNA *Repair Mechanisms.* Academic Press, New York.

HANEL, K. (1988) *Biological Treatment of Sewage by the Activated Sludge Process.* Ellis Horwood, Chichester.

HARPER, D. (1992) *Eutrophication of Freshwaters.* Chapman & Hall, London.

HART, C.W. and FULLER, S.L.H. (Eds) (1974) *Pollution Ecology of Freshwater Invertebrates.* Academic Press, New York.

HARTLEY, J.P. (1979) Biological monitoring of the seabed in the Forties oilfield. In: *Proceedings of a Conference on Ecological Damage Assessment, November, 1979. Arlington, Virginia.* Society of Petroleum Industry Biologists pp. 215–253.

(1982) Methods for monitoring offshore macrobenthos. *Mar. Pollut. Bull.* **13** 150–154.

HASLAM, S.M. (1982) A proposed method for monitoring river pollution using macrophytes. *Env. Technol. Letters* **3** 19–34.

HAWKES, H.A. (1963) *The Ecology of Waste Water Treatment.* Pergamon Press, Oxford.

(1975) River zonation and classification. In: Whitton, B.A. (Ed.) *River Ecology*. Blackwell Scientific Publications, Oxford.

(1983a) Activated sludge. In: Curds, C.R. and Hawkes, H.A. (Eds) *Ecological Aspects of Used-water Treatment, Vol II. Biological Activities and Treatment Processes.* Academic Press, London, pp. 77–162.

(1983b) Stabilisation ponds. In: Curds, C.R. and Hawkes, H.A. (Eds) *Ecology of Used-water Treatment, Vol. II. Biological Activities and Treatment Processes.* Academic Press, London pp. 163–218.

HELLAWELL, J.M. (1977) Biological surveillance and water quality monitoring. In: Alabaster, J.S. (Ed.) *Biological Monitoring of Inland Fisheries.* Applied Science Publishers, London.

(1978) *Biological Surveillance of Rivers—A Biological Monitoring Handbook.* Water Research Centre.

(1986) *Biological Indicators of Freshwater Pollution and Environmental Management.* Elsevier Applied Science Publishers, London.

HELLER, S., FONG, W.F. and CANELLAKIS, E.S. (1976) Induction of a protein inhibitor to ornithine decarboxylase by the end products of its reaction. *Proc. Nat. Acad. Sci. USA* **73**, 1858–1861.

HERBERT, D.W.M., ALABASTER, J.S., DART, M.C. and LLOYD, R. (1961) The effect of China-clay wastes on trout streams. *Int. J. Air Water Poll.* **5** 56–74.

HERBERT, D.W.M., ELKINS, G.H.J., MANN, H.T. and HEMENS, J. (1957) Toxicity of synthetic detergents to rainbow trout. *Water Waste Treat. J.* **6** 394–397R.

HERBERT, D.W.M. and MERKENS, J.C. (1961) The effect of suspended mineral solids on trout. *Int. J. Air Water Poll.* **5** 46–55.

HERBERT, D.W.M. and SHURBEN, D.S. (1965) The susceptibility of salmonid fish to poisons under estuarine conditions. II—ammonium chloride. *Int. J. Air Water Poll.* **9** 89–91.

HERBERT, D.W.M. and WAKEFORD, A.C. (1964) The susceptibility of salmonid fish to poisons under estuarine conditions. I. Zinc sulphate. *Int. J. Air Water Poll.* **8** 251–256.

HERMANUTZ, R.O., MUELLER, L.H. and KEMFERT, K.D. (1973) Captan toxicityto fathead minnows *(Pimephales promelas)* bluegills *(Lepomis macrochirus)* and brook trout *(Salvelinus fontinalis) . J. Fish. Res. Bd Can.* **30** 1811–1817.

HEWLETT, P.S. and PLACKETT, R.L. (1979) *The Interpretation of Quantal Responses in Biology.* Edward Arnold, London.

HIGGINS, I.J. and BURNS, R.G. (1975) *The Chemistry and Microbiology of Pollution.* Academic Press, London.

HILL, I.R., HEIMBACH, F., LEEUWANGH, P. and MATTHIESSEN, P. (1994) *Freshwater Field Tests for Hazard Assessment of Chemicals.* CRC Press, Boca Raton.

HILSENHOFF, W.L. (1987) An improved biotic index of organic stream pollution. *The Great Lakes Entomologist* **20** 31–39.

HIRSCH, E. (1963) Strukturelemente von Alkylbenzolsulfonaten und ihr Einfluss auf das Verhalten von Fischen. *Vom Wass.* **30** 249–259.

HMSO (1983a) *Acute Toxicity Testing with Aquatic Organisms 1981. Methods for Examination of Waters and Associated Materials.* Her Majesty's Stationery Office, London.

(1983b) *Bacteriological Examination of Drinking Water Supplies 1982.* Her Majesty's Stationery Office, London.

(1985) *Methods of Biological Sampling. A Colonisation Sampler For Collecting Macroinvertebrate Indicators of Water Quality in Lowland Rivers, 1983. Methods for the Examination of Waters and Associated Materials.* Her Majesty's Stationery Office, London.

(1990) *Isolation and Identification of* Giardia *cysts,* Cryptosporidium *Oocysts and Free Living Pathogenic Amoebae in Water etc. 1989. Methods for the Examination of Waters and Associated Materials.* Her Majesty's Stationery Office, London.

HOKANSON, K.E.F. and SMITH, L.L. (1971) Some factors influencing the toxicity of linear alkylbenzene sulphonate (LAS) to the bluegill. *Trans. Am. Fish. Soc.* **100** 1–12.

HOLME, N.A. and MCINTYRE, A.D. (Eds) (1984) *Methods for the Study of Marine Benthos.* 2nd ed. IBP Handbook No. 16, Blackwell Scientific Publications, Oxford.

HORAN, N.J. (1990) *Biological Wastewater Treatment Systems—Theory and Operation.* John Wiley & Sons, Chichester.

HORNICK, R.B. (1982) Typhoid fever. In: Evans, A.S. and Feldman, H.A. (Eds) *Bacterial Infections of Humans.* Plenum Publishing Corporation, New York.

HOWARTH, R.S. and SPRAGUE, J.B. (1978) Copper lethality to rainbow trout in waters of various hardness and pH. *Water Res.* **12** 455–462.

