

Waste Management, Bioremediation and Land Restoration

Managing the chemistry and biology of an acid lake by adding nutrients.

A large proportion of the acid neutralizing capacity of soft water lakes is generated within the lake through processes associated with the synthesis and decomposition of organic material. By stimulating the carbon cycle, nutrients can indirectly influence the pH and stimulate the development of a more diverse biotic community. Seathwaite Tarn, an upland lake in the English Lake District (Figure 21), is currently the site of a field experiment designed to test the feasibility of increasing the pH of an acid lake by fertilising with phosphorus. The central objective of the experiment is to develop a system of internal base generation that can be used to buffer the pH of any moderately acid lake. The first phase of the experiment was designed to cover a three year period with one 'pre-treatment' year followed by two years of regular fertilisation.

In 1991, we monitored the physical, chemical and biological characteristics of the untreated lake and produced chemical budgets to quantify the seasonal change in pH. The lake is relatively unproductive (average chlorophyll concentration $< 2 \mu\text{g l}^{-1}$) and the pH typically fluctuates between pH 5 in winter and pH 5.3 in summer. Fertiliser was added to the lake at fortnightly intervals in 1992 and the dosing rate doubled in 1993. Only 75 litres of a concentrated solution of sodium phosphate (Figure 22) was required on each occasion to increase the notional 'epilimnetic' concentration of soluble reactive phosphorus to $15 \mu\text{g l}^{-1}$ in 1992 and $30 \mu\text{g l}^{-1}$ in 1993. Most of the phosphorus added to the lake was rapidly assimilated by the growing phytoplankton. The maximum concentration of phytoplankton chlorophyll increased from $2 \mu\text{g l}^{-1}$ in 1991 to $37 \mu\text{g l}^{-1}$ in 1993. The most immediate effect of fertilisation was a pronounced increase in the assimilative uptake of nitrate nitrogen



Figure 22. Adding the fortnightly dose of concentrated sodium phosphate.

(Figure 23a). In 1991, around $25 \mu\text{E l}^{-1}$ of nitrate nitrogen were left in the lake at the end of the growing season but by the summer of 1993 there was no measurable nitrate in the lake. This assimilative uptake of nitrate was responsible for most of the pH changes noted in 1992 and 1993 (Figure 23b). In 1991, the average pH of the lake was 5.16 and the maximum summer pH was 5.30. In 1993, the average pH was 5.49 and the maximum summer pH had increased to 6.0. The regular additions of phosphorus have had relatively little effect on the qualitative composition of the plankton. A few species known to be characteristic of very unproductive waters have disappeared but there has been a marked increase in the general productivity of the lake. The numbers of filter feeding microcrustacea have increased by almost two orders of magnitude since the lake has been fertilised. Calculations suggest that the grazing rate of the microcrustacea in 1993 was high enough to limit the growth rate of the phytoplankton and greatly accelerate the rate at which organic matter accumulated in the deep sediments.

The treatment described here used less than 6 cubic metres of concentrated phosphate solution. To bring about

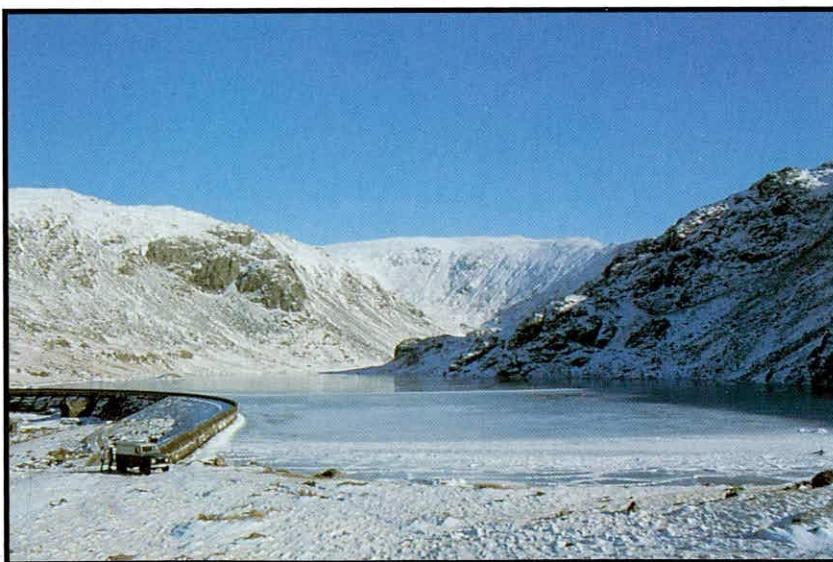


Figure 21. A general view of Seathwaite Tarn.

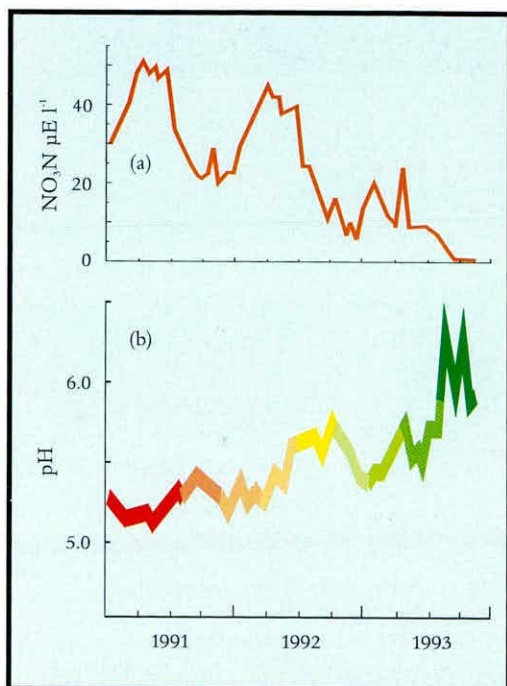


Figure 23. The chemical effects of adding fertiliser in 1992 and 1993.

a) The seasonal change in the concentration of nitrate nitrogen in 1991, 1992 and 1993.

b) The seasonal change in the pH of the lake in 1991, 1992 and 1993.

the same effect using lime would have required 34 tonnes of calcium carbonate and a more complicated system of preparation and dispersion. Like other neutralisation strategies, the addition of fertilizer is likely to be most effective in lakes with long residence times. Seathwaite tarn has a relatively short residence time (3 months) so its winter pH is readily depressed by prolonged periods of heavy rain.

Oiled beach materials

It has been estimated that 2.35 million tonnes of crude oil and derivatives thereof entered the worlds oceans during 1990. Newsworthy events such as tanker accidents cause disastrous localised pollution but these make up only 5% of the total hydrocarbon load to the marine environment. Once in the sea the oil begins to change due to weathering processes and most volatile and

soluble components are lost within a short time. Weathered crude oil can be regarded as a complex mixture of hydrocarbons with varying susceptibility to biological decomposition. Generally the aliphatic compounds are most readily attacked with the aromatic compounds being less susceptible. Tars

and asphaltenes tend to be more recalcitrant with low rates of degradation. The critical stage in the degradation of aromatic compounds is the cleavage of the ring structure. Weathered oil residues are eventually washed up on beaches and it is the responsibility of the local authorities to clean-up and dispose of the oiled beach material (OBM). Landfill sites have been used for disposal in the past but these are now becoming less available and alternative disposal options are being sought.



