discharging polluting effluent between sample visits. The pollution will impact on the most sensitive members of the aquatic community, acting as indicators of pollution. The amount of change in the community will be related to the severity of the incident. Because the community can only be restored to its former diversity by reproduction and immigration, its recovery is likely to be slow. If the intermittent pollution occurs with some frequency, the community will remain impoverished. The biologist will be able to detect such damage and suggest a more detailed surveillance programme, both biological and chemical, to find the source and frequency of pollution.

Thirdly, biological communities may respond to unsuspected or new pollutants in the environment. There are over 70 million registered inorganic and organic compounds of which 8400 chemicals have been comprehensively tested for toxicity.[2] Thousands are discharged to freshwaters, while only some 30 determinands will routinely be tested for given financial constraints in ambient water quality monitoring programmes. If there is a change in the biological community, however, then a wider screening for pollutants can be initiated. A famous example comes from male rainbow trout (*Oncorhynchus mykiss*), caged in the River Lea, north of London, below the point of discharge of effluent from a sewage treatment works, which exhibited marked increases in vitellogenin levels.[3] This compound is normally produced in the liver of female fish in response to the hormone oestradiol and is incorporated into the yolk of developing eggs. Clearly, some chemical in the effluent was behaving like a female hormone and the male trout provided an early warning of a potential problem requiring urgent investigation. Nonylphenols (widely used as anti-oxidants) were suspected of stimulating vitellogenin production in the trout. The effect has since been demonstrated in fish from a number of other rivers. Laboratory experiments have identified three such active compounds causing oestrogenic activity in treated sewage effluent, two naturally occurring hormones and an active ingredient of birth control pills.[3,4] There are many other substances, including DDT and some PCBs, which exert similar effects. For example, abnormalities in the eggs and hatchlings of snapping turtles (*Chelydra serpentina*) from the Great Lakes region of North America were considered to be caused by polychlorinated aromatic hydrocarbons.[5] Increases in testicular cancer and falling sperm counts in the human male may be related to oestrogen-mimics released into the environment,[6] and these endocrine disruptors have long since been of concern and research effort.

Finally, some chemicals are accumulated in the tissues of certain organisms, the concentrations reflecting environmental pollution levels over time. In any particular sample of water, the concentration of a pollutant may be too low to detect using routine methods, but nevertheless will be gradually accumulated within the ecosystem to levels of considerable concern in some species. Metals, organochlorine pesticides, and PCBs have caused particular problems in aquatic habitats and are potential threats to human health. Eels (*Anguilla anguilla*), for example, have historically been used to detect mercury below discharges of sewage effluent.[7]

4.1.2 Pollution Types and Interactions

Pollutants having biological impacts may derive from point sources, often discrete discharges known to the authorities, which have traditionally been the focus for regulatory monitoring. However, the previous example of endocrine disruptors emphasizes that we are not always aware of the ecological effects of compounds coming from apparently well-regulated sewage effluent discharges. This may also be the case with many emerging contaminants such as engineered nanoparticles and personal health care products which are considered later in the chapter. Alternatively, sources of pollution may be diffuse, entering watercourses from land drainage and run-off, such as fertilizers and pesticides applied to agricultural land. Management of our river systems has gained a greater appreciation of the importance of such diffuse sources in recent years with regulation focussing increasingly on catchment-scale processes as opposed to discrete point discharges. In

Europe, the *Water Framework Directive (WFD, 2000/60/EC)* has facilitated this major change in how we evaluate and manage our water environment. Biological monitoring is a crucial tool, given the aquatic communities present in our freshwaters represent a function of the broader catchment morphology, land use and other anthropogenic impacts acting over a range of timescales.

Much pollution is chronic, the watercourse receiving discharges continuously or regularly, and such pollution can generally be reduced to acceptable levels given the right regulatory framework. A greater problem is episodic or accidental pollution, which is unpredictable in space or time. Table 4.1 offers a range of examples of major accidental water pollution events and their biological effects. Such events typically receive much public and scientific attention, which can drastically improve our knowledge of the risks associated with, and biological effects of the pollutants involved. They can also have a major bearing on developing regulation to minimise the risks of such events reoccurring. For example, the Aznalcóllar and Baia Mare events were responsible for a major piece of legislation in Europe (the *Mining Waste Directive: 2006/21/EC*) intended to reduced the risk of subsequent mine and processing waste disasters in Europe. However, the Ajka red mud spill in Hungary highlights that even with appropriate regulatory frameworks, avoidable accidents do still happen (see Table 4.1). One of the recurring themes apparent in many of these acute pollution events is the problem of determining what the specific causal agents are for observed biological impacts amongst a mixture of contaminants. Most polluting effluents are usually a cocktail of potential toxicants and these will interact with each other and naturally occurring substances in a variety of ways. As such, as we aim to understand the effects of pollutants on biological communities we must be aware of interactions between chemical species and how these affect bioavailability and subsequent toxicity to organisms. These are often summarised into three main types of interaction:

(i) *Additive effects*: where the action of combined pollutants is equal to the sum of their individual effects, for example mixtures of zinc and copper, common in many of the mine waste disasters (see Table 4.1), act together additively.
(ii) *Synergistic effects*: where the effect of combined pollutants is greater than the sum of their individual effects. For example, synergistic effects of multiple organophosphate compounds were cited as important in the deaths of grayling (*Thymallus thymallus*) and brown trout (*Salmo trutta*) in the Rhine after the Sandoz chemical spill as individual concentrations of the relevant contaminants were several orders of magnitude below their individual lethal concentrations (see Table 4.1).
(iii) *Antagonistic effects*: where the effects of combined pollutants is less than the sum of their individual effects. For example calcium antagonizes the toxic effect of lead and aluminium, while abundant particulate iron oxides are likely to have been important in limiting the toxicity of chromium and arsenic after the Ajka red mud spill (see Table 4.1).

This review will describe the effects of major types of pollutants on aquatic life and will consider some of the key methods used for assessing the biological impacts of water pollution.

4.2 ORGANIC POLLUTION

Organic pollution results when large quantities of organic matter are discharged into a watercourse to be broken down by microorganisms which utilize oxygen to the detriment of the stream biota. The most important source of organic pollution globally is the discharges associated with untreated or treated sewage. Over 2.5 billion people live without improved sanitation and 780 million with unsafe drinking water sources[16] which poses an extreme risk to human health through parasitic infections and waterborne diseases such as dysentery and cholera; a health burden which falls disproportionately on children.

In countries with established water management infrastructure, sewage treatment works (STWs) can still be enduring sources of instream organic pollution, particularly where infrastructure predates any rapid population growth in the towns they serve. In Europe, the water industry does, however, have to meet increasingly stringent legislation on the quality of discharged effluents (*e.g.* the *Urban Waste Water Treatment Directive* and the demands of the *Water Framework Directive*) which has seen demonstrable improvements in levels of organic pollution in many streams over recent decades. As such, in many catchments the chief sources of organic pollution are more likely to be related to:

(i) Short-term storm pollution events in urban areas that see Combined Sewer Overflows discharge untreated sewage directly into streams and rivers.
(ii) Farm effluents, especially in areas of intensive pastoral farming, which are likely to be diffuse in origin.
(iii) Discharges from other industries such as dairies, breweries and food processing plants.

Biochemical Oxygen Demand is a simple measure of the potential of organic matter for deoxygenating water which is determined in the laboratory by incubating a sample of water for five days at 20 °C and determining the oxygen used. In most of Europe, BOD targets are typically set by the oxygen conditions associated with macro-invertebrates, which are generally most sensitive to organic pollution, with different values for upland and lowland rivers (with values <4 mg l^{-1} or <5 mg l^{-1} respectively for a 'Good' status for upland and lowland streams). However, more stringent standards are enforced for lowland rivers if they support salmonids.[17]

In recent surveys of pollution pressures in catchments across England and Wales, 7.9% of water bodies (with an associated stream length >4500 km) were deemed at risk of not meeting European standards for Biochemical Oxygen Demand (BOD) while 10.5% (>6100 km of river) were deemed at risk of not meeting ammonia standards (which is a decay product of the breakdown of animal and vegetable wastes and common to most organic pollution sources).[18] The distribution of these impacted catchments around major urban centres and some intensive areas of agriculture predominantly in eastern England suggest a range of sources. These data support general trends of chronic, gross organic pollution diminishing greatly in the last three decades, but there is still widespread mild organic pollution in many settings, which can cause enduring impacts to aquatic biota.[19]

Figure 4.1 outlines the general effects of an organic effluent on a receiving stream.[1] At the point of entry of the discharge there is a sharp decline in the concentration of oxygen in the water, known as the oxygen sag curve. At the same time there is a large increase in BOD as the microorganisms added to the stream in the effluent and those already present utilize the oxygen as they break down the organic matter. As the organic matter is depleted, the microbial populations and BOD decline, while the oxygen concentration increases, a process known as 'self-purification', assisted by turbulence within the stream and by the photosynthesis of algae and higher plants. The effluent will also contain large amounts of suspended solids which cut out the light immediately below the discharge, thus eliminating photosynthetic organisms. Suspended solids settle on the stream bed, altering the nature of the substratum and smothering many organisms living within it.

Under conditions of fairly heavy pollution, sewage fungus develops. This is an attached, macroscopic growth containing a whole community of micro-organisms, dominated by *Sphaerotilus natans*, which consists of unbranched filaments of cells enclosed in sheaths of mucilage, and by zoogloea bacteria. Sewage fungus may form a white or light brown slime over the surface of the substratum, or it may exist as a fluffy, fungus-like growth with long streamers trailing into the water.

Protozoans are chiefly predators of bacteria and respond to changes in bacterial numbers. Attached algae are eliminated immediately below the outfall due to the diminished penetration of

Table 4.1 Examples of major short-term aquatic pollution events and their biological impacts.