HOWARTH, W. (1990) *The Law of the National Rivers Authority.* National Rivers Authority and Centre for Law in Rural Areas, University College of Wales, Aberystwyth.

HOWELLS, G. (1990) *Acid Rain and Acid Waters.* Ellis Horwood, Chichester.

HOWELLS, G.D. (1983) The effects of power station cooling water discharges on aquatic ecology. *Water Poll. Control* **82** 10–17.

HUBERMAN, E. and SACHS, L. (1974) Cell-mediated mutagenesis of mammalian cells with chemical carcinogens. *Int. J. Cancer* **13,** 326–335.

HUNTER, J.B., Ross, S.L. and TANNAHILL, J. (1980) Aluminium pollution and fish toxicity. *Water Poll. Control* **79** 413–420.

HURLBERT, S.H. (1971) The nonconcept of species diversity: a critique and alternative parameters. *Ecology* **52** 577–586.

HYNES, H.B.N. (1960) *The Biology of Polluted Waters.* Liverpool University Press.

(1970) *The Ecology of Running Waters.* Liverpool University Press.

ISO (1985) *International Standard 7828. Water Quality Methods. Biological Sampling: Guidance on Hand-net Sampling of Aquatic Macroinvertebrates.* International Standards Organisation.

(1991) *Draft International Standard 9391. Water Quality Sampling in Deep Waters for Macroinvertebrates. Guidance on Use of Colonisation, Qualitative and Quantitative Samplers.* International Standards Organisation.

ISO/BMWP (1979) *Assessment of the Biological Quality of Rivers by a Macroinvertebrate Score.* ISO/TC147/SC5/WG6/N5, International Standards Organisation.

IWEM (1994) *Tertiary Treatment. 2nd* ed. Handbooks of UK Wastewater Practice, Institution of Water and Environmental Management, London.

JACOB, S.T. and ROSE, K.M. (1976) Stimulation of RNA polymerase I, II and III from rat liver by permidine, and specific inhibition of RNA polymerase I by higher spermidine concentrations. *Biochim. Biophys. Acta* **425**, 125–131.

JAMES, A. and EVISON, L. (1979) *Biological Indicators of Water Quality.* John Wiley, Chichester.

JAMES M.MONTGOMERY INC. (1985) *Water Treatment Principles and Design.* John Wiley & Sons, New York.

JEFFRIES, M. and MILLS, D. (1990) *Freshwater Ecology—Principles and Applications.* Belhaven Press, London.

JOHNSTON, R. (1984) Oil pollution and its management. In: Kinne, O. (Ed.) *Marine Ecology Vol. V: Ocean Management. Part 4: Pollution and Protection of the Seas.* John Wiley & Sons, Chichester.

JOLLIFFE, I.T. (1986) *Principal Components Analysis.* Springer-Verlag, New York.

JONES, J.R.E. (1940a) A study of the zinc-polluted river Ystwyth in North Cardiganshire, Wales. *Ann. Appl. Biol.* **27** 3676–3678.

(1940b) The fauna of the river Melindwr, a lead-polluted tributary of the river Rheidol in north Cardiganshire, Wales. *J. Anim. Ecol.* **9** 188–200.

(1949) An ecological study of the river Rheidol, North Cardiganshire, Wales. *J. Anim. Ecol.* **18** 67–88.

(1958) A further study of the zinc-polluted river Ystwyth. *J. Anim. Ecol.* **27** 1–14.

KAESLER, R.L., HERRICKS, E.E. and CROSSMAN, J.S. (1978) Use of indices of diversity and hierarchical diversity in stream surveys. In: Dickson, K.L. and Cairns, J. (Eds) *Biological Data in Water Pollution Assessment: Quantitative and Statistical Analysis.* ASTM Special Technical Publication No. 652. American Society for Testing and Materials, Philadelphia.

KAGI, J.H.R. and NORDBERG, M. (Eds) (1979) *Metallothionein.* Birkhauser Verlag, Basel.

KAISER, K.L.E. (Ed.) (1987) *QSAR in Environmental Toxicology II.* D.Reidel, Dordrecht, Holland.

KEEFE, T.J. and BERGERSEN, E.R. (1977) A simple diversity index based on the theory of runs. *Water Res.* **11** 689–691.

KENDALL, M.G. (1962) *Rank Correlation Methods.* Griffin and Co., London. KEUSCH, G.T. (1982) Shigellosis. In: Evans, A.S. and Feldman, H.A. (Eds)

*Bacterial Infections of Humans.* Plenum Publishing Corporation, New York.

KHAN, M.A.Q. (Ed.) (1977) *Pesticides in Aquatic Environments.* Plenum Press, New York.

KIMERLE, R.A. and SWISHER, R.D. (1977) Reduction of toxicity of linear alkylbenzene sulphonate (LAS) by biodegradation. *Water Res.* **11** 31–37.

KINNE, O. (Ed.) (1984a) *Marine Ecology Volume V: Ocean Management. Part 3. Pollution and Protection of the Seas: Radioactive Materials, Heavy Metals and Oil.* John Wiley & Sons, Chichester.

(1984b) *Marine Ecology. Volume V: Ocean Management: Part 4. Pollution and Protection of the Seas: Pesticides, Domestic Wastes and Thermal Deformations.* John Wiley & Sons, Chichester.

KLEIN, L. (1966) *River Pollution. 3. Control.* Butterworths, London.

KOLKWITZ, R. and MARSSON, M. (1909) Okologie der Tierischen Saprobien. *Int. Rev. Ges. Hydrobiol.* **2** 125–152.

KONEMANN, H. (1981) Quantitative structure-activity relationships in fish toxicology studies. Part 1. A relationship for 50 industrial pollutants. *Toxicology* **19** 209–221.

KORYAK, M., SHAPIRO, M.A. and SYKORA, J.L. (1972) Riffle zoobenthos in streams receiving acid mine drainage. *Water Res.* **6** 1239–1247.

KOTHE, P. (1962) Der 'Artenfehlbetrag', ein Einfaches Gutekriterium une Siene Anwerdung bei Biologischen Vorfluteruntersuchungen. *Deutsche Gewas-serkundl. Mitt.* **6** 60–65.

KUMAGURU, A.K. and BEAMISH, F.W.H. (1981) Lethal toxicity of Permethrin to rainbow trout *S. gairdneri,* in relation to body weight and temperature. *Water Res.* **15** 503–505.