Figure 24. Duplicate plates before and after (left) spraying with catechol. Only the colonies on the left can degrade the ring structure.

The IFE is a member of a consortium including the Institute of Terrestrial Ecology, British Geological Survey and engineers from Sir William Halcrow and Partners (Scotland) Ltd which has been commissioned by the Marine Pollution Control Unit (Dept. of Transport) to:-
i) test the suitability of burial and

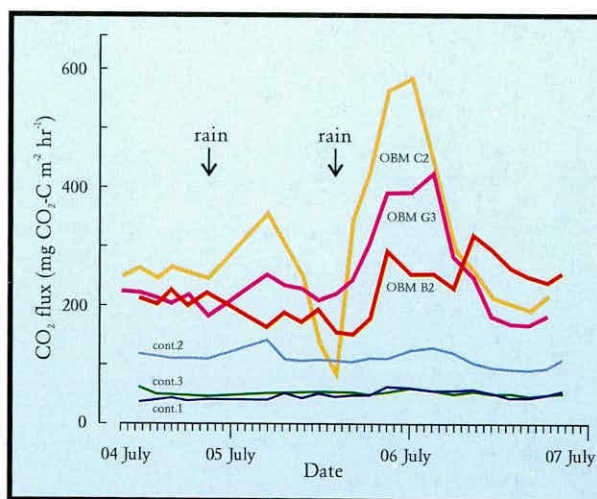


Figure 25. Rate of carbon dioxide emission from three sites on the OBM and three control sites.

landfarming of OBM in sandy coastal environments of low conservation value

ii) to assess if these operations are environmentally acceptable. Field and laboratory trials, including sites where OBM has been dumped, will examine the effects of oil type, time of disposal (winter/summer), the treatment of the dump (landfarming/burial), the potential for groundwater contamination and the effects of different climatic conditions around the British coastline.

The IFE are investigating the response of the microbial communities on exposure to oil residues as determined by changes in community respiration and counts of the number of culturable bacteria. The latter are also tested for the ability to degrade aromatic compounds by spraying the plates with catechol; the colonies capable of degrading the aromatic ring turn yellow due to the production of 2-hydroxymuconic semialdehyde (Figure 24). Whilst this represents only one of several pathways for the cleavage of aromatic ring structures it is frequently plasmid coded and has the potential to be transferred within the bacterial populations. An increase in the incidence of this activity would provide additional evidence for a response to buried oil.

The work is still at an early phase but studies on OBM buried after oil was washed up on the South Wales coast in January 1994 are showing that emissions of carbon dioxide from the dump fluctuate widely over short periods of time and are greatly affected by rainfall (Figure 25). However, the CO₂ flux from the dump was generally greater than that from adjacent 'control' areas. Also organisms capable of degrading catechol were not found in samples taken during February but could be readily isolated from all samples in July.

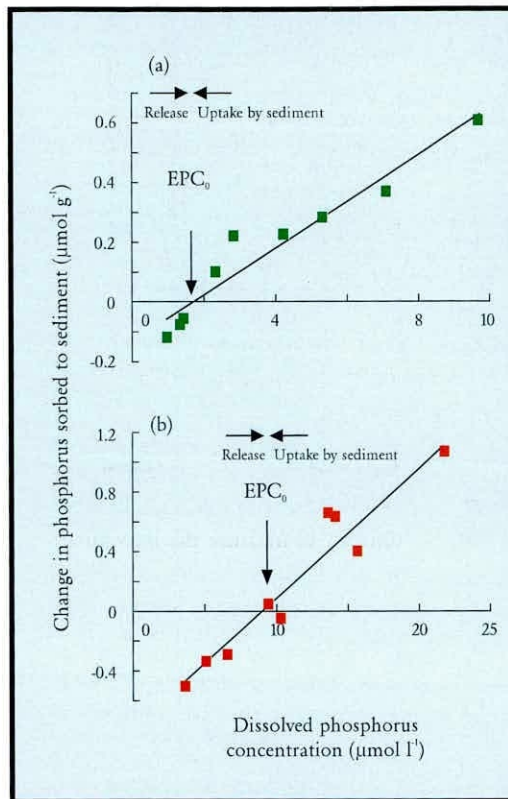


Figure 26. Comparison of the measurement of the equilibrium phosphate concentration for a sediment collected upstream of a sewage outfall (a) compared with at a site downstream (b) of the mixing zone. To convert the concentration to $\mu\text{g/l}$ as P multiply by 31.

The objective of the project is to provide practical protocols for local authorities faced with the problem of coastal oil pollution.

Natural sediments as a source or sink of phosphorus to fresh waters ?

Phosphorus is considered as one of the key nutrients which limit productivity in freshwater ecosystems, whereas nitrogen generally limits productivity in marine systems. The cycling of nitrogen and phosphorus are in fact very different with nitrogen mainly resident in the atmosphere and its cycling mediated by a range of organisms compared with phosphorus which has no abundant gaseous state and is cycled by both biotic and physico-chemical processes.

The forms of dissolved phosphorus range from simple inorganic anions and metal complexes, inorganic polyphosphates to various organic

phosphates and residues originating from the assimilation of phosphorus into living tissues. The analytical methods which measure dissolved phosphorus in water bodies are not generally very selective and so measure a range of molecular forms of phosphorus. Work is in progress to quantify how these operationally defined methods quantify the different fractions, i.e. by molecular or colloidal size to specific groups of compounds. Some parts of these fractions are considered to be biologically available (BAP), i.e. can be utilised directly by the plants and algae. The most important BAP is the inorganic phosphorus predominantly in the form of hydrogen phosphate; this is also the dominant phosphorus constituent of sewage effluent and normally found at increased concentrations in river water at points downstream of sewage discharges. Other sources of phosphorus include the various diffuse inflows from cultivated and uncultivated land; these inputs include both inorganic phosphorus from fertiliser applications and microbial breakdown



Figure 27. Fluvarium at the IFE, River Laboratory, used to measure nutrient fluxes in polluted and unpolluted river sediments; river water from the R. Frome is used to maintain the bed-sediment temperature as close to natural conditions as possible.

of organic matter and organic phosphorus which has been mobilised by incorporation in bacteria tissue and subsequent degradation. With our present knowledge of phosphorus chemistry, it is impossible to generalise on the relative contributions of different phosphorus compounds to fluxes. Instead a pragmatic approach has been taken to measure phosphorus by well defined operational procedures. These include soluble reactive phosphorus, i.e. that phosphorus measured after filtration at $0.45 \mu\text{m}$ by complexation with molybdate in acid conditions to form a coloured molybdo-phosphoric acid complex, total dissolved phosphorus measured by the same procedure after oxidation and digestion with persulphate and total phosphorus measured as total dissolved phosphorus but without filtration of the sample. In addition other methods are available to estimate the biologically available phosphorus using for example iron-oxide stripping to imitate the effects of algae in taking phosphorus out of the water.