Event	*Pollution issue*	*Biological effects*	*Comment*
Sandoz Agrochemical Spill, Schweizerhalle, Switzerland, November 1986	• 5–8 tons of pesticides released into Rhine	• Most aquatic life in immediate downstream reaches killed. • Eels (*Anguilla anguilla* L.) were killed over 560 km downstream.[8]	• Highlights complexity of response and difficulty in identifying causal agents for biological effects in cocktails of pollutants. • Many individual contaminants were below LC_{50} values so synergistic effects may have been important.[8]
Cantara Loop herbicide/pesticide spill, Sacramento River, USA, July 1991	• 70 000 litres of metam sodium, a soil fumigant spilt after train derailment. • Breakdown products include volatile methyl isocyanate.	• 41 mile reach of river affected. One million fish (including 200 000 rainbow trout, *Onchorhynchus mykiss*), tens of thousands of amphibians and crayfish killed. • Widespread damage to riparian trees. Estimated recovery time from <1 year (for periphyton) to 20 years (molluscs).[9]	• Impacts not just confined to instream organisms given the cloud of toxic gas released as the herbicide reacted with stream water. • Disparities in recovery rates between sessile and mobile organisms.
Aznalcóllar mine waste disaster, Spain, April, 1998.	• 6 000 000 m^3 of acidic wastes (rich in As, Cd, Cu, Pb and Zn) released after dam collapse into the Agrio-Guadiamar river system. • Contains the internationally important Doñana wetland site.	• 30 000 tons of fish killed. • Near lethal metal concentrations reported in Greylag Geese (*Anser anser*). • Increased incidence of DNA damage in nestling white storks (*Ciconia ciconia*) and black kites (*Milvus migrans*) at least in part explained by As and metal contamination.[10] • 1241 tonnes of fish killed.[11]	• Long-term studies highlight difficulties in attributing biological effects to disaster in mobile organisms.

Baia Mare mine waste disaster, Romania, January and March 2000.	• Failure of two dams released 10 000 m^3 of cyanide rich gold mine tailings.		• Underlying chronic pollution in the system made assessments of long-term recovery to baseline conditions difficult. • Major cross border disaster
Kingston fly ash spill, Emory River, Tenessee, USA, December, 2008	• 3.8 million cubic meters of fly ash released after storage pond dyke failure. • As, Se, Ba, Hg and V rich. • Fine grained material, physical smothering.[9]	• Physical smothering of streambed retarded recovery of macro-invertebrate communities. • Risk of methylmercury contamination of fish.[12]	• Highlights that physical impacts on aquatic biota can be as significant as chemical changes.
Ajka bauxite processing residue (red mud) spill, Hungary, October 2010	• 1 million m^3 red mud released into tributaries of Danube after pond dyke failure. • High salinity, extreme pH (>13), elevated trace metals/metalloids (*e.g.* Al, As, Cr, V), P-rich.[13]	• Major fish kills in 90 km reach of the Marcal River. • Numerous bioassays showed adverse response in contact with red mud.[14] • Short-term decline in planktonic rotifer diversity and abundance 250 km downstream in the Danube.[15]	• Multiple stressors which may have differing long-term significance: salinity (short term), metal/metalloid availability (medium term) and nutrient enrichment (long term) all likely to be important.

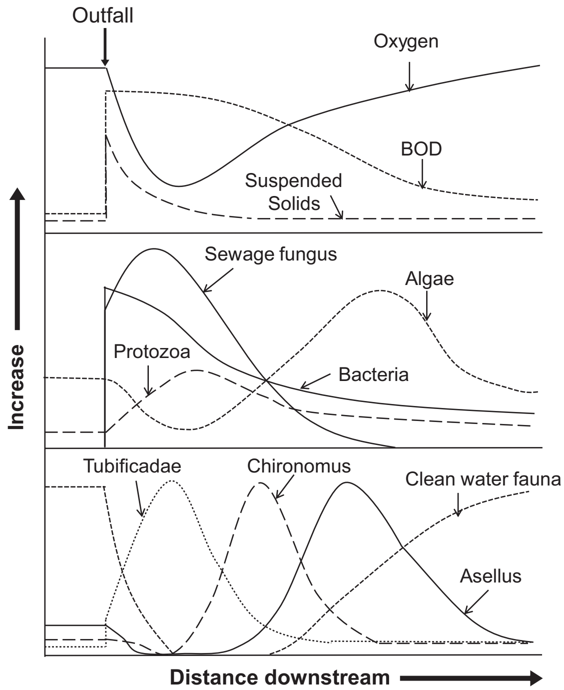

Figure 4.1 Changes in water quality and populations of organisms in a river below a discharge of an organic effluent.

light, but they gradually reappear below the zone of gross pollution, *Stigeoclonium tenue* being the initial colonizer. With the decomposition of organic matter large quantities of nitrates and phosphates are released, stimulating algal growth and resulting in dense blankets of the filamentous green *Cladophora* smothering the stream bed. Similarly, higher aquatic plants (macrophytes) may respond to the increased nutrient concentration, though only *Potamogeton pectinatus* is very tolerant of organic pollution.

Heavy organic pollution affects whole taxonomic groups of macroinvertebrates, rather than individual sensitive species and it is only in conditions of mild pollution that the tolerances of individual species within a group assume significance. The groups most affected are those which thrive in waters of high oxygen content and those which live on eroding substrata, the most sensitive being the stoneflies (*Plecoptera*) and mayflies (*Ephemeroptera*). The differences in tolerances of groups of macroinvertebrates form the basis of methods for monitoring, as will be described later in this chapter.

In the most severe pollution, the tubificid worms, *Limnodrilus hoffmeisteri* and/or *Tubifex tubifex*, are the only macroinvertebrates to survive. The organic effluent provides an ideal medium for burrowing and feeding, while in the absence of predation and competition, the worms build up dense populations, often approaching one million individuals per square metre of stream bed.

These worms contain the pigment haemoglobin, which is involved in oxygen transport, and they can survive anoxic conditions for up to four weeks. As conditions improve slightly downstream, the larvae of the midge *Chironomus riparius*, which also contains haemoglobin, thrives in dense populations and, as the water self-purifies, other species of this large family of flies appear, the proportion of *Chironomus riparius* gradually declining. Below the chironomid zone, the isopod crustacean *Asellus aquaticus* becomes numerous, especially where large growths of *Cladophora* occur. At this point, molluscs, leeches and the predatory alder fly (*Sialis lutaria*) may also be present in some numbers. As self-purification progresses downstream the invertebrate community diversifies, though some stonefly and mayfly species, which are sensitive even to the mildest organic pollution, may not recolonize the stream.

Fish are the most mobile members of the aquatic community and they can swim to avoid some pollution incidents. In conditions of chronic organic pollution they are absent below the discharge, reappearing in the *Cladophora*/*Asellus* zone, the tolerant three-spined stickleback (*Gasterosteus aculeatus*) being the first to take advantage of the abundant invertebrate food supply. Organic pollution is usually most severe in the downstream reaches of rivers and may prevent sensitive migratory species, such as Atlantic salmon (*Salmo salar*) and sea trout (*S. trutta*), from reaching their pollution-free breeding grounds in the headwaters.

4.3 EUTROPHICATION

There has been at least a doubling of reactive nitrogen at the Earth's surface since the widespread adoption of the Haber–Bosch process for fixing atmospheric nitrogen into ammonia.[20] Processing of ammonia for chemical fertilizers and their subsequent application on agricultural land has contributed to one of the major anthropogenic impacts on freshwater systems since the second half of the 20th century. Alongside this dramatic increase in nitrogen in freshwater systems, the global phosphorus cycle has been amplified by around 400% by human activities.[21]

It has already been described how the release of nutrients during the breakdown of organic matter stimulates the growth of aquatic plants. This addition of nutrients to a waterbody is known as eutrophication. Nitrogen and phosphorus are the two nutrients most implicated in eutrophication and, because growth is normally limited by phosphorus rather than nitrogen, it is the increase in phosphorus which stimulates excessive plant production in freshwaters. Beyond agricultural fertilizers, the main sources of excess nutrients to surface waters include phosphorus from STW, the washing of manure from intensive farming units into water, the burning of fossil fuels which increases the nitrogen content of rain and the felling of forests which causes increasing erosion and run-off.

4.3.1 Nutrient Pollution in Lakes

The concentrations of nitrate and phosphate in the water of Ardleigh Reservoir, a eutrophic waterbody in East Anglia, are shown in Figure 4.2.[22] Note that the concentration of nitrate increases during the late winter when fertilizer is applied to growing crops and is washed into streams feeding the reservoir in large amounts. By contrast, the concentration of phosphate peaks in late summer, when low flows in the feeder streams consist largely of treated sewage effluent while, at this time of year, much phosphate is released from the reservoir sediments into the water.[22]

Table 4.2 lists the guidelines for assessing the trophic status of a waterbody. Peak phosphorus concentrations in Ardleigh Reservoir were some 250 times the minimum concentration for assigning a waterbody as eutrophic, while peak concentrations of nitrogen were 10 times the minimum. One of the major biological effects of eutrophication is the stimulation of algal growth. As eutrophication progresses, there is a decline in the species diversity of the phytoplankton and a change in species dominance as overall populations and biomass increase.

Figure 4.3 illustrates the seasonal changes in biomass of the dominant groups of algae in Ardleigh Reservoir.[22] Typical of temperate lakes is the early peak of diatoms (*Bacillariophyta*), followed by a late spring peak of green algae (*Chlorophyta*). Eutrophic lakes are characterized by enormous summer growths of cyanobacteria, in the case of Ardleigh Reservoir mainly *Microcystis aeruginosa*.