LACK, T.J. (Ed.) (1984) *Environmental Protection: Standards, Compliance and Costs*. Ellis Horwood, Chichester, for Water Research Centre.

LAURIE, R.D. and JONES, J.R.E. (1938) The faunistic recovery of a lead-polluted river in north Cardiganshire, Wales. *J. Anim. Ecol.* **1** 272–289.

LEE, G.F. (1973) Chemical aspects of bioassay techniques for establishing water quality criteria. *Water Res.* **7** 1525–1546.

LEMLIN, J.S. (1980) The value of ecological monitoring in the management of petroleum industry discharges. *Water Sci. Technol.* **13** 437–464.

LESTER, W.F. (1975) Polluted river: River Trent, England. In: Whitton, B.A. (Ed.) *River Ecology*. Blackwell Scientific Publications, Oxford, pp. 489–513.

LETTERMAN, R.D. and MITSCH, W.J. (1978) Impact of mine drainage on a mountain stream in Pennsylvania. *Environ Poll.* 53–73.

LEWIS, J.R. (1972) Problems and approaches to baseline studies in coastal communities. In: Ruivo, M. (Ed.) *Marine Pollution and Sea Life.* Fishing News Books for FAO, pp. 401–404.

LINDAHL, P. and CABRIDENC, R. (1978) Molecular structure-biological properties relationships in anionic surface-active agents. *Water Res.* **12** 25–30.

LITCHFIELD, J.T. (1949) A method for rapid graphic solution of time-per cent effect curves. *J. Pharmac. Exp. Ther.* **97** 399–408.

LITCHFIELD, J.T. and WILCOXON, F. (1949) A simplified method of evaluating dose-effect experiments. *J. Pharmac. Exp. Ther.* **96** 99–113.

LLOYD, R. (1960) Toxicity of zinc sulphate to rainbow trout. *Ann. Appl. Biol.* **48** 84–94.

(1961) Toxicity of mixtures of zinc and copper sulphates to rainbow trout *(Salmo gairdneri* Richardson). *Ann. Appl. Biol.* **49** 535–538.

(1965) Factors that affect the tolerance of fish to heavy metal poisoning. In: *Biological Problems in Water Pollution.* US Public Health Service, Washington, 99-WP-25, pp. 181–187.

(1972) Problems in determining water quality criteria for freshwater fisheries. *Proc. R. Soc. Lond.* **B180** 429–449.

(1991a) Interpretation and application of ecotoxicological data: II. Complex effluents. In: Abel, P.D. and Axiak, V. (Eds) *Ecotoxicology and the Marine Environment.* Ellis Horwood, Chichester.

(1991b) Interpretation and application of ecotoxicological data: I. Single chemicals. In: Abel, P.D. and Axiak, V. (Eds) *Ecotoxicology and the Marine Environment*. Ellis Horwood, Chichester.

LLOYD, R. and ORR, L.D. (1969) The diuretic response by rainbow trout to sublethal concentrations of ammonia. *Water Res.* **3** 335–344.

LUNDGREN, D.G., VESTAL, J.R. and TABITA, F.R. (1972) The microbiology of mine drainage pollution. In: Mitchell, R. (Ed.) *Water Pollution Microbiology*. Wiley Interscience, New York.

MACEK, K.J. and SLEIGHT, B.H. (1977) Utility of toxicity tests with embryos and fry of fish in evaluating hazards associated with the chronic toxicity of chemicals to fishes. In: *Aquatic Toxicology and Hazard Evaluation. Proceedings of the First International Symposium on Aquatic Toxicology.* American Society for Testing and Materials, Philadelphia, STP No. 634.

MAFF (1991) *Code of Agricultural Practice for the Protection of Water*. Ministry of Agriculture, Fisheries and Food, London.

MAITLAND, P.S. (1980) The habitats of British Ephemeroptera. In: Flannagan, J. F. and Marshall, K.E. (Eds) *Advances in Ephemeroptera Biology.* Plenum Press, New York.

(1990) *Biology of Fresh Waters.* 2nd ed. Blackie and Sons, Glasgow.

MAKI, A.W. and BISHOP, W.E. (1979) Acute toxicity studies of surfactants to *Daphnia magna* and *Daphnia pulex. Environ. Contam. Toxicol.* **8** 599–612.

MAKI, A.W. and DUTHIE, J.R. (1978) Summary of proposed procedures for the evaluation of aquatic hazard. In: *Estimating the Hazard of Chemical Substances to Aquatic Life.* ASTM Special Technical Publication No. 657, American Society for Testing and Materials, Philadelphia.

MANCE, G. (1987) *Pollution Threat of Heavy Metals in Aquatic Environments.* Elsevier Applied Science, London.

MARA, D.D. (1988) *Waste Stabilisation Ponds.* Pergamon Press, Oxford.

MARCHETTI, R. (1965) The toxicity of nonylphenol ethoxylate to the developmental stages of the rainbow trout, *S. gairdneri* Richardson. *Ann. Appl. Biol.* **55** 425–430.

MARGALEF, R. (1958) Information theory in ecology. *Gen. Syst.* **3** 36–71.

MARKING, L.L. (1977) Method for assessing additive toxicity of chemical mixtures. In: Mayer, F.L. and Hamelink, J.L. (Eds) *Aquatic Toxicology and Hazard Evaluation. Proceedings of the First International Symposium on Aquatic Toxicology, Philadelphia.* American Society for Testing and Materials, STP 634, pp. 99–108.

MASON, C.F. (1991) *Biology of Freshwater Pollution.* 2nd ed. Longman, London.

MATA, L.J. (1978) *The Children of Santa Maria Cauque: a Prospective Field Study of Health and Growth.* MIT Press, Cambridge.

MAUGH, T.H. (1978) How many chemicals are there? *Science* 1**99** 162.

MCENROE, W.D. and HEALY, S.R. (1977) Ornithine decarboxylase may function as an initiation factor for RNA polymerase I. *Science* **195**, 505.

MCINTOSH, R.P. (1967) An index of diversity and the relation of certain concepts to diversity. *Ecology* **48** 392–404.

MCKIM, J.M. (1977) Evaluation of tests with early life stages of fish for predicting long-term toxicity. *J. Fish. Res. Bd Can.* **34** 1148–1154.