Once phosphorus enters water bodies it is involved in many cycles. Recent research at the Institute has focused on the interactions of phosphorus with sediments, both in suspension and in the river bed. Inorganic phosphorus has a high affinity for sediments and there is generally either a net uptake or release of phosphorus depending on the concentration in the overlying water, capacity of the surface sediment

for phosphorus and biological productivity at the surface. It is difficult to measure the individual invasion and evasion fluxes and much easier to measure the net flux of phosphorus which, after all, determines the impact of the sediment on the nutrient status of the water. In the laboratory this may be done by enhancing or depleting the concentration of soluble inorganic phosphorus in contact with the sediment and then measuring the net uptake or release rate of soluble reactive phosphorus.

The interaction of inorganic phosphorus with suspended sediment is relatively fast over the first four hours of contact but then slows down but continues over 24 h and longer. Various kinetic equations have been applied to quantifying the results and have proved successful. In contrast, the kinetics of uptake and release on flat river bed sediments is slow with the interaction of the phosphorus molecules thought to occur in the surface layer of the sediment and controlled by diffusion processes. It has been possible to understand the flux in these situations using a parameter derived from the measurement of the adsorption isotherm at 10°C termed here the equilibrium phosphate concentration, EPC_0 (Figure 26). This was first used by soil scientists to measure the concentration of phosphorus in solution which, if placed in contact with a soil, produced no net flux of

phosphorus. For a river sediment it is the concentration of soluble reactive phosphorus in the water in contact with a sediment at which there is no net transfer of phosphorus to or from the sediment. As a sediment is exposed to higher concentrations of inorganic phosphorus, the EPC_0 increases so that when the concentration of phosphorus in the water decreases for whatever reason, a release of phosphorus from the sediment occurs. The response of the sediment has been found to depend on the difference between the EPC_0 and the dissolved phosphorus concentration with the flux of phosphorus increasing as the concentration profile increases.

Different methods have been applied to utilise the information on the equilibrium phosphate concentration of the sediment to estimate fluxes and the release and uptake rates of phosphorus in different conditions. An experimental channel housed in the fluvarium at the River Laboratory (Figure 27) has been used over the last year to measure net phosphorus fluxes to river sediments collected from polluted rivers from different regions of England. The object of the study is to determine the role of the sediments in controlling the phosphorus concentrations in the water, particularly in situations where the water has been subject to a high external loading of phosphorus from sewage treatment. Ancillary measurements on the sediment have been made including particle size distributions, wet analysis of the sediments, BAP and EPC_0 . The chemical data, in conjunction with the fluvarium studies, are used to model the kinetics of the release and uptake of phosphorus to the bed sediment and applied to understanding the changes in the phosphorus concentration in rivers downstream of sewage inputs and how the response changes with the seasons.

Environmental Pollution

Statistical appraisal of the Harmonised Monitoring Scheme

The Harmonised Monitoring Scheme (HMS) was established by the Department of Environment (DoE) in 1974 to monitor river water quality in Britain within a consistent framework of lowland sites. The scheme covers physical determinands such as pH, determinands measuring oxygen, nutrients, the major anions and cations, detergents, heavy metals, pesticides, polychlorinated biphenyls, phenolics, polyaromatic hydrocarbons, bacteria and non-metals such as fluoride. By 1992 the HMS data for England and Wales

totalled over 2.35 million measurements taken at about 200 sites. These data constitute the best long-term records for river water quality in Britain.

Water sample collection and laboratory analyses were carried out by the regional water authorities until privatisation of the water industry in 1989, when responsibility moved to the National Rivers Authority (NRA). The DoE have continued responsibility for the computer database where the records reside. At the behest of the NRA and following the 1992 report by the Royal Commission on Environmental Pollution, the IFE carried out a

statistical appraisal of the HMS data for England and Wales.

The first phase of the appraisal was to catalogue the massive contents of the database. Site and determinand directories were produced detailing the measurements made in each year together with summary statistics.

The second phase looked in detail at the quality of data for seventeen determinands, namely average and instantaneous flow, ammoniacal nitrogen, free ammonia, chloride, biochemical oxygen demand (BOD), percentage oxygen saturation, dissolved oxygen, nitrite, nitrate, orthophosphate, total phosphorus, total phosphate, cadmium, copper, zinc and the pesticide Gamma-BHC. The precision of estimation of annual mean quantity was judged against a target error of 20%. The target was achieved for chloride concentration, percentage oxygen saturation, dissolved oxygen and nitrate concentration. BOD fell a bit short of the target and the others failed by a large margin. Notwithstanding, the data for thirteen of the seventeen determinands were found to be adequate for assessing long-term change at many sites because this was more a function of numbers of years of monitoring than precision of estimation in any one year. The percentage of sites showing improvement and deterioration is illustrated in Figure 28.

Some additional questions were addressed in the second phase. A comparison of adjacent sites was undertaken with a view to reducing the number in the scheme. Regional variation in levels of pollutants was looked at and recommendations were made for improved recording and future analysis of the data.

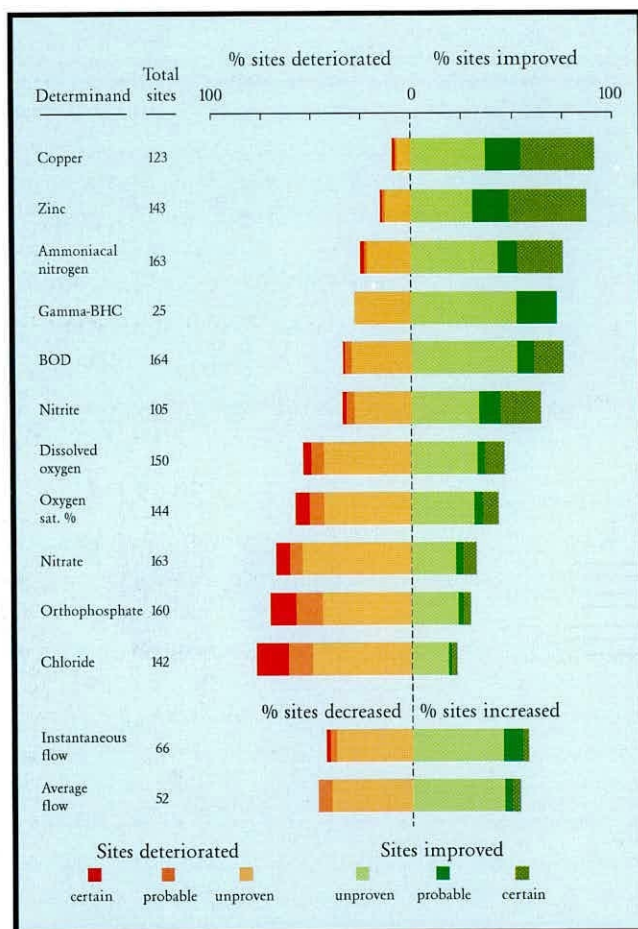


Figure 28. The percentage of Harmonised Monitoring Scheme sites which have improved or deteriorated between the late 1970's and the early 1990's for thirteen determinands.