The algal blooms associated with excessive amounts of nutrients have other consequences for the aquatic ecosystem. The macrophyte swards of many lakes have been eliminated as the light is

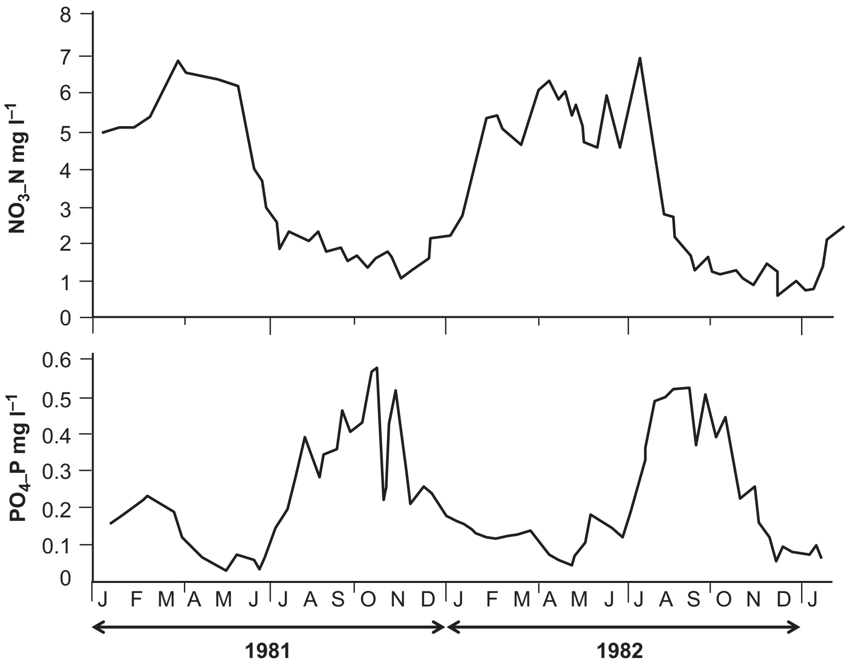

Figure 4.2 Concentrations (mg l^{-1}) of nitrate and phosphate in Ardleigh Reservoir, eastern England, over two years.[22]

Table 4.2 Eutrophication survey guidelines for lakes and reservoirs.

	Oligotrophic	*Mesotrophic*	*Eutrophic*
Total phosphorus (μg l^{-1})	<10	10–20	>20
Total nitrogen (μg l^{-1})	<200	200–500	>500
Secchi depth (m)	>3.7	3.7–2.0	<2.0
Hypolimnetic dissolved oxygen (% saturation)	>80	10–80	<10
Chlorophyll a (μg l^{-1})	<4	4–10	>10
Phytoplankton production (g C m^{-2} d^{-1})	7–25	75–250	350–700

reduced on the lake bed, preventing photosynthesis of germinating plants. Growths of epiphytic algae on the leaf surface may also restrict light uptake by aquatic plants so that they become scarce in the lake. Zooplankton use macrophyte canopies as refuges from fish. Without macrophytes, they are very vulnerable and this itself can accelerate the eutrophication process as the grazing pressure on phytoplankton is reduced in the absence of zooplankton, allowing denser algal blooms to develop.

The Norfolk Broads of eastern England are a series of shallow lakes, formed during medieval times when peat workings became flooded, and famous for their rich flora of Charophytes (stoneworts) and aquatic angiosperms, supporting a diverse assemblage of invertebrates. During the 1960s a rapid deterioration set in as nutrient concentrations increased and a survey of 28 of the main broads in 1972–73 revealed that eleven were devoid of macrophytes and only six had a well developed aquatic flora. The invertebrate fauna was similarly impoverished. Since then the situation has deteriorated further with additional losses of macrophytes and the Norfolk Broads now have some of the highest total phosphorus concentrations of world freshwater lakes.[23]

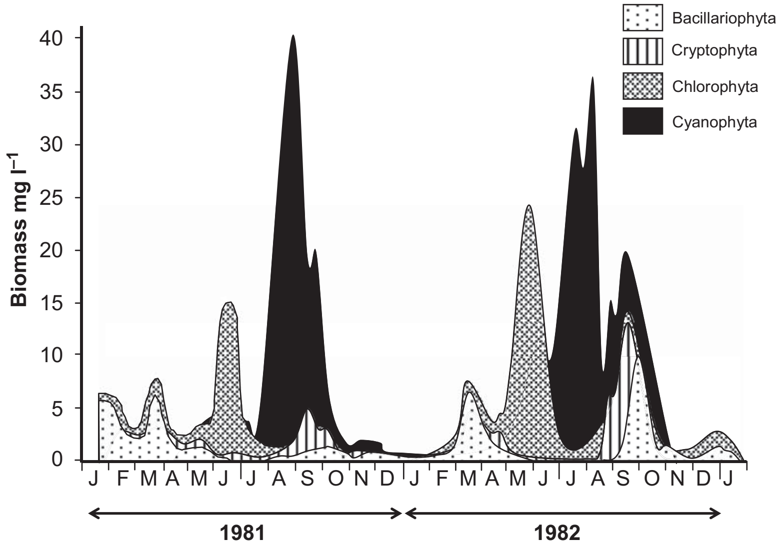

Figure 4.3 Seasonal variation in phytoplankton composition and total biomass (mg l^{-1} wet weight) in Ardleigh Reservoir, eastern England, over two years.[22]

It appears that shallow lakes have two alternative stable states over a range of nutrient concentrations.[24] They may either have clear water, dominated by aquatic vegetation, or turbid water with high algal biomass. The clear water, macrophyte community may be stabilized by luxury uptake of nutrients, making nutrients unavailable to plankton, by secreting chemicals to prevent plankton growth, and by sheltering large populations of grazing zooplankton which eat planktonic algae. Fish predation of zooplankton is reduced in the structured macrophyte sward.[25] The turbid water phytoplankton community may be stabilized by an early growing season, shading the later germination of macrophytes, and by producing large, inedible algae, which also acquire carbon dioxide more easily, especially at high pH. An absence of macrophytes increases the vulnerability of herbivores to predation by fish in the unstructured environment, while the predatory pike (*Esox lucius*) declines without cover for ambushing its prey, allowing even larger populations of planktivorous fish to survive.[26]

Fish communities also change as oligotrophic lakes become more eutrophic. Cold water fish with high oxygen requirements, such as salmonids and white-fish, are replaced by less demanding cyprinids, the commercial value of the fishery declining. Some algae at high densities produce toxins which kill fish. In the Norfolk Broads *Prymnesium parvum* has caused several large fish kills over the last four decades. The sudden collapse of an algal bloom may result in rapid deoxygenation of the water and large kills of fish. Cyanobacteria may also produce potent poisons which can induce rapid and fatal liver damage at low concentrations. Livestock, dogs and wildlife have been killed. Toxins do not always occur in blooms and can be highly variable with time, making them difficult to predict, detect and monitor.[27]

4.3.2 Nutrient Enrichment in Rivers and Groundwaters

The problems of eutrophication are not restricted to standing waters. Around 10 000 km of streams in England and Wales are deemed at risk of failing WFD guidelines for nitrate, the vast majority situated in areas of intensive lowland agriculture in eastern England.[18] Over 25 000 km of stream (which equates to 43% of all water bodies) were deemed at risk of phosphorus pollution which highlights the extent of the nutrient pollution problem.[18] The geographic distribution of streams impacted by excess phosphorus covers the same agricultural areas as those impacted by nitrate pollution, but also most major urban centres, reflecting a range of point and diffuse urban sources.[18]

Nitrate-rich drinking water presents potential health problems. In particular, babies under six months of age who are bottle fed may develop methaemoglobinaemia (blue baby syndrome), the nitrate in their milk being reduced to nitrite in stomach acids and then oxidizing ferrous ions in the haemoglobin of their blood, so lowering its oxygen carrying capacity. The disease only occurs when bacteriologically impure water, containing nitrate levels approaching 100 mg l^{-1}, is supplied, and is usually only an issue with water supply from private wells. It is worth cautioning however that a number of authors are questioning the simple association between nitrate and infant methaemoglobinaemia, and considering nitrate as a co-factor in one of several causes of the disease.[28] The *EU Drinking Water Directive (80/778/EEC)* standard for drinking water recommends that nitrates should not exceed 50 mg NO_3 l^{-1}, but concentrations in some lowland areas of Britain frequently exceed 100 mg NO_3 $^{-1}$. Recent studies have also warned of a potential "timebomb" with nitrate concentrations in the UK still rising in many areas despite efforts to curb nutrient pollution (notably the *EU Nitrates Directive* and WFD). Areas of eastern England with intensive agriculture, chalk lithology with a thick unsaturated zone and thin or absent superficial deposits are particularly susceptible.[29] This may lead to some strategically important aquifers becoming unsuitable for potable water supply unless they are treated. Furthermore, where river systems are predominantly fed by groundwater in these intensive agricultural areas, we may see similarly slow recoveries in nutrient status of the surface waters.[30]

4.3.3 Managing Nutrient Pollution

It is possible to reverse eutrophication although, in many cases, expensive remedial measures have proved much less successful than was hoped.[31] Methods include 'bottom-up' (nutrient control) and 'top-down' (biomanipulation) approaches. Bottom-up approaches involve controlling inputs of phosphorus because this element is normally limiting to plant growth and much of it in freshwaters is derived from point sources (*e.g.* sewage treatment works), whereas nitrogen enters the aquatic ecosystem diffusely, *via* land drainage. Phosphorus releases from STW have reduced in recent years with legislative pressure and increased investment in treatment technologies, while recent attempts to control diffuse agricultural releases in Europe has seen the setting of Nitrate Vulnerable Zones (NVZs) in areas with sensitive geology. This involves setting controls on the quantities and timings of fertilizer additions, and regulating the storage of silage to minimise risk of runoff. These source minimisation measures have been coupled with a range of control mechanisms to manage releases from land. Modifications to agricultural practice, such as the planting of buffer strips of grassland, woodland or wetland at field margins can help minimise nutrient loss from fields. Similarly, sediment detention ponds, the reinstatement of riparian wetlands (which may also serve dual benefits for flood control) and the installation of sub-surface gravel drainage from areas of farm hardstandings have also been recommended. Many researchers are also investigating novel, low-cost nutrient stripping technologies such as waste ferric oxides from coal mine drainage systems, which effectively minimise phosphorus release, with the pelletised ferric oxide suitable for deployment as a slow-release fertilizer when filter systems are routinely maintained.[32]