MCLEAY, D.J. and BROWN, D.A. (1974) Growth stimulation and biochemical changes in juvenile coho salmo *(Oncorhynchus kisutch)* exposed to bleached kraft mill effluent for 200 days. *J. Fish. Res. Bd Can.* **31** 1043–1049.

MCLOUGHLIN, J. and BELLINGER, E.G. (1993) *Environmental Pollution Control: An Introduction to Principles and Practice of Administration*. Graham & Trotman, London.

MEEHAN, T. and STRAUB, K. (1979) Double-stranded DNA stereoselectively binds benzo(a)pyrene diol epoxides. *Nature* **277**, 410–412.

MEHRLE, P.M. and MAYER, F.L. (1980) Clinical tests in aquatic toxicology: state of the art. *Environ. Health Perspectives* **34** 139–143.

MENHINICK, E.P. (1964) A comparison of some species-individuals diversity indices applied to samples of field insects. *Ecology* **45** 859–861.

MER (1996) One in ten ABS and ABV ships detained in Australia. *Marine Engineers' Review,* February, p. 7.

METCALFE, J.L. (1989) Biological water quality assessment of running waters based on macroinvertebrate communities: history and present status in Europe. *Environ. Poll.* **60** 101–139.

METCALFE-SMITH, J.L. (1994) Biological water quality assessment of rivers: use of macroinvertebrate communities. In: Calow, P. and Petts, G.E. (Eds) *The Rivers Handbook, Vol. II.* Black well Scientific Publications, Oxford.

MILLER, E.C. and MILLER, J.A. (1981) Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. *Cancer* **47**, 2327–2345.

MOORE, M.N. (1991) Cellular reactions to toxic environmental contaminants in marine molluscs. In: Abel, P.D. and Axiak, V. (Eds). *Ecotoxicology and the Marine Environment.* Ellis Horwood, Chichester.

MOORE, R.J. (1978) Is *Acanthaster planci* an r-strategist? *Nature* **271** 56–57.

MORIARTY, F. (1984) Persistent contaminants, compartmental models and concentrations along food chains. In: Rasmussen, S. (Ed.) *Ecotoxicology. Proceedings of the Third Oikos Conference. Ecol. Bull.* (Stockholm) **36** 35–45.

MOSEY, F.E. (1983) Anaerobic processes. In: Curds, C.R. and Hawkes, H. (Eds) *Ecology of Used-water Treatment, Vol. II. Biological Activities and Treatment Processes.* Academic Press, London, pp. 219–260.

Moss, B. (1988) *Ecology of Fresh Waters—Man and Medium. 2n*d ed. Blackwell Scientific Publications, Oxford.

MOUNT, D.I. and STEPHAN, C.E. (1967a) A method for establishing acceptable toxicant limits for fish—malathion and butoxyethanol ester of 2, 4-D. *Trans. Am. Fish. Soc.* **96** 185–193.

(1967b) Chronic toxicity of copper to the fathead minnow *(Pimephales promelas)* in soft water. *J. Fish. Res. Bd Can.* **26** 2449–2457.

MUDRACK, K. and KUNST, S. (1986) *Biology of Sewage Treatment and Water Pollution Control.* Ellis Horwood, Chichester.

MUIRHEAD-THOMPSON, R.C. (1987) *Pesticide Impact on Stream Fauna, with Special Reference to Aquatic Invertebrates.* Cambridge University Press, Cambridge.

MURPHY, P.M. (1978) The temporal variability in biotic indices. *Environ. Poll.* **17** 227–236.

MUSIAL, C.E., ARROWOOD, M.J., STERLING, J.R. and GERBA, C.P. (1987) Detection of *Cryptosporidium* in water using polypropylene cartridge filters. *Appl. Environ. Microbiol.* **53,** 687–692.

NICOLSON, N. (1993) *An Introduction to Drinking Water Quality.* Institution of Water and Environmental Management, London.

NORRIS, R.H. and GEORGES, A. (1993) Analysis and interpretation of benthic macroinvertebrate surveys. In: Rosenberg, D.M. and Resh, V.H. (Eds) *Freshwater Biomonitoring and Benthic Macroinvertebrates.* Chapman & Hall, New York.

NORRIS, R.H., LAKE, P.S. and SWAIN, R. (1982) Ecological effects of mine effluents on the South Esk river, Tasmania. III—benthic macroinvertebrates. *Austr. J. Mar. Freshwater Res.* **33** 789–809.

NOY, J. and FEINMESSER, A. (1977) The use of wastewater for agricultural irrigation. In: Shuval, H.I. (Ed.) *Water Renovation and Reuse.* Academic Press, New York.

NRA (1990) *Toxic Blue-green Algae.* National Rivers Authority Water Quality Series No. 2. Her Majesty's Stationery Office, London.

(1991) *The Quality of Rivers, Canals and Estuaries in England and Wales: Report of the 1990 Survey.* National Rivers Authority Water Quality Series No. 4. Her Majesty's Stationery Office, London.

(1992) *Water Pollution Incidents in England and Wales 1991.* National Rivers Authority Water Quality Research Series No. 7. Her Majesty's Stationery Office, London.

(1993) *Water Pollution Incidents in England and Wales 1992.* National Rivers Authority Water Quality Research Series No. 13. Her Majesty's Stationery Office, London.

(1994a) *Water Pollution Incidents in England and Wales 1993.* National Rivers Authority Water Quality Series No. 21. Her Majesty's Stationery Office, London.

(1994b) *Implementation of the EC Freshwater Fish Directive: Water Quality Requirements for the Support of Fish Life.* National Rivers Authority Water Quality Series No. 20. Her Majesty's Stationery Office, London.

NRIAGU, J.O. and SIMMONS, M.S. (1983) *Toxic Contaminants in the Great Lakes.* John Wiley & Sons, New York.

NSCA (1992) *1992 Pollution Handbook.* National Society for Clean Air and Environmental Protection, Brighton.

NUTTALL, P.M. and PURVES, J.B. (1974) Numerical indices applied to the results of a survey of the macroinvertebrate fauna of the Tamar catchment (south-west England). *Freshwater Biol.* **4** 213–222.

ODONELL, A.R., MANCE, G. and NORTON, R. (1984) *A Review of the Toxicity of Aluminium in Fresh Water.* Technical Report No. 197, Water Research Centre.

ODUM, H.T., CANTLON, J.E. and KORNICKER, L.S. (1960) An organisational hierarchy entropy and ecosystem evolution, and the meaning of a species-variety index. *Ecology* **41** 395–399.