Pollution, eutrophication and Bassenthwaite Lake

IFE has included the long-term sedimentary record of biological and geochemical changes to Bassenthwaite Lake (a Cumbrian NNR) in its water quality review for National Rivers Authority, North West Region. This lake has a large catchment in a region of volcanics and slates, with numerous old mines and quarries; other land perturbations include the building of the Penrith-Cockermouth railway, Thirlmere Dam and Keswick by-pass. Treated sewage effluent from Keswick also enriches the lake.

The lake has been studied over the last few years but earlier data are only infrequent. Long-term environmental history and evidence as to the natural lake state was lacking and has been assessed from the palaeolimnology of a short sediment core; dating, by lead-210, has identified several periods of increased accumulation in the top 27 cm, or last c.100 years.

Catchment disturbance and sediment deposition

Although the surface sediment is very

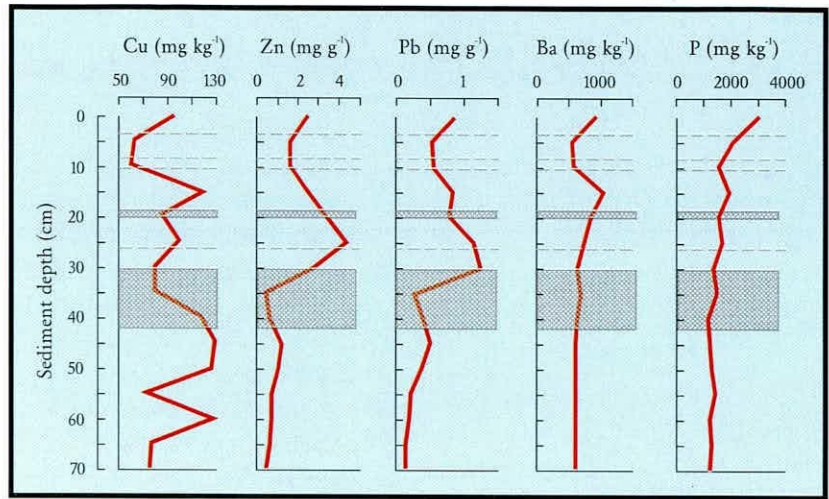


Figure 29. Concentration changes in certain minerals, together with total phosphorus, in the recent sediments of Bassenthwaite Lake in the north-west of the English Lake District. Lithology (.....) organic, older material is more minerogenic and susceptibility indicates considerable ferrimagnetic alteration within the grey clay layer (30 - 42 cm), indicating changes in source material. Organic carbon <5% (except in the top 5 cm) confirms a higher clay concentration due to more catchment disturbance than in other local lakes.

Lead, copper and zinc have been mined on this catchment since the 1200's (19thC mechanization leaving sizeable spoil heaps), most remaining mines closed as post-war prices declined; high metal levels at 25-30 cm (mid-1800's to

c. 1920) reflect this. The clay layer (30-42 cm, Figure 29) is not directly correlated with mine waste as metal concentrations do not rise, as in Coniston sediments related to copper mining. The top of the clay dates from pre-1890 (pre-Thirlmere Dam construction which seems to have made little impact on deposition in Bassenthwaite) so probably much of the clay layer reflects local railway construction c. 1850, which has diluted mining inputs. A dated clay band at 19-20.5 cm suggests changed wartime agricultural practices, while increased sediment accumulation of

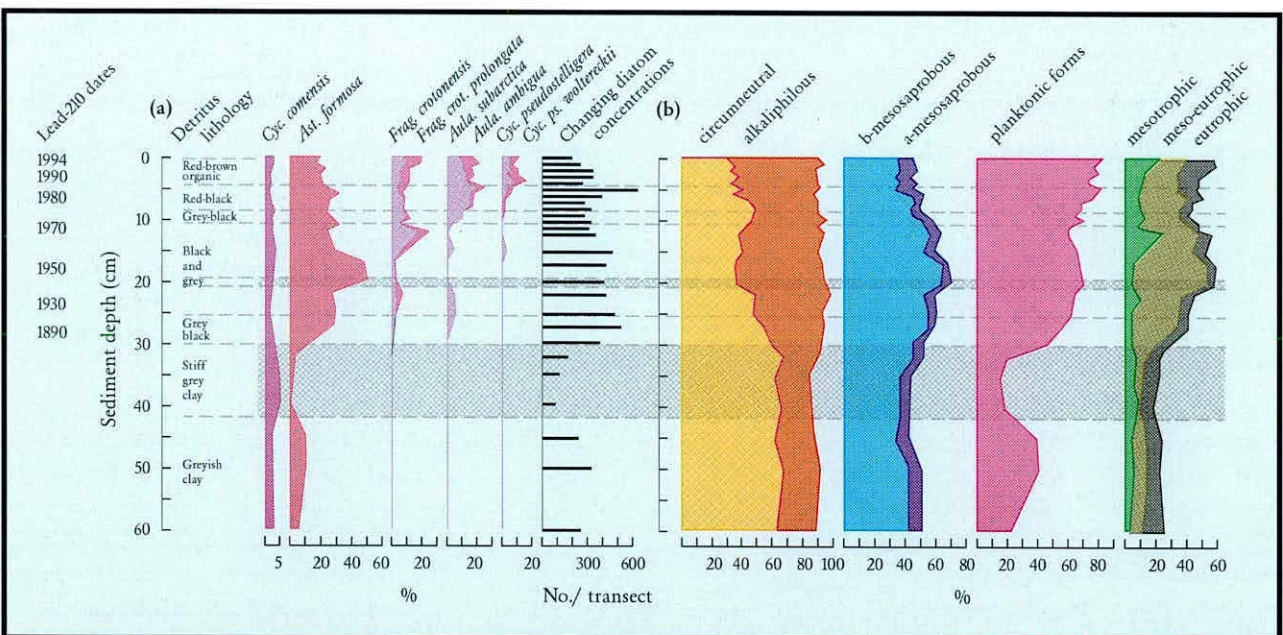


Figure 30. Percentage sediment profiles of certain indicator diatoms plus overall changes in alkalinity (circumneutral to alkaliphilous), water quality (b- and a- mesosaprobous) and nutrient level (mesotrophic and eutrophic) indicators. Timescale and lithological changes (----) are also indicated.

the mid 1970's, with lower water and organic content, reflects the 1973-4 roadworks alongside the lake and north of Keswick. Barium was mined locally 1939-47 and into the 1960's and concentrations are highest in sediments dated 1944-60.