In some lentic systems (a non-flowing or standing body of freshwater such as a lake or pond) affected by eutrophication, biomanipulation has seen the management (or complete removal) of planktivorous fish, allowing the re-establishment of populations of the larger herbivorous zooplankton, which graze algae.[33] Fish removal has led to increases in water clarity, encouraging the growth of macrophytes. Once the macrophyte stable state has developed it should be possible, indeed it is desirable, to reintroduce fish, along with the predatory pike. Biomanipulation technology, however, is still being refined and the clear water phase may only be temporary, several lakes showing increased turbidity after the first few years of management. Some lakes have not responded as predicted and it appears that an increase in herbivorous wildfowl prevent the development of macrophytes.[34] The control of phosphorus is in most cases a necessary prerequisite to biomanipulation, which is likely to be most effective in shallow waters where macrophytes are a major component of the ecosystem. This can incorporate removal of phosphorus-rich surface sediments or iron addition to increase the phosphorus sorption capacity of the sediment.[35]

4.4 ACIDIFICATION

4.4.1 Acidification from Above: Sulfur and Nitrogen Oxides

The threats posed by acid rain and associated acidification of freshwaters have been documented from direct monitoring as early as the 1920s in Scandinavia, while by reconstructing past environments through analysing diatom communities in lake sediment cores these trends can be detected even earlier. For example studies of the diatom remains in cores of sediment from lochs in south-west Scotland have indicated that progressive acidification began around 1850, with an increase in *Tabellaria binalis* and *T. quadriseptata*, both species characteristic of acid waters.[36]

Acidification also represents an excellent example of a major global environmental pollution problem which, through a combination of legislation, technological improvements and reactive management, is now on trajectory that is at least tentatively positive in many parts of the world. Coal-fired power stations and metal smelters produce much of the polluting oxides of sulfur and nitrogen responsible for the problem, but domestic and other industrial sources are also significant, as are the exhausts of vehicles, particularly for nitrogen oxides (NO_x) (see also Chapters 7 and 8). The acids either fall directly into waterbodies as wet or dry deposition or are washed in from vegetation and soils within the catchment. The effects acidification has are largely a function of the bedrock geology of the receiving catchments, with three broad categories of water which differ in acidity:

(i) Those which are permanently acid, with a pH less than 5.6, low electrical conductivity, and an alkalinity close to zero. Such conditions occur in the headwaters of streams and in lakes, where the soils are strongly acid, or in the outflows of peat bogs.
(ii) Those which are occasionally acid, where pH is normally above 5.6 but because they have low alkalinity (usually less than 5.0 mg l^{-1} $CaCO_3$) the pH may drop below 5.6 periodically. These include streams and lakes in upstream areas of low conductivity on rocks unable to neutralize acid quickly. Such waters may show episodes of extreme acidity, for instance during snowmelt or following storms. These may be very damaging to aquatic life but the infrequency of acid events makes the problem difficult to detect.
(iii) Those which are never acid, the pH never dropping below 5.6 and the alkalinity always above 5 mg l^{-1} $CaCO_3$.

Much of northern and western Britain has a solid geology consisting of granites and acid igneous rocks; there is little or no buffering capacity. The situation is exacerbated in those catchments which have been extensively planted with conifer forests. The sulfate ion is very mobile and transfers acidity very efficiently from soils to surface waters. The nitrate ion behaves similarly but is normally quickly taken up by plant roots.

The effects of acidity on aquatic organisms are both direct (*e.g.* direct changes to ion regulation across the gill epithelium) and indirect, for example the increased availability of metal ions, notably aluminium which increases in solubility as pH falls. Table 4.3 provides a generalized summary of

Table 4.3 Sensitivities of aquatic organisms to lowered pH.

pH	*Effects*
6.0	Molluscs and most crustaceans disappear. White moss increases.
5.8	Salmon, char, trout and roach die; sensitive insects, phytoplankton and zooplankton die.
5.5	Whitefish, grayling die; crayfish lose calcium from exoskeleton
5.0	Perch, pike die, fish eggs do not hatch.
4.5	Eels, brook trout die.
4.0	Mayflies and frogs begin to die.

the sensitivity of aquatic organisms to lowered pH based on studies in Scandinavian and North American lakes. Changes in the community begin at pH 6.5 and most species have disappeared below pH 5.0 leaving just a few species of tolerant insects and some species of phyto and zooplankton. Considerable research has been directed towards the effects of acidification on fish because of their economic and recreational importance. The biological impacts caused by acidification became fully acknowledged after the extent of damage to salmonid populations in Scandinavia was revealed. For example, by 1975 it was recognised that over half a total of 2850 lakes in southern Norway had lost their brown trout (*Salmo trutta*) populations.[37]

The effect of acidity on fish is mediated *via* the gills. The blood plasma of fish contains high levels of sodium and chloride ions and those ions which are lost in the urine or from the gills must be replaced by active transport, against a large concentration gradient, across the gills. When calcium is present in the water it reduces the egress of sodium and chloride ions and the ingress of hydrogen ions. The main cause of mortality in acid waters is the excessive loss of ions such as sodium which cannot be replaced quickly enough by active transport. When the concentrations of sodium and chloride ions in the blood plasma fall by about a third, the body cells swell and extracellular fluids become more concentrated. To compensate for these changes, potassium may be lost from the cells, but, if this is not eliminated quickly from the body, depolarization of nerve and muscle cells occurs, resulting in uncontrolled twitching of the fish prior to death.

Aluminium has been shown to be toxic to fish in the pH range 5.0–5.5 and, during episodes of acidity, aluminium ions are frequently present in high concentrations. This can be particularly pronounced where water is draining conifer plantations or areas with extensive clay minerals in superficial deposits. Aluminium ions apparently interfere with the regulation by calcium of gill permeability so enhancing the loss of sodium in the critical pH range. They also cause clogging of the gills with mucus and interfere with respiration. The early developmental stages of fish are particularly sensitive to acidification[38,39] and it is thought that aluminium may interfere with the calcification of the skeleton of fish fry resulting in a failure of normal growth. This failure in recruitment results in a gradual decline of the fish population to extinction. Many invertebrate species, such as crustaceans, molluscs and caddis are absent from acidified streams even though their food supply may be present. Physiological stress is the most likely cause. Aquatic invertebrates need to actively take up sodium, chloride, potassium and calcium ions for survival. Uptake is dependent on external concentrations. In acid waters, ion concentrations may be too low while hydrogen and aluminium ions become dominant in the water. These ions are small and mobile and may be transported in instead of essential ions, upsetting the normal ionic balance, leading to death. Aluminium in acidic conditions has been shown to damage the ion-regulatory organs of caddis larvae, disrupting osmoregulation and leading to increased mortality. However the sensitivity to aluminium has been shown to differ between species.[40]

The simplification of the aquatic ecosystem due to acidification can also cascade to higher levels of the food chain, such as birds and mammals. The decline of the dipper (*Cinclus cinclus*), which feeds mainly on aquatic invertebrates was linked with acidification in Welsh upland streams.[41]A decline in pH of 1.7 units on one river over the period 1960–1984 resulted in a 70–80% decline in the dipper population.[41] If fish populations are eliminated from headwaters of streams by acidification, then the piscivorous otter (*Lutra lutra*) may not use them. In general, however, acidification results in a reduction in the carrying capacity for otters rather than a decrease in distribution.[42]

4.4.2 Recovery from Acidification

Amendments to the *US Clean Air Act* in the 1970s and 1980s and the *UNECE Convention on Long-range Trans-boundary Air Pollution* in 1979 mandated many industrial nations to radically cut their acidifying emissions. *Source control*, *via* the use of lime based scrubbers (which can generate useful gypsum products after stripping sulfur out of flue gases), the use of coal with a lower sulfur content and more efficient coal burning power stations has seen an observed fall in sulfur emission and deposition in many affected areas. SO_2 emissions in the UK have fallen by 85% from peak values in the 1970s and 1980s with an associated decline in sulfur deposition rates by at least 50%.[43] In the USA, emissions have fallen by around 80% relative to 1980 values. In addition to such pollution prevention, *reactive management* for acidification is based around direct liming of catchments and is practiced in many catchments in Scandinavia and North America.

Reduced atmospheric sulfate loadings have been accompanied by recovery in stream chemistry in most monitored areas, albeit falls in instream sulfate loadings have typically been less than the falls in deposition rate, suggesting some buffering or lag in response due to previously deposited sulfates in soils.[44] An important resource for assessing the longer term environmental recovery comes from long-term monitoring encompassing biological as well as chemical analysis tools. In the UK, the Acid Waters Monitoring Network (now the Upland Waters Monitoring Network) have maintained monitoring of deposition rates, stream chemistry as well as a suite of biological monitoring tools including diatoms, macro-invertebrates and macrophytes for over twenty five years at a broad geographic range of sites. The response such biological data show is more complex than that apparent in chemical parameters. Typical patterns include community shifts in diatom assemblages incorporating decline of acid indicating diatoms and an increase in those more indicative of less acidic conditions. An increasing number of acid-sensitive macro-invertebrates and a proportional rise in predatory species such as predatory caddisfly (notably *Cyrnus* sp. or *Polycentropus* sp.) and stoneflies (*Siphonoperla torrentium* and *Isoperla grammatica*) were also noted.[45] The more frequent presence of aquatic macrophytes sensitive to acid conditions (*e.g. Hyocomium armoricum, Chara virgata* and *Callitriche hamulata*) were observed at the majority of UWMN rivers and lakes where alkalinity had increased suggesting strong chemical drivers, while the appearance of juvenile brown trout has been documented in some systems once acid neutralising capacity approached or exceeded positive values.[45] However the biological recovery is often gradual, highly variable between catchments, and there are obvious lags in biological recovery after improvements in water quality. Biological recovery is always likely to be more complex than chemical recovery given limitations to the dispersal of acid-sensitive species and the fact that there may be certain chemical thresholds that elicit a biological response (*e.g.* some macrophytes utilise dissolved organic carbon from the water column in photosynthesis and only occur when this is present in less acid conditions) as opposed to a steady linear response.[45] Furthermore, many affected catchments are still subject to acid flushing events which can limit the recovery of more sensitive taxa, while recovery may also be impacted by land use. For example, there is evidence that chemical and ecological recovery is slower in areas of upland coniferous forestry in Wales.[43] One related side-effect of the decrease in mineral acidity in upland settings is the rise in organic acidity associated with increased release of dissolved organic carbon to surface waters. This has been a consistent phenomenon in recovering upland settings with organic-rich (peat) soils and may have implications for downstream biota, notably through increased water colour and reduced UV-B penetration through the water column.