OESCH, F. (1982) Chemical carcinogenesis by polycyclic aromatic hydrocarbons. In: Nicolini, C. (Ed.) *Chemical Carcinogenesis.* Plenum Publishing, New York, pp. 1–24.

OPEN UNIVERSITY (1993) *Environmental Control and Public Health: Water. T 237, Unit 7: Water Supply and Sewage Treatment.* Open University, Milton Keynes.

ORMEROD, S.V., BOOLE, P., MCCAHON, C.P., WEATHERLY, N.S., PASCOE, D. and EDWARDS, R.W. (1987) Short term experimental acidification of a Welsh stream: comparing the biological effects of hydrogen ions and aluminium. *Freshwater Biol.* **17** 341–356.

PAGENKOPF, G.K., Russo, R.C. and THURSTON, R.V. (1974) Effect of complexation on toxicity of copper to fishes. *J. Fish. Res. Bd Can.* **31** 462–465.

PANTLE, R. and BUCK, H. (1955) Die biologische uberwachung der Gewasser und die Darstellung der Ergebrisse. *Besondere Mitteilugen zum Deutschen Gewasserkundlichen Jahrbuch* **12** 135–143.

PARR, W. (1994) Water quality monitoring. Ch. 7 In: Calow, P. and Petts, G.E. (Eds) *The Rivers Handbook,* Vol. 2. Blackwell, Oxford.

PASCOE, D. and CRAM, P. (1977) The effect of parasitism on the toxicity of cadmium to the three-spined stickleback, *Gasterosteus aculeatus. J. Fish Biol.* **10** 467–472.

PATTEN, B.C. (1962) Species diversity in net plankton of Raritan Bay. *J. Mar. Res.* **20** 57–75.

PEARSON, R.G. and JONES, N.V. (1975) The colonisation of artificial substrate by stream macroinvertebrates. *Progr. Water Technol.* **7** 497–504.

PEARSON, T.H., GRAY, J.S. and JOHANNESSEN, P.J. (1983) Objective selection of sensitive species indicative of pollution-induced change in benthic communities. 2. Data analyses. *Mar. Ecol. Prog. Ser.* **12** 237–255.

PERKINS, E.J. (1974) *The Biology of Estuaries and Coastal Waters.* Academic Press, London.

(1979) The effects of marine discharges on the ecology of coastal waters. In: James, A. and Evison, L. (Eds) *Biological Indicators of Water Quality.* John Wiley & Sons, Chichester.

PERRING, F.H. and MELLANBY, K. (Eds) (1977) *Ecological Effects of Pesticides. Linnean Society Symposium No. 5.* Academic Press, London.

PERSOONE, G. and DE PAUW, N. (1979) Systems of biological indicators for water quality assessment. In: Ravera, O. (Ed.) *Biological Aspects of Freshwater Pollution.* Pergamon Press, Oxford.

PHILLIPS, J. (1972) Chemical processes in estuaries. In: Barnes, R.S.K. and Green, J. (Eds) *The Estuarine Environment.* Applied Science Publishers, London.

PIELOU, E.C. (1984) *The Interpretation of Ecological Data.* John Wiley & Sons, New York.

PITCAIRN, C.E.R. and HAWKES, H.A. (1973) The role of phosphorus in the growth of *Cladophora. Water Res.* **7** 159–71.

POFF, N.L. and MATTHEWS, R.A. (1986) Benthic invertebrate community structural and functional response to thermal enhancement in the Savannah river and a coastal plain estuary. *Archiv. fur Hydrobiol.* **106,** 119–137.

PORTER, E. (1973) *Pollution in Four Industrialised Estuaries.* Her Majesty's Stationery Office, London.

PRESTON, F.W. (1948) The commonness and the rarity of species. *Ecology* **29** 254–283.

PRESTON, M.R. (1989) Marine Pollution. In: Riley, J.P. (Ed.) *Chemical Oceanography,* Vol. 9. Academic Press, New York.

PRICE, A.R.G. and ROBINSON, J.H. (1993) The 1991 Gulf War: coastal and marine environmental consequences. *Mar. Poll. Bull.* **27**.

RAABE, E.W. (1952) Uber den 'Affinititatswert' in der Planzensoziologie. Vegetatio. *Haag* **4** 53–68.

RADFORD, D.S. and HARTLAND-ROWE, R. (1971) Subsurface and surface sampling of benthic invertebrates in two streams. *Limnol. Oceanogr.* **16** 114–120.

RAFAELLI, D.G. and MASON, C.F. (1981) Pollution monitoring with meiofauna, using the ratio of nematodes to copepods. *Mar. Poll. Bull.* **12** 158–163.

RAHEL, F.J. (1982) Population differences in acid tolerance between yellow perch, *Perca flavescens,* from naturally acidic and alkaline lakes. *Can. J. Zool.* **61** 147–152.

RAO, V.C. and MELNICK, J.L. (1986) *Environmental Virology.* Van Nostrand Reinhold UK, Wokingham.

REID, D.A. (1993) Regulation of non-point source water pollution in Scotland. In: Thomas, P. (Ed.) *Water Pollution Law and Liability.* Graham & Trotman and International Bar Association, London, pp. 91–104.

REISH, D.J. (1973) The use of benthic animals in monitoring the marine environment. *J. Environ. Plann. Poll. Control* **1** 32–38.

(1984) Domestic wastes. In: Kinne, O. (Ed.) *Marine Ecology Vol. V: Ocean Management Part 4: Pollution and Protection of the Seas.* John Wiley & Sons, Chichester.

REISH, D.J. and OSHIDA, P.S. (1986) *Manual of Methods in Aquatic Environmental Research. Part 10. Short-term Static Bioassays.* FAO Fisheries Technical Paper No. 247. FAO, Rome.

RESH, V.H. and MCELRAVY, E.P. (1993) Contemporary quantitative approachesto biomonitoring using benthic macroinvertebrates . In: Rosenberg, D.M. and Resh, D.H. (Eds) *Freshwater Biomonitoring and Benthic Macroinvertebrates.* Chapman & Hall, New York.

REYNOLDS, K.A. and ROSE, B. (1991) New approaches for the detection of human enteric viruses in shellfish and harvesting waters. *Florida J. Publ. Health* **3** 29–37.