Changes in nutrient levels and lake biota

Changing diatoms indicate distinct environmental changes within the 0-60 cm section (Figure 30), with a steady increase in diatom plankton from 20%, mainly of low nutrient indicators (*Cyclotella comensis*, *Synedra nana*), to over 60%, including a series indicative of increasing nutrients (*Asterionella formosa*, *Fragilaria crotonensis*, *Aulacoseira subarctica*, *A. ambigua* and *Cyclotella pseudostelligera*). Diatom populations increased over the period, with an obvious decrease in the clay section. *A. ambigua* (a eutrophic diatom) only occurs in post-1987 plankton samples and was assumed to be a recent arrival, however our studies show that this taxon arrived c.1890; small short-term population increases occurring c.1921, 1937, and the mid-1950's, before the current post-1981 expansion. The thin silicification of many of these diatom valves, as in *Cyclotella pseudostelligera* fo. *woltereckii*, suggests that population responses to other plant nutrients have caused periodic silica depletion.

The plankton increase coincides with the enhanced levels of total and soluble reactive phosphorus, between 0-26 cm (post-1914). Humic acid bound P (unavailable for growth) became significant in post-1957 deposits. TP rises in more recent sediments and, while chemical levels may be modified by diagenesis, diatoms indicative of these increased nutrient levels have been more abundant since 1980; pollution-tolerant taxa also increased. Such changes suggest a trend contrary to any lake management strategy.

Metal-particle interactions

The Land-Ocean Interaction Study (LOIS) is a major Community Programme, involving NERC Institutes and Universities. One of IFE's contributions is a collaborative study with the University of Plymouth into metal-particle interactions in rivers and estuaries. This work has benefitted from the participation of a visiting Brazilian worker (Dr J R Ferreira), funded by a Marie Curie Fellowship from the EU.

Metals occur in the aquatic environment in many different chemical forms, the distribution among which determines their

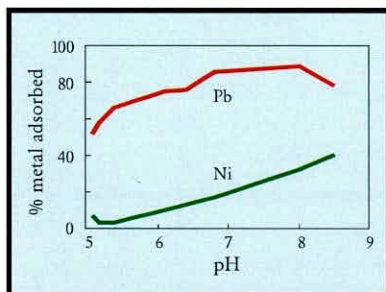


Figure 31. Dependence on pH of the adsorption of lead (Pb) and nickel (Ni) by suspended particles from the River Ouse. To obtain the results, the particles were separated from the solution by centrifugation, and residual metal concentrations were determined with an Inductively-Coupled Plasma / Mass Spectrometer (ICP-MS).

availability to biota, and their retention and transport. Interactions with suspended particles can exert a powerful control on metal chemistry, and the project is concerned with the measurement and modelling of these interactions. The study is focused on the Ouse and Trent river/estuary system, with the aim of understanding how metal chemistry changes as fresh water mixes with sea water.

At IFE we have made measurements of the adsorption of metals by samples of suspended particulates from the

River Ouse. The extent of adsorption is found to vary with pH (solution acidity) and among metals. Figure 31 shows that lead is more strongly adsorbed than nickel and that both metals bind more strongly as pH increases. In a simplified way, the adsorption of metals can be considered in terms of two competing chemical reactions;



Here P represents the particle, initially with an electric charge of Z-, H+ is a hydrogen ion and M+z is the metal ion. When the concentration of H+ is high (low pH), the first reaction predominates, and there is relatively little PZ- available to react with the metal. At higher pH (lower H+ concentration), the second reaction comes to the fore, and metal adsorption is favoured. The change in charge with pH is shown by the results in Figure 32, which were obtained from acid-base titrations of the particles.

Future work in the project will examine adsorption by samples from other sites in the river-estuary system. We shall also attempt to relate the metal adsorption to the different types of particles present in the samples, and to their charging behaviour.

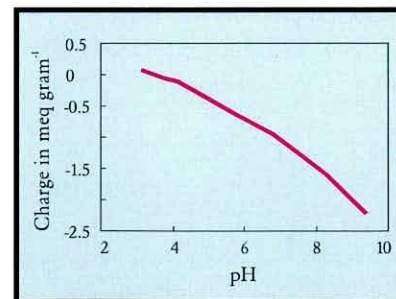


Figure 32. Net electrical charge on River Ouse particles as a function of pH. As the pH is increased, hydrogen ions desorb from the particles according to reaction E1, and the particle charge becomes more negative.

Modelling upland water chemistry

The chemical composition of a surface water is a principal factor in determining its ecology. Understanding of how the chemistry changes on both short and long

laboratory data characterizing the chemistry of natural organic matter, which is the dominant solid phase in acid upland soils. This chemical sub-model (WHAM) was described in last year's IFE Annual Report.

CHUM identifies two forms of soil

to capture most of the variation, in terms of both magnitude and timing. It also reproduces reasonably well variations in the concentrations of major cations (Na^+ , Mg^{2+} , Ca^{2+}), major anions (Cl^- , NO_3^- , SO_4^{2-}), and aluminium.

CHUM can be used to investigate and predict the influences of acid rain and changes in climate on upland waters. In addition, it can be applied to trace elements, by exploiting the extensive database of the WHAM sub-model; in this context the behaviours of heavy metals and radionuclides can be explored.

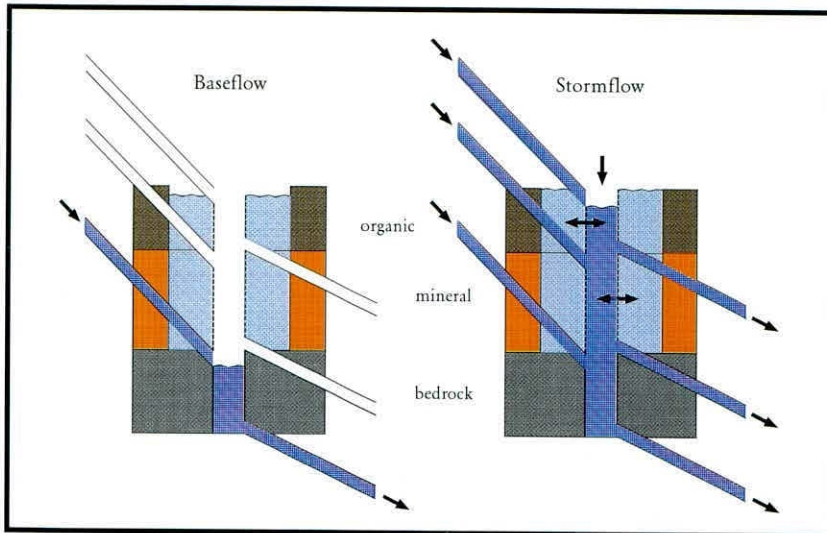


Figure 33. A soil/rock column as represented by CHUM, showing water levels under baseflow and stormflow conditions. The soil horizons are organic (brown), mineral (orange) and bedrock (dark grey). Water is mobile (dark blue) or immobile (light blue). The sloping "pipes" transfer water between adjacent columns on the hillslope, or to the stream.

timescales is necessary in order to interpret ecological observations, and to predict future changes. The Chemistry of the Uplands Model (CHUM) has been developed at IFE in order to calculate flow-dependent water chemistry in upland catchments of the United Kingdom, on the basis of the hydrological, chemical and biological processes that modify rainwater as it passes through the plant-soil-rock system.