Despite the marked reduction in acidifiying emissions, there remain enduring challenges associated with acidification. The slow biological recovery and problems of short-term acid flush events require continued monitoring efforts, while the effects of NO_x emissions require particular attention given legislation has failed to reduce vehicle derived NO_x emissions as quickly as the SO_2 from industrial sources. Furthermore, most of the studies described above are from the North America and north western Europe. The world's largest coal burning nation, China, is currently experiencing rising SO_2 and NO_x emissions. While the former are anticipated to fall in coming years, recent modelling studies suggest that any fall in SO_2 deposition will be negated by rapidly increasing NO_x around China's main industrial cities.[46]

4.4.3 Acidification from Below: Acid Mine Drainage

The other major mechanism for the release of acids to freshwater systems arises from the problems of mine drainage. The process of mining exposes hitherto confined sulfide minerals, such as pyrite (FeS_2) or galena (PbS_2), to oxygen, water and chemosynthetic bacteria. These bacteria assist in the oxidation of sulfide minerals which both releases metals into solution and forms sulfuric acid. As such, mine drainage can have pH as low as 2 or 3 and can contain a cocktail of toxic metals and metalloids, most commonly arsenic, cadmium, copper, lead and zinc as well as iron which is less directly toxic. If the surrounding bedrock is not carbonate-rich to buffer the pH, such discharges can result in the extermination of much of the biota in the watercourse into which it flows. The problem usually arises after mine closure when pumping operations cease and water levels begin to rise and react with sulfide minerals in the mine voids. Mine drainage affects 19 000 km of streams and 73 000 ha of lakes in the USA alone,[47] around 10% of all rivers in England and Wales and is responsible for around half of all known emissions of cadmium, zinc and lead to the water environment of England and Wales.[48] The location of many former orefields in mountainous areas of otherwise high amenity value and quality compounds the issues. Furthermore, facilities storing fine mine wastes (tailings), despite legislative progress, can still cause localised devastation to aquatic systems if there are failures in retaining walls (see Table 4.1).

It is worth noting that while mining activity can lead to conditions that either facilitate or cause direct toxicity to aquatic organisms, in many cases it is the physical effects of streambed smothering that are most prominent. For example the impacts of amorphous iron oxyhydroxides which readily precipitate from iron-rich coal mine drainage prevent oxygen circulation through benthic sediments and have been cited as the causal agent (rather than direct toxicity) for impoverished invertebrate communities in some mine-impacted streams.[49] Similar physical smothering mechanisms may also be apparent at rivers impacted by siltation (*e.g.* from unregulated mining or quarrying) or sites affected by alkali pollution (*e.g.* chlor-alkali wastes or steel slag drainage).

4.5 TOXIC CHEMICALS

4.5.1 Modes of Action of Toxic Chemicals

Some aspects of toxic pollution have already been mentioned but it is now appropriate to describe the modes of action of toxic chemicals. Some of the major types of toxic compounds, which are not mutually exclusive, are:

(i) Metals, such as zinc, copper, mercury, cadmium.
(ii) Organic compounds, such as pesticides, herbicides, polychlorinated biphenyls (PCBs), phenols; organometals such as methylmercury.
(iii) Gases, such as chlorine, ammonia.
(iv) Anions, such as cyanide, sulfate, sulfite.
(v) Acids and alkalis (*e.g.* caustic soda).

There are a number of terms in regular use in the study of toxic effects:

(i) *Acute*: causing an effect (usually death) within a short period.
(ii) *Chronic*: causing an effect (lethal or sub-lethal) over a prolonged period of time.
(iii) *Lethal*: causing death by direct poisoning.
(iv) *Sub-lethal*: below the level which causes death but which may affect growth, reproduction or behaviour so that the population may eventually be reduced.
(v) *Cumulative*: the effect is increased by successive doses.

A schematic of a typical toxicity curve is given in Figure 4.4. The median periods for survival are plotted against a range of concentrations. The lethal concentration (LC) is used where death is the criterion of toxicity and has traditionally been the focus of toxicity studies. The number indicates the percentage of animals killed at that concentration and it is also usual to indicate the time of exposure. Thus 96-hour LC_{50} is the concentration of toxic material which kills fifty per cent of the test organism in ninety six hours. The incipient level is usually taken as the concentration at which fifty per cent of the population can live for an indefinite period of time. Where effects other than death are being sought, for example respiratory stress or behavioural changes, the term used is the effective concentration (EC) which is expressed in a similar way, *e.g.*, 96-hour EC_{50}. Other common terms used include the no observed effects concentration (NOEC), which assumes there is a threshold concentration below which no adverse effect is expected (*i.e.* response at lower concentrations is not significantly different from unexposed control treatments). Statistical testing of exposure concentrations against control concentrations (*i.e.* unexposed treatments), with the null hypothesis that there is no significant difference in average concentration will yield the NOEC along with the lowest observed effect concentration (LOEC). The latter is the lowest average concentration where response differs significantly from control treatments (see Figure 4.4).[50] The arithmetic mean of these two values gives the maximum allowable toxicant concentration (MATC).

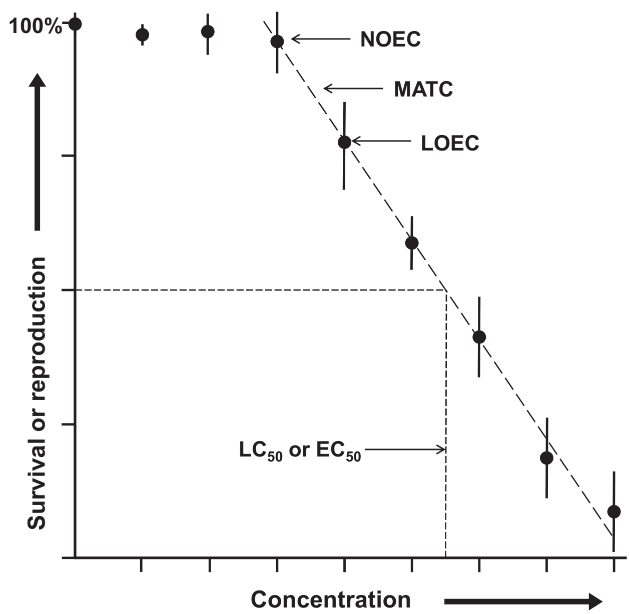

Figure 4.4 A schematic diagram of a typical toxicity curve. (Adapted from ref. 51).

While there is much discussion about the statistical validity of such measures, they are widely used by regulators in many settings.[50]

There has been a large amount of data collected on the acute toxicity of chemicals, especially to fish and invertebrates,[52] and this has undoubtedly been of great value in elucidating the mechanisms of toxicity. However the value of these data for practical river management is more questionable. Incidents resulting in large mortalities of fish and other organisms are usually accidents over which the river manager has no control. He or she can merely assess the damage and perhaps restock when conditions improve. In addition, detailed information on toxicity of a range of compounds is available for only a few test organisms, such as rainbow trout or *Daphnia*, and it is well known that even closely related species may show very different responses to particular pollutants. It is the sub-lethal effects of pollutants which are of particular concern in many field situations, for low levels of pollutants may result in the gradual loss of populations, without any overt signs of a problem.

Experiments on sub-lethal effects are more difficult to carry out because they invariably take longer and individuals under test may respond very differently to low levels of pollution. Furthermore, the reaction to pollutants may vary over the lifetime of an organism, early developmental stages often being more susceptible. It is therefore necessary to study the entire lifecycle of an organism under experimental conditions to find the weak link in its response to pollution and such long-term experiments, possibly over several generations, are essential to discover any carcinogenic, teratogenic or mutagenic effects of pollutants. Sub-lethal effects may be manifested at the biochemical, physiological, behavioural or life cycle level.[1] For example, novel experiments using electrical recordings from the nose (electro-olfactograms) and videos of the behavioural response of Pacific salmon (*Oncorhynchus kisutch*), demonstrated how the detection of salmonid olfactory cues was impaired due to copper exposure well below aquatic life standards[53] which could have a bearing on predator avoidance. Although it is possible in the laboratory to demonstrate small effects at very low levels of pollution, for example in biochemistry or on growth, it is essential to show that these are likely to reduce the fitness of an organism in its natural environment and are not merely within the organism's range of adaptation. Nevertheless these sub-lethal effects may be quite subtle and can be measured long before any outward toxic effects are manifested. They can be used as *biomarkers*, to show that an organism has been exposed to contaminants at levels which exceed the normal detoxification and repair capabilities.[54,55] A *biomarker* can be defined as a xenobiotically-induced variation in cellular or biochemical components or processes, structure, or functions that is measurable in a biological system or sample. Biomarkers can be used to predict what concentrations of a pollutant are likely to cause damage, rather than merely to measure concentrations when damage has been noted.

The liver enzymes (mixed-function oxidases) which metabolize xenobiotics are often used as biomarkers. In the Belgian River Meuse a once common fish, the barbel (*Barbus barbus*), is now scarce. The activities of three liver enzymes were strongly correlated with concentrations of PCBs in the liver. At high PCB levels there were marked alterations in the liver ultrastructure, with a change in mitochondrial membranes and an excessive growth of rough endoplasmic reticulum.[56] High PCB concentrations also reduced reproductive success in barbels,[57] which would explain the decline of fish populations in the Meuse. Organisms which are regularly subjected to toxic pollutants may develop tolerance to them. This may be achieved either by functioning normally at high concentrations of pollutants or by metabolizing and detoxifying pollutants.

Algae living in streams receiving mine drainage are highly tolerant to metals and this adaptation has been shown to be genetically determined. Metal tolerance has also been observed in invertebrates from metal contaminated streams, and in fish. Exposure stimulates the production of metallothioneins, low molecular weight proteins containing sulfur-rich amino acids which bind and detoxify some metals.