RITTMAN, B.E. (1992) Innovations in biological processes for pollution control. Ch. 10 In: Mitchell, R. (Ed.) *Environmental Microbiology.* John Wiley, New York.

ROCH, M., MCCARTER, J.A., MATHESON, A.T., CLARK, M.J.R. and OLAFSON, R.W. (1982) Hepatic metallothionein in rainbow trout *(Salmo gairdneri)* as an indicator of metal pollution in the Campbell river system. *Can. J. Fish. Aquat. Sci.* **39** 1596–1601.

ROSENBERG, D.M. and RESH, D.H. (Eds) (1993) *Freshwater Biomonitoring and Benthic Macroinvertebrates.* Chapman & Hall, New York.

ROYAL COMMISSION ON ENVIRONMENTAL POLLUTION (1972) *Third Report: Pollution in some British Estuaries and Coastal Waters.* Her Majesty's Stationery Office, London.

RUSSELL, D.H. (1971) Drug stimulation of putrescine and spermidine synthesis. Relationship to enhancement of ribonucleic acid synthesis. *Biochem. Pharmacol.* **20** 3481–3484.

(Ed.) (1973) *Polyamines in Normal and Neoplastic Growth.* Raven Press, New York.

RYE, R.P. and KING, E.L. (1976) Acute toxic effects of two lampricides to twenty-one freshwater invertebrates. *Trans. Am. Fish. Soc.* **105** 322–326.

SANDERS, B. (1990) Stress proteins: potential as multitiered biomarkers. In: McCarthy, J.F. and Shugart, L.R. (Eds) *Biomarkers of Environmental Contamination.* Lewis Publishers, Boca Raton.

SANDERS, H.O. (1970) Toxicities of some herbicides to six species of freshwater crustaceans. *J. Water Poll. Contr. Fed.* **42** 1544–1550.

SATTAR, S.A. (1981) Virus survival in receiving waters. In: Goddard, M. and Butler, M. (Eds) *Viruses and Wastewater Treatment.* Pergamon Press, Oxford.

SCHNOOR, J.L. (1982) Field validation of water quality criteria for hydrophobic pollutants. In: *Aquatic Toxicology and Hazard Assessment. Proceedings of the 5th Annual Symposium on Aquatic Toxicology. Philadelphia.* American Society for Testing and Materials, STP No. 737.

SCULLION, J. and EDWARDS, R.W. (1980) The effect of coal industry pollutants on the macroinvertebrate fauna of a small river in the South Wales coalfield. *Freshwater Biol.* **10** 141–162.

SHANNON, C.E. and WEAVER, E. (1949) *The Mathematical Theory of Communication.* University of Illinois Press, Urbana, pp. 82–83, 104–107.

SHAW, T.L. and BROWN, V.M. (1974) The toxicity of some forms of copper to the rainbow trout. *Water Res.* **8** 377–382.

SHIELLS, G.M. and ANDERSON, K.J. (1985) Pollution monitoring using the nematode-copepod ratio—a practical application. *Mar. Poll. Bull.* **16 62**–68.

SHUBERT, E. (1984) *Algae as Ecological Indicators.* Academic Press, London.

SHUVAL, H.I. (Ed.) (1977a) *Water Renovation and Reuse.* Academic Press, New York.

(1977b) Health considerations in water reuse. In: Shuval, H.I. (Ed.) *Water Renovation and Reuse.* Academic Press, New York.

SIMPSON, E.H. (1949) Measurement of diversity. *Nature* **163** 688.

SINGH, A. and MCFETERS, G.A. (1992) Detection Methods for waterborne pathogens. Ch. 6 In: Mitchell, R. (Ed.) *Environmental Microbiology,* John Wiley, New York.

SITTIG, M. (1975) *Environmental Sources and Emissions Handbook.* Noyes Data Corporation, New Jersey.

SKALSKI, J.R. (1981) Statistical inconsistencies in the use of no-observed effect levels in toxicity testing. In: Branson, D.R. and Dickson, K.L. (Eds) *Aquatic Toxicology and Hazard Assessment. Proceedings of the Fourth Annual Symposium on Aquatic Toxicology.* American Society for Testing and Materials STP No. 737. Philadelphia.

SKIDMORE, J.F. (1965) Resistance to zinc sulphate of the zebrafish (*Brachydanio rerio* Hamilton-Buchanan) at different phases of its life history. *Ann. Appl. Biol.* **56** 47–53.

(1970) Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. *J. Exp. Biol.* **52** 481–494.

SLADE, J.S. and FORD, B.J. (1983) Discharge to the environment of viruses in waste water, sludges and aerosols. In: Berg, G. (Ed.) *Viral Pollution of the Environment.* CRC Press, Boca Raton.

SLADECEK, V. (1979) Continental systems for the assessment of river water quality. In: James, A. and Evison, L. (Eds) *Biological Indicators of Water Quality.* JohnWiley , Chichester.

SMITH, J.E. (Ed.) (1968) *'Torrey Canyon' pollution and marine life.* Cambridge University Press, Cambridge.

SMITH, L.L. and OSEID, D.M. (1972) Effects of hydrogen sulphide on fish eggs and fry. *Water Res.* **6** 711–720.

SMITH, L.L., BRODERIUS, S.J., OSEID, D.M., KIMBALL, G.L. and KOENST, W. M. (1978) Acute toxicity of hydrogen cyanide to freshwater fishes. *Arch. Environ. Contam. Toxicol.* **7** 325–337.

SNEATH, P.H.A. and SOKAL, R.R. (1973) *Numerical Taxonomy.* W.H.Freeman, San Francisco, pp. 141–145.

SOLBÉ, J.F. DE L.G. (1973) The relation between water quality and the status of fish populations in Willow Brook. *Water Treatm. Exam.* **22** 41–61.

SOLBÉ, J.F. DE L.G. and FLOOK, V.A. (1975) Studies on the toxicity of zinc sulphate and of cadmium sulphate to stone loach, *Neomacheilus barbatulatus* (1.) in hard water. *J. Fish Biol.* **7** 631–637.

SORBER, C.A. (1983) Removal of viruses from wastewater and effluents. In: Berg, G. (Ed.) *Viral Pollution of the Environment.* CRC Press, Boca Raton.

SORENSEN, T. (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons. *Biol. Skr. (K. denske indensk. Selske. N.S.)* **5** 51–34.

