Fresh water is the planet's most valuable natural resource and the challenge is to reconcile the needs for water in human activities with the requirements for healthy aquatic ecosystems. Management of this resource must be based upon the best available scientific knowledge, including the factors controlling water quantity, water quality and freshwater fisheries. All three areas interact and the IFE's research programme is directed towards understanding the key processes involved and then using this information to develop predictive models to aid the practical management of this vital, but fragile resource.

Programme 4 Freshwater Resources

Earlier growth of a planktonic diatom over 50 years: perturbation, pattern and process

Patterns of long-term change in Lake District lakes

Lakes experience perturbation produced by local changes in the catchment, such as increased nutrient loading, year-to-year variation in the weather and global climate change. The effects of these changes, modified by lake sensitivity, can be detected in long-term records. For example, a striking feature of a 50-year record from four lake basins in Cumbria is the earlier growth of the dominant phytoplankter in the spring, the diatom *Asterionella formosa*, in two of the basins: North

Long-term records allow the effects of environmental perturbation on ecosystem function to be detected.

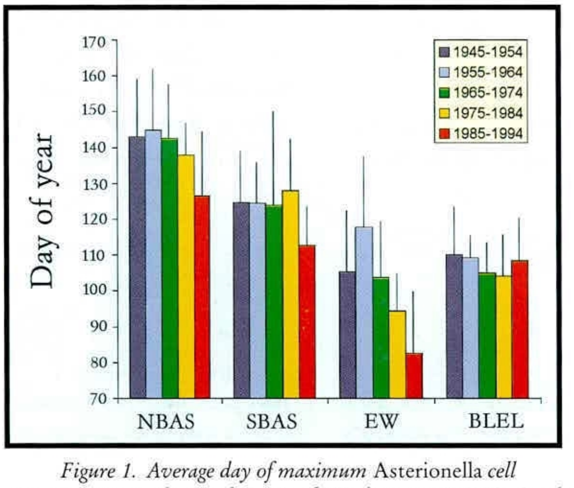

Figure 1. Average day of maximum Asterionella *cell concentration in the North Basin of Windermere (NBAS) South Basin of Windermere (SBAS) Esthwaite Water (EW) and Blelham Tarn (BLEL) for 10-year blocks between 1945 and 1994; error bars show one standard deviation.*

Basin of Windermere and Esthwaite Water (Figure 1).

Linking pattern to process

The cause of this pattern is more difficult to determine. Climate change has resulted in slightly higher water temperatures in early spring now

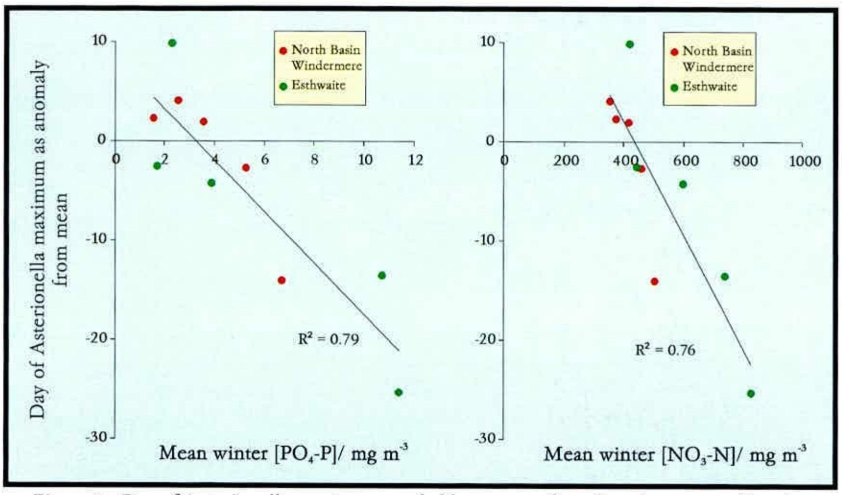

Figure 2. Day of Asterionella *maximum, scaled by average day of maximum, as a function of early winter concentrations of phosphate and nitrate in Esthwaite Water and the North Basin of Windermere.*

compared to 50 years ago, but there appears to be no link between this and the earlier spring growth of *Asterionella* in the North Basin of Windermere or Esthwaite Water. Changes in the catchment have increased the availability of the nutrients, phosphate and nitrate, over the 50-years. The day of cell maximum, when scaled by the average day of maximum for the two basins, occurs significantly earlier as the winter concentration of phosphate and nitrate has increased (Figure 2). The precise process involved has not yet been confirmed but it is possible that nutrient enrichment allows faster growth of *Asterionella* towards the end of the spring bloom, producing an earlier population maximum. The analysis underlines the value of long-term records to detect the effect of environmental perturbation on lake performance and to understand the processes responsible for the changes.

Nutrient enrichment has led to the earlier growth of a planktonic alga.

Advances in modelling phytoplankton dynamics using PROTECH-C

Modelling phytoplankton responses to environmental change has been a major objective of the Institute since its creation. The stimulus for this activity has been a series of management issues, relating either to the projection of algal water quality in new, artificial water bodies or to the effects of major changes in the operation of existing installations. The problems posed have invoked (mainly, though not exclusively) the probabilities of bloom occurrence: what is the biomass capacity and what are the risks of dominance by toxic cyanoprokaryotes?