With the rapid advances in molecular biology and bioinformatics in recent years, new tools and approaches for toxicity screening are being generated. These focus on identifying the cellular response pathways, that when sufficiently perturbed by toxicants at environmentally-realistic exposure levels, are likely to result in adverse health effects.[58] Such adverse cellular responses have been identified for metal pollution (using metallothionein response) and hydrocarbon pollution (based on cytochrome P4501A, a protein which is involved in the detoxification of hydrocarbons)[59] amongst other groups of toxicants. The application of technologies developed in the pharmaceutical industry for drug development allows large numbers of chemicals to be screened each day to assess their molecular, biochemical or cellular effects. The large sample sizes permitted (compared to traditional approaches) also allows for the screening of many more chemicals, tested to a range of endpoints across different lifestages, species and genetic diversity within species. As such, these tools are going to reveal much about the effects of pollutants on aquatic organisms and will underpin regulatory tools for setting environmental thresholds in the 21st century.

4.5.2 Bioaccumulation and Biomagnification

Of particular concern to environmental toxicologists are those compounds which accumulate in tissues, especially some metals and Persistent Organic Pollutants (POPs) such as organochlorines (pesticides and PCBs). A chemical is said to *bioaccumulate* if it builds up in living organisms at concentrations higher than those in the surrounding environment. From often undetectable concentrations in water, organisms may accumulate levels of biological significance if the chemicals are difficult to break down and are absorbed at a rate greater than they are excreted from an organism. Furthermore, if these chemicals appear in progressively higher concentrations through the food chain they are said to have undergone *biomagnification*.

There have been various notorious examples of where such biomagnification in aquatic systems has even led to major public health issues. For example, the release of mercury, used as a catalyst in the production of acetaldehyde, from the Chisso Corporation chemical works into the enclosed Minamata Bay in south-west Japan led to methyl-mercury accumulation in shellfish and fish and the subsequent poisoning of at tens of thousands of people in the mid-20th century.[60] Methylmercury is readily absorbed across the intestine and into the bloodstream unlike elemental mercury and in this case caused severe neurological damage. It can also pass across the placenta and can severely affect the development of unborn children.[60] Prior to the Minamata disaster, Japan had already been subject to a major industrial pollution disaster in the early half of the 20th century, with the release of cadmium from metal mining into rivers in Toyoma Prefecture in north-east Japan. This cadmium accumulated in stream and floodplain sediments of the Jinzū River, areas widely used for rice cultivation. Cadmium is readily taken up by some cultivars of Asian rice (*Oryza sativa*)[61] and led to a major public health disaster known as "itai itai" disease; metal poisoning characterised by renal damage and weakening of bones. In both cases, a population with a narrow dietary range who were over-reliant on a particular local food source which became contaminated were severely affected.

Perhaps the most documented biomagnification example that spans numerous sites and trophic levels comes from monitoring the effects of PCBs on wild populations of vertebrates in the Great Lakes region of North America. In Green Bay, Lake Michigan, Forster's Terns (*Sterna forsteri*) exhibit impaired reproduction. The incubation is extended, few eggs hatch, the chicks have lower body weight and their livers are larger than normal. They show a high incidence of congenital deformities. The parents are inattentive at nesting and this further reduces their reproductive success. It was concluded, following a detailed toxicological analysis, that those PCB congeners which induce the enzyme aryl hydrocarbon hydroxylase (AHH) were the only contaminants present in sufficient amounts to cause the observed effects on eggs and chicks. More than 90% of the effect could be explained by two pentachlorobiphenyls. The behavioural abnormalities in adults were caused by total PCBs.[62] In addition to the Forster's Tern, six other species of fish-eating bird from the Great Lakes have exhibited growth deformities and physiological defects.[63] These symptoms have only been observed in the past three decades and it is considered that, in earlier years, symptoms were masked by the effects of DDE, which thinned eggs to such an extent that they did not survive long enough for abnormalities to be expressed. The biomagnification factor of PCBs from water to top carnivores may be as high as twenty-five million times.[64] Studies on humans also give cause for concern. Behavioural and neurological disorders have been found in the children of mothers who eat modest amounts of fish caught in the Great Lakes.[65] Parallel studies on rats have shown that these behavioural changes are still measurable two generations after exposure.[65]

There has been considerable, detailed research on PCBs in the Great Lakes but the problem, of course, is not confined to that region. For example, evidence suggests that PCBs have been largely responsible for the decline of the otter over large areas of Western Europe, in some places to extinction. PCBs are known to affect the reproductive success of mammals, being powerful endocrine disruptors. There is a strong relationship between the mean amount of PCBs in tissues of otters and the extent of the population decline, relationships which do not hold for other contaminants.[66] Using vitamin A in liver as a biomarker, a strong negative correlation was found between concentrations of this vitamin and PCB concentrations, which coincided with the incidence of disease,[67] the PCBs presumably affecting the immune system. A relationship between PCB concentrations and the incidence of disease has also been reported in harbour porpoises (*Phocoena phocoena*).[68]

With the restrictions placed on the manufacture and use of PCBs, concentrations have declined in tissues of otters in Britain to average levels in the early 1990s which are unlikely to have adverse health effects (see Figure 4.5).[69] The species has shown a considerable increase in range in rivers in lowland England from where it had been absent for four decades, and otters are now beginning to penetrate into the city of London. Similar studies in Sweden (see Figure 4.5) have also shown positive trends in otter populations coinciding with decreases in environmental and otter PCB concentrations.[70]

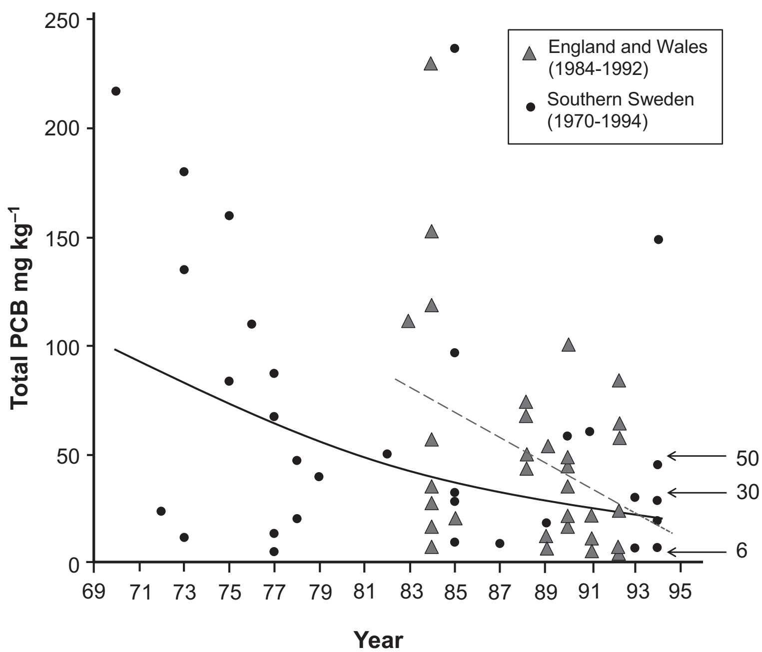

Figure 4.5 PCB concentrations (mg kg^{-1} lipid) in tissues of otters collected in England and Wales between 1984 and 1992[69] and Southern Sweden between 1971 and 1994.[70] (Trendlines taken from original sources). The arrow at 50 mg kg^{-1} illustrates the concentration of PCBs causing reproductive failure in mink (*Mustela vison*), that at 30 mg kg^{-1} is a more stringent standard based on the precautionary principle. The more stringent standard of 6 mg kg^{-1} is based on dose effects levels on vitamin A deficiency.

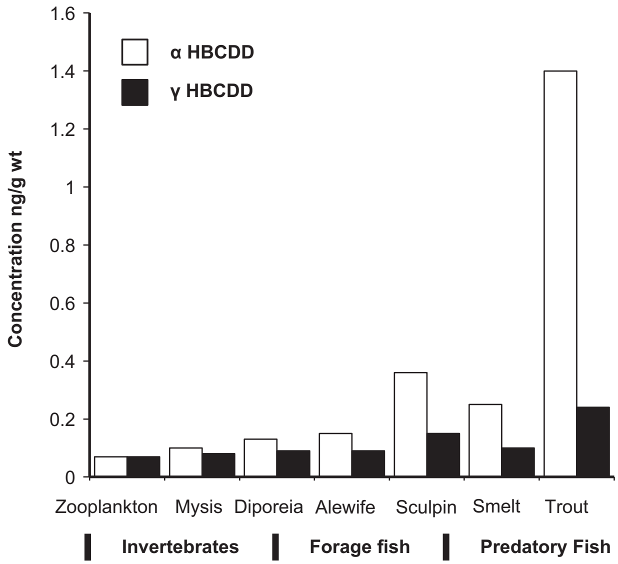

Figure 4.6 Biomagnification of HBCDD in Lake Ontario foodchain.[71] Data show two isomers of HBCDD, highlighting persistence of αHBCDD.

However, PCBs represent just one example of a group of chemicals, which through careful study, we now have an understanding of their behaviour across entire aquatic and terrestrial food chains. International agreement to manage such persistent, bioaccumulative and toxic substances comes *via* the Stockholm Convention on POPs, to which new substances are added on a regular basis (see also Chapter 18). A recent example includes the flame retardant HBCDD (hexabromocyclododecane), widely used in electronics, furniture and polystyrene tiles in construction materials. Studies have shown the biomagnification of HBCDD at levels similar to some PCBs (see Figure 4.6).[71] Like many POPs, HBCDD is *lipophilic*, so accumulates in fatty tissue, with concentrations in higher predators far exceeding those at lower trophic levels. Despite recommendations for a worldwide ban, HBCDD is still widely used and manufactured in some countries and is generally showing rising trends in the environment in media ranging from lake sediments to human breast milk.[72,73] As with many other POPs, given they accumulate over long timescales in higher trophic levels, the need for long-term monitoring of ecosystem effects will remain an area of great concern in the future.