SOUTHWARD, A.J. and SOUTHWARD, E.C. (1978) Recolonisation of rocky shores in Cornwall after use of toxic dispersants to clean up the 'Torrey Canyon' spill. *J. Fish. Res. Bd Can.* **35** 682–706.

SOUTHWOOD, T.R.E. (1978) *Ecological methods.* 2nd ed. Chapman & Hall, London.

SPRAGUE, J.B. (1969) The measurement of pollutant toxicity to fish. I—Bioassay methods for acute toxicity. *Water Res.* **3** 793–821.

(1970) The measurement of pollutant toxicity to fish. II—Utilising and applying bioassay results. *Water Res.* **4** 3–32.

(1971) The measurement of pollutant toxicity to fish. III—Sublethal effects and 'safe' concentrations. *Water Res.* **5** 245–266.

(1973) The ABCs of pollutant bioassay using fish. In: *Biological Methods for the Assessment of Water Quality.* ASTM Special Technical Publication No. 528, American Society for Testing and Materials, Philadelphia, pp. 6–30.

SPRAGUE, J.B., ELSON, P.F. and SAUNDERS, R.L. (1965) Sublethal copper-zinc pollution in a salmon river—a field and laboratory study. *Int. J. Air Water Poll.* **9** 531–543.

SPRAGUE, J.B. and RAMSAY, B.A. (1965) Lethal effects of mixed copper and zinc solutions for juvenile salmon. *J. Fish. Res. Bd Can.* **22** 425–432.

STEBBING, A.R.D. (1979) An experimental approach to the determinants of biological water quality. *Phil. Trans. R. Soc. Lond.* **B286** 465–481.

STEELE, J.H. (1979) The uses of experimental ecosystems. *Phil. Trans. R. Soc. Lond.* **B286** 583–595.

STENDAHL, D.H. and SPRAGUE, J.B. (1972) Effects of water hardness and pH on vanadium lethality to rainbow trout. *Water Res.* **16** 1479–1488.

STEPHAN, C.E. (1977) Methods for calculating an LC50. In: Mayer, F.L. and Hamelink, J.L. (Eds) *Aquatic toxicology and Hazard Evaluation. Proceedings of the First International Symposium on Aquatic Toxicology, Philadelphia.* American Society for Testing and Materials, STP No. 634, pp. 65–84.

STIFF, M.J. (Ed.) (1980) *River Pollution Control.* Ellis Horwood, Chichester, forWater Research Centre .

STIRN, J. (1981) *Manual of Methods in Marine Environmental Research. Part 8. Ecological Assessment of Pollutant Effects.* FAO Fish. Tech. Paper 209: FAO, Rome.

SUTCLIFFE, D.W. (1967) Sodium regulation and adaptation to fresh water in gammarid crustaceans. *J. Exp. Biol.* **46** 359–360.

(1983) *Acid Precipitation and its Effects on Aquatic Systems in the English Lake District (Cumbria).* Freshwater Biological Association Annual Report No. 50, Freshwater Biological Association, Ambleside, pp. 30–62.

SUTCLIFFE, D.W. and CARRICK, T.R. (1973) Studies on mountain streams in the English Lake District. I. pH, calcium and the distribution of invertebrates in the River Duddon. *Freshwater Biol.* **3** 437–462.

SWARTS, F.A., DUNSON, W.A. and WRIGHT, J.E. (1978) Genetic and environmental factors involved in increased resistance of brook trout to sulphuric acid solutions and mine-acid polluted waters. *Trans. Am. Fish. Soc.* **107** 651–677.

SWEDMARK, M., BRAATEN, B., EMANUELSSON, E. and GRANMO, A. (1971) Biological effects of surface active agents on marine animals. *Mar. Biol.* **9** 183–201.

SWISHER, R.D., O'ROURKE, J.T. and TOMLINSON, H.D. (1964) Fish bioassays of linear alkylate sulphonate (LAS) and intermediate biodegradation products. *J. Amer. Oil-chem. Soc.* **41** 746–752.

THATCHER, T.O. (1966) The comparative lethal toxicity of a mixture of hard ABS detergent products to eleven species of fishes. *Int. J. Air Water Poll.* **10** 585–590.

THATCHER, T.O. and SANTNER, J.F. (1967) *Acute Toxicity of LAS to Various Fish Species. Proceedings of the 21st Industrial Waste Conference,* Purdue University Engng. Ext. Ser. **121** 996–1002.

THORP, V.J. and LAKE, P.S. (1973) Pollution of a Tasmanian river by mine effluents. II—Distribution of macroinvertebrates. *Int. Rev. Ges. Hydrobiol.* **58** 885–892.

THURSTON, R.V., PHILLIPS, G.R., RUSSO, R.C. and HINKINS, S.M. (1981) Increased toxicity of ammonia to rainbow trout *(Salmo gairdneri)* resulting from reduced concentrations of dissolved oxygen. *Can. J. Fish. Aquat. Sci.* **38** 983–988.

TOOBY, T.E. (1978) A scheme for the evaluation of hazards to non-target aquatic organisms from the use of chemicals. *Proceedings of the Fifth Symposium of the European Weed Research Society,* pp. 287–294.

TSUBAKI, T. and IRUKAYAMA, K. (1977) *Minamata Disease.* Elsevier, Amsterdam.

TYLER, A.V. (1965) Some lethal temperature relations of two minnows of the genus Chrosomus. *Can. J. Zool.* **44** 349–364.

TYLER, P.A. and BUCKNEY, R.T. (1973) Pollution of a Tasmanian river by mine effluents. I—Chemical evidence. *Int. Rev. Ges. Hydrobiol.* **58** 873–883.

UNDERWOOD, A.J. (1994) Spatial and temporal problems with monitoring. In: Calow, P. and Petts, G.E. (Eds) *The Rivers Handbook, Vol. II.* Blackwell Scientific Publications, Oxford.

UNEP (1985) *Mediterranean Action Plan.* Co-ordinating Unit for Oceans and Coastal Areas of the United Nations Environment Programme, Athens.

(1989a) *Environmental Data Report.* 2nd ed. Blackwell, Oxford.

(1989b) *State of the Mediterranean Marine Environment.* MAP Technical Reports Series No. 28. United Nations Environment Programme, Athens.

(1991) *Environmental Data Report.* 3rd ed. Blackwell, Oxford.