The principal processes considered in CHUM are the chemical interactions of solutes with the solids of different soil horizons, plant uptake of nitrogen, and weathering reactions of bedrock. Of these, the description of solid-solution chemical interactions represents an advance over other dynamic catchment-scale models. It is based on a wealth of published

water. One is immobile, held by the soil solids, while the other is mobile, controlled by gravity. The immobile water is in chemical equilibrium with the soil solids, and exchanges solutes with the mobile water, depending upon time of contact and exchange rate constants. CHUM conceptualizes a catchment as a series of soil columns, as depicted in Figure 33. Each column consists of two soil columns and an underlying permeable bedrock zone. The mobile water can flow vertically from one horizon to another, and laterally between columns or into a stream or lake. The model is run on a daily time-step, driven by inputs of rainwater, dissolved solutes, and dry-deposited sulphur.

Figure 34 shows calculated and observed flow and pH for a stream in the Lake District. The model is able

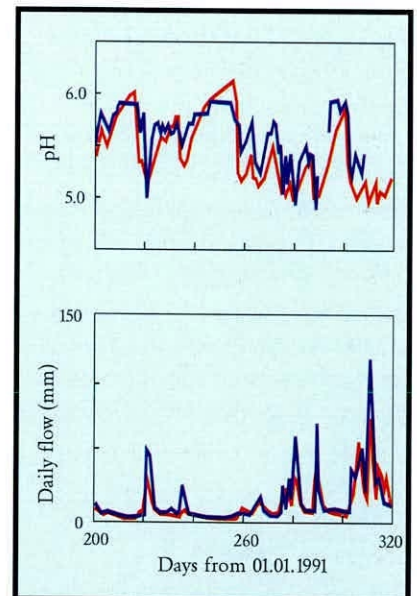


Figure 34. Observed (blue) and CHUM-simulated (red) pH and flow in Tarn Head Beck, Cumbria, for a 120-day period (July-November) in 1991.

Environmental Risks and Hazards

Physiological stress in fish during toxicological procedures: a potentially confounding factor

Deleterious effects of chronic or repeated stress on growth, reproductive function and immunocompetence in fish are demonstrably linked to the activity of the pituitary-interrenal axis and to circulating levels of cortisol. In all vertebrates, stressful stimuli activate the hypothalamic-pituitary-adrenal/interrenal (HPA/I) axis, resulting in rapid elevation of circulating corticosteroid levels. As a component of the broad range of adaptive changes occurring during exposure to stressful conditions, elevated corticosteroid levels are believed to bestow adaptive advantage upon individuals exposed to acute challenge. Paradoxically, elevated corticosteroid levels are also a causal factor in many of the most damaging effects of chronic stress, or of repeated acute stress. Fish are extremely sensitive to environmental stress, and while severe chronic stress and attendant immunosuppression can result in an increased likelihood of disease outbreaks and ultimately mortality, single or intermittent episodes of acute stress can also have profound effects on normal functioning of the fish. An increasing body of evidence suggests that stress may modulate the response of an animal to toxicological challenge by influencing the physiological systems responsible for processing organic and inorganic contaminants.

Fish are widely used as a test organism for defining the toxicity of chemicals to aquatic animals. While it is the case that aquatic toxicological test procedures have been

standardised to reduce inter-laboratory variation and thus increase confidence in results, little or no attention has been directed towards the effect of the procedures themselves on the physiology of the test organisms. Considering the potential impact of unaccounted-for modulation of detoxification mechanisms on the accurate assessment of the toxicity of compounds, a study was designed to address the degree to which widely-used test procedures, in the absence of attendant exposure to test compounds, elicit HPI activation in fish. The degree of stress inherent in the procedures comprising a fish LC_{50} study was assessed by measuring plasma cortisol levels in rainbow trout. Aspects of the 96 h LC_{50} protocol which were found to evoke corticosteroidogenic activity in the test fish included transfer from holding tanks to test tanks, tank transfer during semi-static procedures, and disturbance associated with water quality measurement. There was evidence for acclimation of the fish to repetitive stimuli such as tank transfer but, overall, the data suggest that the 96 h test protocols evoke a state of chronic stress in the test population (see Figure 35). The removal of fish from test vessels during longer-term (6-week) studies was also found to evoke a response in fish remaining within the vessel, suggesting that these conditions may expose test fish to a regime of intermittent periods of acute stress. Therefore, the possibility that physiological stress may interfere with the accurate estimation of toxicological endpoints was examined. In three independent LC_{50} tests the toxicity of the arylmethane dye, malachite green, to rainbow trout was found to be markedly and consistently reduced when the degree

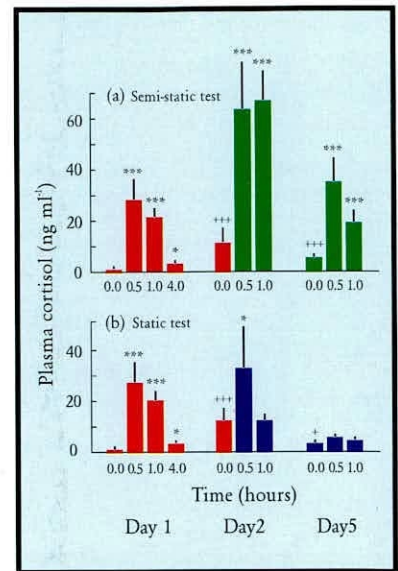


Figure 35. Plasma cortisol levels in rainbow trout during semi-static (a) and static (b) test procedures. Fish were sampled immediately prior to transfer from the acclimation tanks to the test tanks (0 h) on day 1 (red) and immediately prior to and at intervals following pH and dissolved O_2 measurement on days 2 and 5, with (a) (green) or without (b) (blue) additional tank-to-tank transfer. The first 5 data points are common to both groups. Each value is the mean \pm SE, $n = 8$. Asterisks indicate levels significantly different from the 0 h sample on each day; * $p < 0.05$; *** $p < 0.001$. + indicates significantly different from 0 h sample on day 1; + $p < 0.05$; +++ $p < 0.001$.

of disturbance and handling during the test period was minimised. These data indicate that many key elements of commonly-employed fish toxicology protocols are inherently stressful, and that the degree of stress experienced by test fish during toxicological procedures may influence the outcome of the test. Further work may be advisable to determine whether present protocols for acute and chronic toxicity tests are implemented with adequate regard for the possible effects of stress.

Aeromonas salmonicida in the aquatic environment

Aeromonas salmonicida subsp. *salmonicida* is the causative agent of furunculosis in salmonids and related diseases in other species. Infected fish have been shown to shed large numbers of virulent bacteria into the surrounding water. The importance of released cells for the transmission of this disease over long distances is poorly understood. Certain gram-negative bacteria have been shown to enter a physiological state under low nutrient conditions in which they become non-culturable but remain viable (NCBV). The basis of this state is an inability to re-culture these organisms after exposure to low nutrient conditions. Therefore, the presence of NCBV *A. salmonicida* cells in lake water would not be detected by standard techniques that are based on isolation. This may explain how outbreaks of furunculosis can occur in fish populations that apparently have not come in contact with the pathogen. Early warning through detection is therefore of some commercial importance.