4.6 THERMAL POLLUTION

4.6.1 Anthropogenic Impacts on Thermal Regime

Cooling water discharges from electricity generating stations are the main sources of pollution by heat. Such effluents also contain a range of chemical contaminants which, though small in relation to the volume of cooling water, may in fact have a greater impact on the ecology of the receiving stream.[74] Other anthropogenic activity can also impact on the thermal regime of streams, notably forestry operations, with some studies showing increases of over 7 °C in mean monthly maximum water temperatures in experimental catchments after felling of riparian trees (trees located near rivers, streams or lakes).[75] The location of much plantation forestry in headwater areas with low flow and therefore low thermal capacity can exacerbate such problems.

An increase in temperature alters the physical environment, in terms of both a reduction in the density of the water and its oxygen concentration, while the metabolism of organisms increases. Cold water species, especially of fish, are very sensitive to changes in temperature and they will disappear if heated effluents are discharged to the headwaters of streams. As the temperature increases, the oxygen consumption and heart rate of a fish will increase to obtain oxygen for increased metabolic processes but, at the same time, the oxygen concentration of the water is decreased. For example, at 18 °C, carp (*Cyprinus carpio*) can survive in an oxygen concentration as low as 0.5 mg l^{-1}, whereas at 35.8 °C the water must contain 1.5 mg l^{-1}. The swimming speeds of some species declines at higher temperatures, *e.g.* trout at 19 °C, making them less efficient predators. Resistance to disease may also change. The bacterium *Chondrococcus columnaris* is innocuous to fish below 10 °C but it invades wounds between 10 and 21 °C while it can invade healthy tissues above 21 °C. On the other hand, some organisms have the ability to adapt to altered thermal regime and there may even be some benefits in terms of increased productivity in some areas receiving heated effluents. Change in thermal regime can also lead to invasive or non-native species colonising areas and either out-competing the native biota or forming large colonies. In some cases this has caused major management problems, such as with zebra mussels (*Dreissena polymorpha*) clogging water discharge or intake pipes.

4.6.2 Climate Change

Much contemporary research is assessing the current and future impacts of climate change on the river environment. These impacts encompass direct changes to thermal regime with rising air temperatures in addition to the predicted increased incidence of extreme hydrological events, be they drought or flood. Flood events play a crucial role in the dynamic equilibrium of lotic systems (flowing freshwater systems such as rivers or streams) and connectivity of instream habitats with floodplain environments. However, frequent extreme events can reduce the habitat complexity of riparian environments while they may also be associated with chemical conditions challenging to instream biota (*e.g.* acid flushing events in upland settings). Of potentially greater ecological significance are the pressures associated with low flow periods which are likely to provide more frequent challenges to water managers in the future. Encouragingly, there are already examples of effective management strategies to deal with such circumstances. For example, the River Tyne in northern England, has seen numbers of Atlantic Salmon (*Salmo salar*) and Sea Trout (*Salmo trutta*) return to pre-industrial levels in recent decades. However, during recent drought years (1995, 2003, 2005) the river has been subject to major fish kills in summer months when returning salmon aggregate in estuarine reaches awaiting suitable flow conditions to progress to headwater spawning grounds.[76] Low flow conditions impart stress on various aspects of the aquatic ecosystem through:

(i) Reducing concentrations of dissolved oxygen.
(ii) Increasing temperature.
(iii) Reducing dilution of perennial pollutant sources (*e.g.* STW point discharges).
(iv) Reducing the volume of water which minimises refuge areas from predators.

An integrated management system in the Tyne catchment sees automated monitoring of dissolved oxygen and temperature in estuarine reaches of the catchment inform environment managers as to the timing of critical poor water quality conditions. This prompts the controlled release of water from a major headwater reservoir. Fish count stations above the tidal limit demonstrate the success of this scheme with large numbers of salmonids migrating upstream after the release, akin to natural flood conditions.[76]

The challenges posed by a changing climate should therefore be viewed alongside other interacting pressures on freshwater systems such as increased abstraction and the discharge of polluted effluents.

Indeed a recent study in upland waters contaminated by mine pollution (notably Zn) in the Rocky Mountains showed a greater sensitivity of benthic invertebrate communities to increased UV-B exposure (another potential stress associated with more frequent low flow periods), which was suggested to be a consequence of the costs associated with tolerance to Zn pollution.[77] Such complex interactions are likely to be a common feature of the response of aquatic biota to changing climate.

4.7 RADIOACTIVITY

Chemically, radionuclides behave in the same way as their non-radioactive isotopes but, if they accumulate up the food chain the radioactive isotopes have much greater significance. Radionuclides come mainly from fall-out from weapons testing and the effluent from nuclear power stations. Because ionizing radiation is highly persistent in the environment, causing cancer and genetic disorders in humans, it has always attracted special concern and the release of radionuclides is strictly monitored and controlled.

Most of our information on the effects of radionuclide release has arisen out of research in the aftermath of the Chernobyl nuclear power station disaster in the Ukraine in April, 1986 (see also Chapter 19). The subsequent spread and deposition of radionuclides, (notably caesium (^{137}Cs) and strontium (^{90}Sr) which have half lives in the region of thirty years), over large areas of western Europe emphasized how potentially damaging and long-lived such pollution can be.

Research has highlighted the pathways by which aquatic organisms and potentially humans can be exposed to ionising radiation after fallout. Caesium behaves similarly to potassium and is soluble in typical surface waters (especially ion-poor upland waters) although once deposited on land, transfer to surface waters can take many years. Studies in the aftermath of the Tohoku earthquake/Fukushima Dai-ichi nuclear disaster in Japan in March 2011 suggested less than 1% of the deposited radiocaesium (^{134}Cs and ^{137}Cs) was transported out of affected catchments in the nine months following the disaster.[78] This highlights the need for long-term monitoring of the fate of ^{137}Cs through aquatic food webs. In studies on Finnish lakes in the months immediately after the Chernobyl disaster, planktivorous fishes such as whitefish (*Coregonus lavaretus*) had the highest concentrations suggesting rapid bioconcentration of ^{137}Cs in plankton and fish with the shortest food chain. Over time however and after 1987, activity concentrations suggested biomagnification of ^{137}Cs across trophic levels, with the piscivorous pike (*Esox lucius*) having activity concentrations 1.5–3.2 times higher than perch (*Perca fluviatilis*), which feeds primarily on zooplankton. These concentrations were in turn 1–2 times higher than planktivorous whitefish.[79] These studies also highlight that activity concentrations in perch and pike exceed human consumption guidelines (600 Bq kg^{-1}) twenty years after the Chernobyl disaster.

Because wild caught freshwater fish feature little in the diet of the vast majority of the local human population it was considered that the Chernobyl accident presented no cause for concern to human health away from the vicinity of the reactor. After Fukushima however, there have been concerns raised about a potential exposure pathway for humans from rice, given extensive use of river water for irrigating rice paddy fields in catchments affected by the fallout and the fact that Cs is readily taken up from soil solutions by many plants.

However, other species within the aquatic ecosystem are more exclusively piscivorous. The impact of fallout after Chernobyl has been demonstrated in studies of otter (*Lutra lutra*) from the UK. In south west Scotland in particular, an area of soft waters and with high elevation terrain heavily affected by Chernobyl fall-out, average radioactivity in otter faeces (spraints) were more than six times that in less affected areas of Wales (up to a maximum of 79 500 Bq kg^{-1} in one sample).[80] Unfortunately, there was no pre-Chernobyl sample but levels were still high in the following January, as has been found for other biological materials from this area.[80]

In highly contaminated areas around Chernobyl there has been extensive documentation of mutagenic effects of ionising radiation on aquatic life. These include increased incidence of aneuploidy (a chromosome in excess or missing) in channel catfish (*Ictalurus punctatus*), Crucian carp (*Carassius carassius*), carp (*Cyprinus carpio*) and tench (*Tinca tinca*) and increased DNA damage in channel catfish.[81] However, at lower dose rates further afield it is more challenging to disentangle the signal of ionising radiation from other mortality factors and environmental pressures; a common theme in ecotoxicology. New approaches to screening molecular level response to ionising radiation may be able to assist in partitioning such DNA damage in the future as has recently been demonstrated in marine mussels (*Mytilus edulis*).[82]

4.8 OIL

Compared to the marine situation (see Chapter 2), comparatively little work has been done on the effects of oil in freshwater ecosystems. Nevertheless the chronic pollution of freshwaters with hydrocarbons is widespread. Much of it derives from diffuse urban sources such as petrol and oil washed from roads together with the illegal discharge of engine oil. In England and Wales, the Environment Agency report on average 3000 pollution incidents involving oils and fuels each year,[83] and there have been various initiatives in recent years to minimise such incidents. These include educational initiatives aimed at better handling and storage of oils through to Sustainable Urban Drainage Systems (SuDS) to manage diffuse urban sources and prevent discharge of contaminated runoff into surface waters.

The water soluble components of crude oils and refined products may prove toxic to freshwater animals though the prediction of toxic effects is rather difficult owing to the complex chemical nature of discharges. American studies have shown eggs and young stages of organisms are especially vulnerable. In a study using the tree frog *Hyla cinerea*, hatching success of eggs was not influenced by the presence of oil but growth rates and metamorphosis were.[84] A study of pearl dace (*Margariscus margarita*) in pools contaminated with diesel fuel demonstrated severe pathological changes and a failure in reproduction which would eventually lead to the extermination of this fish population.[85]

In general terms, the aliphatic compounds of oils are relatively innocuous while the aromatic hydrocarbons, such as benzene, xylene and toluene are highly toxic. There are also marked species differences in susceptibility to these compounds, further adding to the difficulties of making predictions about toxicity. Some components of oils, such as PCBs and lead, will accumulate in tissues. Emulsifiers and dispersants, used to clean up spillages, are themselves often highly toxic as has been demonstrated in marine settings after the Deepwater Horizon spill.[86] The surface active agents which they contain make cell membranes more permeable and increase the penetration of toxic compounds into organisms. In this way mixtures of oils and dispersants are often more toxic than either applied separately.

The physical properties of floating oil are a special threat to higher vertebrates, especially aquatic birds because contamination reduces buoyancy through damage to the waterproofing of plumage, while oil may be ingested during subsequent preening. A further problem is the tainting of flesh, especially of fish, which is detectable to the human palate at very low levels of contamination and renders fish inedible. The major sources of taint are light oils and the middle boiling range of crude oil distillates but there are a number of other sources, such as exhaust from outboard motors, waste from petrochemical factories, refinery wastes, and all crude oils.