UNEP/FAO/IAEA (1989) *Test of the Acute Lethal Toxicity of Pollutants to Marine Fish and Invertebrates.* Reference Methods for Marine Pollution Studies No. 43. United Nations Environment Programme, Nairobi.

UNITED NATIONS (1978) *Mediterranean Action Plan and the Final Act of the Conference of Plenipotentiaries of the Coastal States of the Mediterranean Region for the Protection of the Mediterranean Sea.* United Nations, New York.

(1980) *Conference of Plenipotentiaries of the Coastal States of the MediterraneanRegion for the Protection of the Mediterranean Sea against Pollution from Land-based Sources. Final Act and Protocol* . United Nations, New York.

(1984) *Protocol Concerning Mediterranean Specially Protected Areas.* United Nations, New York.

VARLEY, M. (1967) *British Freshwater Fishes: Factors Affecting their Distribution.* Fishing News (Books), London.

VAUGHN, J.M. and LANDRY, E.F. (1983) Viruses in soils and groundwaters. In: Berg, G. (Ed.) *Viral Pollution of the Environment.* CRC Press, Boca Raton.

WARD, G.S. and PARRISH, P.R. (1982) *Manual of Methods in Marine Environment Research. Part 6. Toxicity Tests.* FAO Fish. Tech. Paper No. 185. FAO, Rome.

WASHINGTON, H.G. (1984) Diversity, biotic and similarity indices. A review with special reference to aquatic ecosystems. *Water Res.* **18** 653–694.

WATTON, A.J. and HAWKES, H.A. (1984) Studies on the effects of sewage effluent on gastropod populations in experimental streams. *Water Res.* **18** 1235–1248.

WEATHERLEY, A.H., BEAVERS, J.R. and LAKE, P.S. (1967) The ecology of a zinc-polluted river. In: Weatherley, A.H. (Ed.) *Australian Inland Waters and their Fauna: Eleven Studies.* Australian National University Press, Canberra, pp. 252–278.

WEGL, R. (1983) Index fur die Limnosaprobitat. (Index for limnosaprobity.) *Wasser und Abwasser* **26** 1–175.

WHITE, J.B. (1978) *Wastewater Engineering.* Edward Arnold, London.

WHITTAKER, R.H. (1965) Dominance and diversity in land plant communities. *Science* **147** 250–260.

WHITTON, B.A. (1970) Biology of *Cladophora* in freshwaters. *Water Res.* **4** 457–476.

(Ed.) (1975) *River Ecology.* Blackwell Scientific Publications, Oxford.

(1979) Algae and higher plants as indicators of river pollution. In: James, A. and Evison, L. (Eds) *Biological Indicators of Water Quality.* John Wiley, Chichester.

(1980) Zinc and plants in rivers and streams. In: Nriagu, J.D. (Ed.) *Zinc in the Environment. Part II. Health Effects.* John Wiley, New York.

WHITTON, B.A. and SAY, P.J. (1975) Heavy Metals. In: Whitton, B.A. (Ed.) *River Ecology.* Blackwell Scientific Publications, Oxford, pp. 286–311.

WHITTON, B.A., SAY, P.J. and JUPP, B.P. (1982) Accumulation of zinc, cadmium and lead by the aquatic liverwort *Scapania. Environ. Poll. B.* **3** 299–316.

WHITTON, B.A., SAY, P.J. and WEHR, J.D. (1981) Use of plants to monitor heavy metals in rivers. In: Say, P.J. and Whitton, B.A. (Eds) *Heavy Metals in Northern England: Environmental and Biological Aspects.* University of Durham, Department of Botany.

WILCHER, L.S. (1993) From flammable to fishable: the story of improving US water quality, Ch. 2 In: Thomas, P. (Ed.) *Water Pollution Law and Liability.* Graham & Trotman and International Bar Association, London, pp. 25–34.

WILDISH, D.J. (1972) Acute toxicity of polyoxyethylene esters and polyoxyethylene ethers to *Salmo salar* and *Gammarus oceanicus. Water Res.* **6** 759–762.

WILHM, J.L. and DORRIS, T.C. (1968) Biological parameters for water quality criteria. *Bioscience* **18** 477–481.

WILLIAMS, C.B. (1964) *Patterns in the Balance of Nature, and Related Problems in Quantitative Ecology.* Academic Press, New York, pp. 14–31, 147–192.

WILLIAMS, E.T. (1971) Principles of clustering. *Ann. Rev. Ecol. Syst.* **2**, 202–326.

WINNER, R.W. and FARRELL, M.P. (1976) Acute and chronic toxicity of copper to four species of *Daphnia. J. Fish. Res. Bd Can.* **33** 1685–1691.

WOOD, L.B. (1982) *The Restoration of the Tidal Thames.* Adam Hilger Ltd, Bristol.

WOODIWISS, F. (1964) The biological system of stream classification used by the Trent River Board. *Chemistry and Industry,* **11** 443–447.

YANG, K.S., DEUTSCH, J. and GELBOIN, H.V. (1978) Benzo(a)pyrene metabolism: activation and detoxification. In: Gelboin, H.V. and Ts'O P.O.P. (Eds) *Polycyclic Hydrocarbons and Cancer. Vol. 1*. Academic Press, New York.

YANG, K.S., MCCOURT, D.W., LEUTZ, J.L. and GELBOIN, H.V. (1977) Benzo(a)-pyrenediolepoxide: mechanism of enzymatic formation and optically-active intermediates. *Science* **196,** 1199–1202.

YOUNG, D.D. (1980) River pollution control by quality objectives. In: Stiff, M.J. (Ed.) *River Pollution Control.* Ellis Horwood, Chichester, for Water Research Centre.

ZAHN, R.K. (1991) Primary deoxyribonucleic acid damage produced in the marine environment by pollution: assessment and possible consequences. Ch. 11 in: Abel, P.D. and Axiak, V. (Eds) *Ecotoxicology and the Marine Environment,* Ellis Horwood, Chichester.

ZAHN, R.K., ZAHN-DAIMLER, G., MULLER, W.E.C., MICHAELIS, M.L., KURELEC, B., RIJAVEC, M., BATEL, R. and BIHARI, N. (1983) DNA damage by PAH and repair in a marine sponge. *Sci. Total. Environ.* **26** 137–156.

ZAMAN, V. and AH KEONG, L. (1982) *Handbook of Medical Parasitology.* ADIS Health Science Press, New York.

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