We have shown conclusively that *A. salmonicida* can enter a NCBV state and, although non-culturable, maintained their cellular integrity, plasmid and genomic DNA, and RNA; in addition changes in fatty acid profiles and cell size were detected. The survival pattern of *A. salmonicida* in sterile aquatic systems is generally predictable with the organisms becoming unculturable after 5-6 days in fresh water. However, amendment of microcosms with a nutrient, or eutrophication of lake water, was found to extend the period of culturability up to 25 days.

It has been shown that the recovery of *A. salmonicida* on minimal media

containing glucose was entirely dependent on the presence of arginine and methionine. We tested the effect of both amino acids on culturability in amended sterile freshwater systems. In the absence of any amino acid supplements, the survival pattern over 60 days was no different to our previous studies over short time courses of 25 days. However, the survival in microcosms receiving both amino acids was significantly enhanced. In these systems non-culturability was never attained. Single supplements of each amino acid enhanced survival to a lesser extent than the combined amendment.

Common throughout these experiments, which created variable responses with respect to overall culturability, was the universal physiological response of the cells with a reduction in size and rounding up of cell shape (commonly associated with 'stress'). It is clear from these

results that arginine and methionine prevented or at least postponed the entry into non-culturability. Despite this 'stress' response, cells amended with the two amino acids remained culturable. It would appear therefore that the reduction in size and morphological change cannot be taken as an indicator of non-culturability although it may be a significant step in that direction. Furthermore, the amended cells were significantly smaller than those that received no amendment, yet at that size ($< 1 \mu\text{m}$) they remained culturable (Figure 36). It is apparent that both amended and non-amended cells undergo reductive cell division i.e. the cells are smaller after each division. The difference between the two treatments could be due to the amended cells undergoing more reductive cycles. Further work is focusing on the pathogenicity of the 'dwarf' cells.

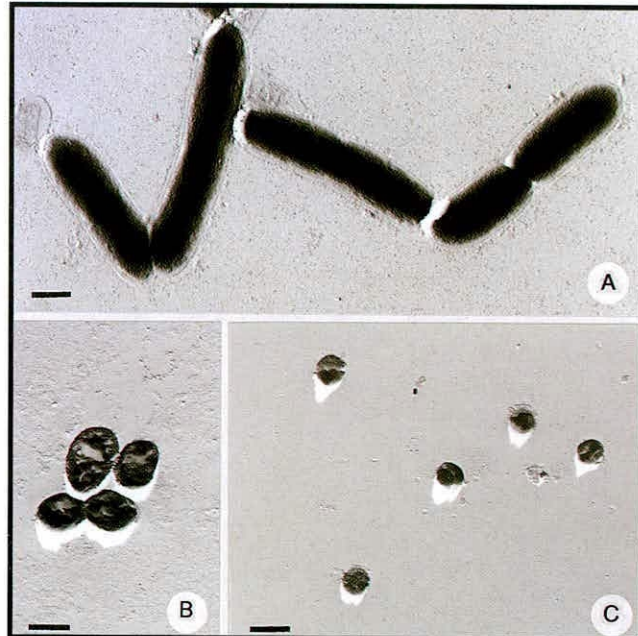


Figure 36. Electron micrographs of cells of *Aeromonas salmonicida*: A, Cells recovered from an overnight culture, washed in sterile water and representing the inoculum on day 0; B, Cells recovered directly from unamended lakewater after 50 days; C, Cells recovered directly from lakewater amended with both amino acids after 50 days; The size bar represents $1 \mu\text{m}$

Impact of rainbow trout on Loch Leven

In April 1993 and in March 1994, some 40,000 rainbow trout (*Oncorhynchus mykiss* Walbaum) with an average weight of 0.25 kg, were introduced to the 4-m mean depth Loch Leven (Kinross, Scotland) to bolster an ailing sport fishery based hitherto on brown trout (*Salmo trutta* L.). During 1994 the phytoplankton exhibited the following four characteristics (Figure 37): very high lakewide mean and maximum chlorophyll *a* levels (86 and 236 $\mu\text{g l}^{-1}$

respectively), the occurrence of the maximum in summer, and the small size of the dominant species - *Oscillatoria subtilissima* Kutz. (ca 1.5 μm diameter). These features had not been recorded in combination in any year since the late 1960s. In the period 1970-1993, mean pigment concentrations rarely exceeded 50 $\mu\text{g l}^{-1}$ and usually came within the range 30-40 $\mu\text{g l}^{-1}$ (Figure 38). Annual maxima occasionally exceeded 100 $\mu\text{g l}^{-1}$ but these usually occurred in winter or early spring with small diatoms dominating. Meanwhile, large cyanobacteria such as *Anabaena flos-*

aquae often dominated the summer scene.

We attribute this phytoplankton reversion to sixties character to firstly, the poor showing of the filter-feeding cladoceran *Daphnia hyalina* (Leydig). In many of the years for which we have zooplankton data, it achieved peak densities of 45 individuals l^{-1} , whereas the maxima in 1993 and 1994 were only 25 and 20 individuals l^{-1} respectively, and the animal was virtually absent from the water column in the late 1960s. However, we suggest that the ultimate cause of this change is due to a 'top-down' cascade effect of the *Oncorhynchus* introduction: these fish have increased predation on the cladoceran, and as this animal appears to feed preferentially on the smaller phytoplankton elements here, small algae have burgeoned - as they did in the late 1960s, although whether fish predation was the cause of low *Daphnia* numbers then is not known. Then, as in 1994, the availability of phosphorus - including phosphate ions released from the sediments - was important in supporting (from 'bottom-up') the dense crops of cyanobacteria in summer.

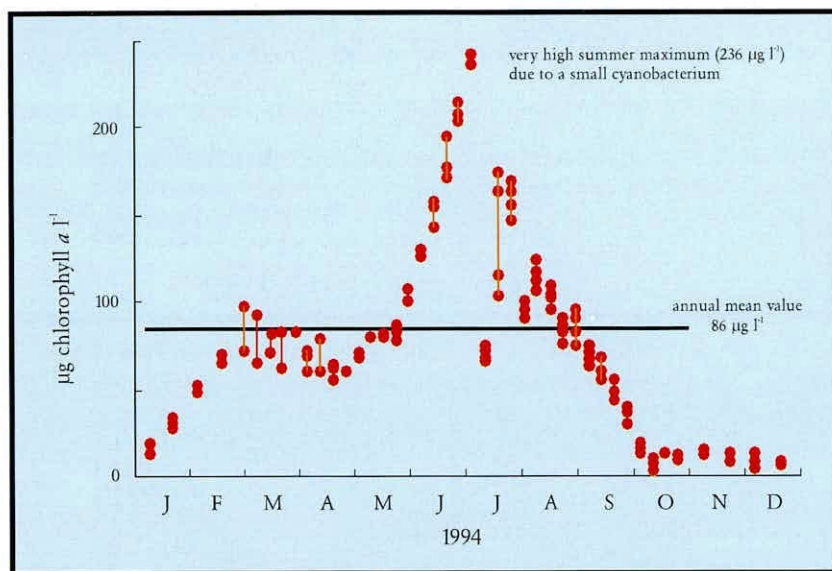


Figure 37. The main features of phytoplankton development in Loch Leven during 1994. Vertical lines join pigment values obtained from sites sampled on the same day.