4.9 EMERGING CONTAMINANTS

In recent years there has been increasing concern about the impacts of so called 'emerging contaminants' on the environment. These encompass a broad range of synthetic compounds being increasingly used in everyday life and released into the environment generally without prior systematic risk assessments of their potential long-term effects on the terrestrial or aquatic environment. While the presence of these novel materials in the environment does not necessarily indicate negative effects on aquatic organisms, there is an urgent need to improve our knowledge base to accurately assess such risks. The key emerging contaminants include human and veterinary pharmaceuticals (and their metabolites), personal health care products, flame retardants (the example of HBCDD we considered previously) and engineered nanoparticles. Some of these have been released to the environment (notably medicines) for many decades, but it is only in recent years that improved analytical techniques have permitted direct measurement of them in a range of environmental matrices.

4.9.1 Nanomaterials

Nanotechnology concerns the development and exploitation of technologies of the size order 0.1–100 nm in diameter, and promises to be an area of scientific innovation and economic growth over coming decades. While we are aware of the very harmful effects of fine combustion-derived particulates (ultrafines) on pulmonary systems of mammals and humans, due to the novelty and rapid growth in nanotechnology applications, we have yet to gain a detailed understanding of potential hazards associated with the presence of engineered nanoparticles (ENPs) in the aquatic environment. Key sources and pathways for engineered nanoparticles to reach freshwater systems are summarised in Table 4.4. The most commonly cited ENP aquatic pollution problem concerns silver nanoparticles, which have been widely adopted in recent decades as antibacterial/antifungal agents in medicinal applications, biotechnology and textiles. The latter represents a key potential pathway into aquatic systems, where antibacterial textiles, such as the burgeoning market for antibacterial leisurewear, release silver ENPs during washing cycles into wastewater treatment systems. There they could be of potential significance to microbial communities essential for waste water purification. The presence of silver nanoparticles has been established in a range of wastewater treatment sites in various studies with the potential for a portion to pass through these systems highlighted. The key pathways by which aquatic biota could be adversely affected by ENP include direct ingestion, transfer across the body wall or *via* epithelial boundaries such as gills or olfactory organs.[87] The ingestion pathway may be exacerbated given ENPs aggregate to microalgae

Table 4.4 Examples of engineered nanoparticle sources and pathways to the aquatic environment.[89,90]

Nanoparticle	*Application*	*Important pathways to the aquatic environment*
Silver (n-Ag)	Antibacterial/ antifungal agent in textiles; medicinal uses.	Domestic washing of n-Ag containing textiles releases n-Ag into wastewater; sewage sludge application to land.
Nano titanium dioxide (n-TiO_2)	Paints; whitening agent; cosmetics and sunscreens.	Personal Health Care Products → wastewater effluents; industrial effluents; localised high concentrations in bathing waters.
Nano zinc oxide (n-ZnO)	UV filter in cosmetics.	Personal health care products → wastewater.
Fullerenes (C60)	Cosmetics, bactericides, drug delivery systems.	Personal health care products → wastewater.
Single walled carbon nanotubes (SWCNT)	Superconductors, optical and storage devices, fuel cells, catalysts.	Industrial effluents; poorly regulated disposal (*e.g.* landfill leachate).

and could cause bioconcentration of ENPs from the water column for zooplanktonic grazers such as copepods and cladocerans.[88]

Numerous workers have undertaken laboratory toxicity tests of ENPs on various common test species such as *Daphnia magna* and *Onchorynchus mykiss* which typically show a low acute toxicity.[90] However, silver nanoparticles have been demonstrated to accumulate in bacterial cell membranes and cause cell death,[90] while significant inhibition of growth of the commonly used aquatic plant bioassay, *Lemna minor* (Common Duckweed), has been observed at exposure rates of the same order of magnitude as those modelled in wastewater treatment facilities (but higher than those observed thus far in environmental settings).[91] Titanium oxides have been shown to inhibit photosynthesis in algae and fullerenes have been implicated in causing oxidative stress in the brains of juvenile Largemouth Bass (*Micropterus salmoides*) at low concentrations.[90,92]

However, most toxicological screenings of engineered nanoparticles are accompanied by various precautionary notes on findings.[88,90] Traditional standard toxicity tests are designed for ionic compounds in solution, whereas the behaviour of ENPs in the environment is governed by complex interactions with other chemical species, notably humic substances, which bind to particle surfaces and potentially limit bioavailability, as well as agglomeration and sedimentation which is difficult to replicate in laboratory tests. As such, there are widespread calls for new systematic approaches to exposure and effects studies of ENPs using a range of test species, appropriate environmental matrices and ENP concentrations, and tests on a range of organisms proceeding to various end-points to identify sub-lethal as well as lethal concentrations. Such work will play a key role in assessing the long-term risks of ENPs to the aquatic environment.

4.9.2 Human and Veterinary Medicines

We have already considered the example of oestrogenic pollution on rainbow trout which highlighted the importance of biomonitoring in identifying potential pollution impacts that would not be recognised by routine chemical monitoring alone. In recent years considerable research effort has been invested in assessing the effects of a range of emerging human and veterinary drugs on the water environment. As with oestrogenic compounds, it is the use, adsorption, metabolism and excretion of pharmaceuticals, be they prescribed or illicit, into sewage treatment systems, that provide the principal pathway for environmental exposure. Some drugs and metabolites will at least in part pass through treatment systems and into surface waters, while the application of sewage sludge to land as an agricultural amendment provides a major secondary pathway whereby compounds initially partitioned onto solid phases in sewage treatment works can be leached into the aquatic environment. Other exposure pathways can include release during manufacture and during disposal of unused medicines.[90] Pharmaceuticals such as hormones, steroids, antibacterials and illicit psychoactive drugs pose particular problems given they are designed to interact with human or animal receptors and can therefore trigger a biological response (and likely behavioural response) at incredibly low concentrations in the environment. Impacts have been documented for some common pharmaceuticals, for example, ibuprofen, widely used as an anti-inflammatory, has been shown to negatively impact the growth of aquatic plants such as *Lemna minor*, while stimulating cyanobacterial growth.[93] However, for many emerging substances such as illicit drugs, studies are establishing their presence in surface waters,[94] but we do not know yet what the long-term impacts on wildlife and humans are likely to be. An additional challenge posed by human and veterinary pharmaceuticals are the numerous transformation products or degradates that arise from parent chemicals either in the body or in the environment, some of which can be more toxic than the parent compound.[90] Therefore research is needed not only to test the impacts of an individual compound, but also to identify and assess the toxicity of degradates, which some recent legislation explicitly demands (*e.g.* the *EU Pesticides Directive: 91/414/EEC*). As with ENPs, traditional short-term toxicity tests may not be particularly appropriate for pharmaceuticals given exposure to medicinal products and degradates is likely to chronic, over very long periods of time, albeit at low concentrations.

4.10 BIOLOGICAL MONITORING OF POLLUTION IN FRESHWATERS

4.10.1 Laboratory Monitoring Techniques

The biological assessment of pollutants includes both laboratory and field techniques.[1] In the laboratory, the most widely used methods are toxicity tests for the preliminary screening of chemicals, for monitoring effluents to determine the extent of risk to aquatic organisms, and, for those effluents which are toxic, to determine which component is causing death so that it can receive special treatment. The simplest type of test is the static test in which an organism is placed in a standard tank in the water under investigation for 48–96 h. There are normally a series of tanks with test water of different dilutions. More sophisticated techniques involve the periodic replacement of test water or indeed continuous flow systems. Fish have traditionally been used as test organisms. In the United States, the main test species have been fathead minnows (*Pimephales promelas*) and bluegill sunfish (*Lepomis macrochirus*). Much toxicity work in Britain has been with rainbow trout but the tropical harlequin (*Rasbora heteromorpha*) has become increasingly popular because it is smaller and has a similar sensitivity to pollutants. Fish require large volumes of clean water for maintenance and, because tests need replicating, much space is needed. Furthermore there are obvious ethical objections to using vertebrates for routine toxicological assessments. There has therefore been much research effort in developing other test organisms across a range of trophic levels in whole effluent toxicity tests. The planktonic crustaceans *Daphnia magna* and *D. pulex* are widely used for they are easily cultured, have a high reproductive rate and are sensitive to a range of pollutants. Other invertebrates that have been used include the resilient brine shrimp *Artemia salina,* tubificid worms, zebra mussels *Dreissena polymorpha*, whose water filtration rates are measured, and the flagellate *Euglena gracilis*, in which movement is the measure of toxicity.[95–97] Bacterial tests are also available, of which one, the Microtox® test, is commercially available. It utilizes the bioluminescence of the marine bacterium *Allivibrio fischeri*, the reduction in light output being the measure of toxicity. The test is sensitive, precise and reproducible. In many applications a range of test organisms are used not only to reflect impact on different trophic levels, but potentially different environmental compartments, such as sediments, soils and waters.[14]

The revolution in molecular testing and bioinformatics highlighted previously will see major changes in the way we approach toxicity testing in the future. These new approaches should address the increasingly complex issues faced such as the differing exposure scenarios required for some emerging contaminants, testing mixtures of contaminants, assessing susceptibility at different lifestages and understanding the mechanisms of toxicity.

4.10.2 Field Monitoring Techniques

Fish and macroinvertebrate communities have been used as tools for monitoring the health of streams and rivers by monitoring agencies for over thirty years in some parts of Europe and North America. Macroinvertebrates have generally been the most favoured group for monitoring in the UK given they are relatively easy to study, are crucial components of aquatic food chains, sensitive to many pressures and their short lifespan (typically months to years) along with lack of mobility means that changes in community can be a used as a good indicator of impacts. Indeed community analysis in its simplest form can provide useful insight into invertebrate response to pollution on a site by site basis. For example, diversity indices take into account the number of species within the collection (species richness) and the relative abundance of species within the collection (evenness). It is argued that a community from an unstressed, *i.e.*, pollution free, environment will contain a large