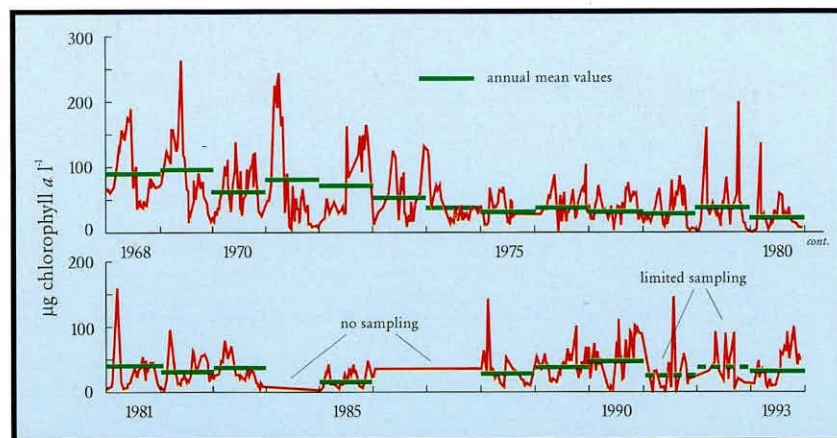


Figure 38. Variation in phytoplankton biomass (expressed as chlorophyll *a* concentration) in Loch Leven from the late 1960s to 1993.

Long-term environmental monitoring at freshwater sites

Construction of the Channel Tunnel terminal site at Folkestone involved a considerable degree of landscaping and earthmoving (Figure 39). The environmental impact programme initiated by the developers (Eurotunnel) and subsequently run by their contractor Transmanche-Link was designed to monitor ecological conditions in both terrestrial and aquatic habitats before, during and after the main periods of construction between 1987 and 1993.



Figure 39. Folkestone Terminal site under construction. (Copyright Eurotunnel courtesy of QA photos).

The three main streams in the area, the Saltwood and Seabrook, which enter the sea west of Folkestone, and the Pent Stream, which passes through the town itself, arise at about 50m above sea level, are short (<6.5 km) and fall quickly to the sea. The catchments contain wooded reaches, pastures and urban areas. Samples were taken at intervals between source and mouth of each stream.

The fact that the environmental assessments were funded over a number of years (nine to date) has provided an ideal opportunity to examine long-term changes in both faunal and physical conditions. (see Figure 40 which illustrates changes in the Saltwood stream from top SW1 to bottom SW3).

Most changes were related to ‘natural’ events (formation of debris dams and spates at Saltwood 2) and ‘anthropogenically induced’ mobilization of fine sediments from disturbed areas in the catchment, which were not all directly related to Channel Tunnel activities. These disturbances were associated with, topsoil clearance, pipe-line crossings and quarrying. Other impacts arose from saline drainage and oil contamination. The variations in faunal richness over the study period are shown in Figure 41 for all regularly examined sites.

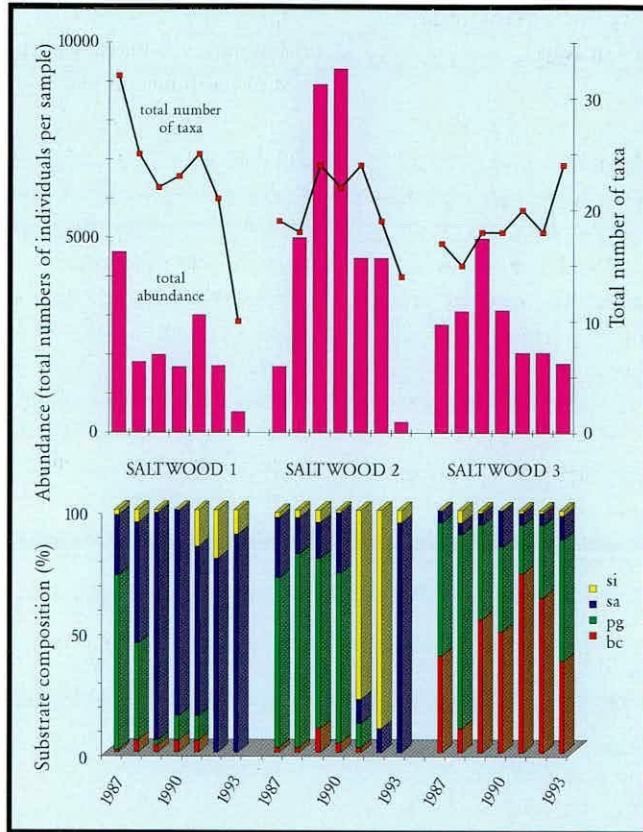


Figure 40. The annual variation in faunal abundance and taxon richness at three sites on the Saltwood Stream together with associated changes in the proportions of substrate categories (bc boulders/cobbles, pg pebbles/gravel, sa sand, si silt on the stream bed).

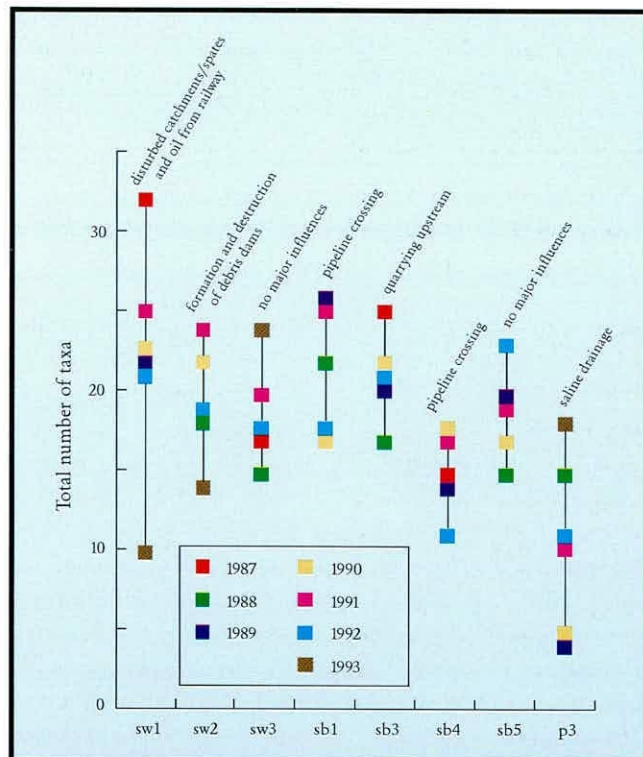


Figure 41. The annual variation in the number of taxa (family level) recorded at sites on the Saltwood (sw), Seabrook (sb) and Pent (p) streams