The tangible response has been the development of a modelling philosophy (abbreviated, as is fashionable in these matters, to a slightly stylised acronym, PROTECH), based on a fundamental knowledge of the natural control of *in situ* algal growth. This has been crucial, for it has always been recognised that the development of algal populations is a net outcome of a complex series of gain and loss processes, conspicuously involving photosynthetic carbon fixation and nutrient resource harvesting on the one hand and removal of live cells by outwash, by sedimentation, and by planktonic grazers. Many years of field experimentation had yielded a series of credible, validated, first-order dilution functions to describe the various losses, invoking where appropriate, size-dependent sinking rates, feeder-specific food selection, growth and individual ingestion rates and these were written into the debit routines. On the positive side, population anabolism was approached on the supposition that algae grow not as a function of the supply of its requirements but, rather, that they do so at the maximum allowed by the resource in weakest supply. At the heart of all the models based on PROTECH is a trio of regressions which predict (i) how fast

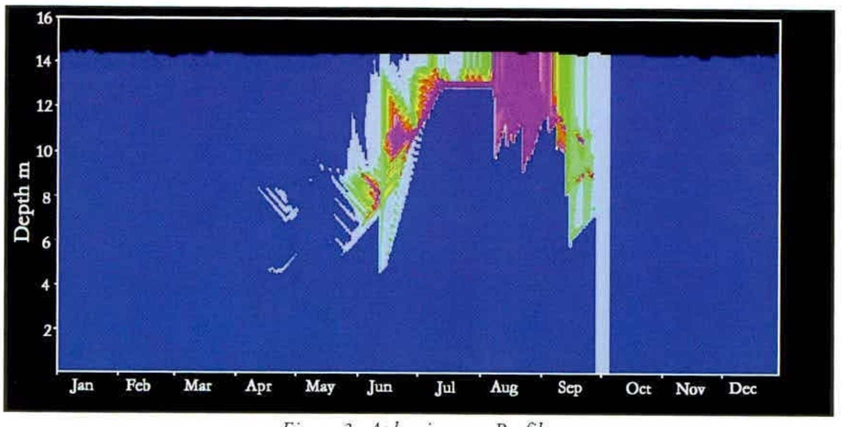

Figure 3. Aphanizomen Profile.

each alga can replicate itself under constant ideal conditions, (ii) how that species-specific rate responds to temperature and (iii) how the species-specific temperature-dependent replication rate responds to truncation of the underwater light field. These regressions were assembled by fitting curves to experimental data. The routines solve for each of up to eight species of algae the maximal daily rate of replication, subject to an audit that establishes whether there are still nutrients present in sufficiency to sustain it and subject to the adjustments due to the species-specific rates of biomass loss.

The rest of PROTECH is the construction of the changing growth medium – the shape of the basin, the accommodation of heating and cooling, hydraulic exchanges and so on – and the means of logging the accumulating or depleting components. The original model was created for a uniformly-mixed layer; PROTECH2 was built for the exclusive use of the then National Rivers Authority, to simulate cyanoprokaryote growth in relation to thermal stratification; PROTECH-C is a new, general model which incorporates routines for multiple abstraction at depth (more like a reservoir, in fact), for incorporating particulate exchanges between surface sediment and water column (making it possible to simulate sediment focussing and the entrainment of interstitial

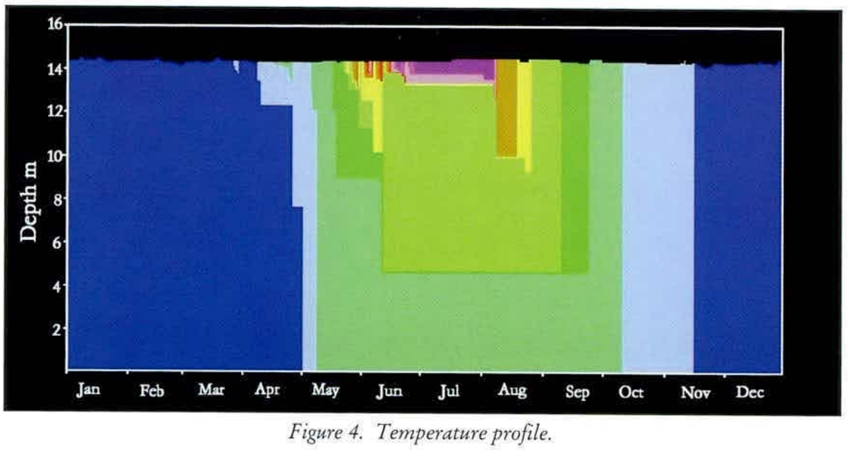

Figure 4. Temperature profile.

water) and for working in the currency of carbon (allowing the simulation of BOD accumulation and redox drift). The latest challenge accepted is to apply the PROTECH philosophy to the downstream increase in river phytoplankton and to relate the simulation back to correspondences with the time-series pertaining to fixed sampling points along the river.

The most exciting, though hitherto unexplored, potential of PROTECH-C is as a research tool. If it can re-construct acceptable simulations of phytoplankton responses to a variable environment, what can it do against environments contrived to provide test-beds of ecological theories? For instance, the model can be used to measure the rates of competitive exclusion in species-rich assemblages and to see how different starting conditions might produce different outcomes. It is certainly possible to use

PROTECH-C is set up to test the mechanisms of biodiversity.

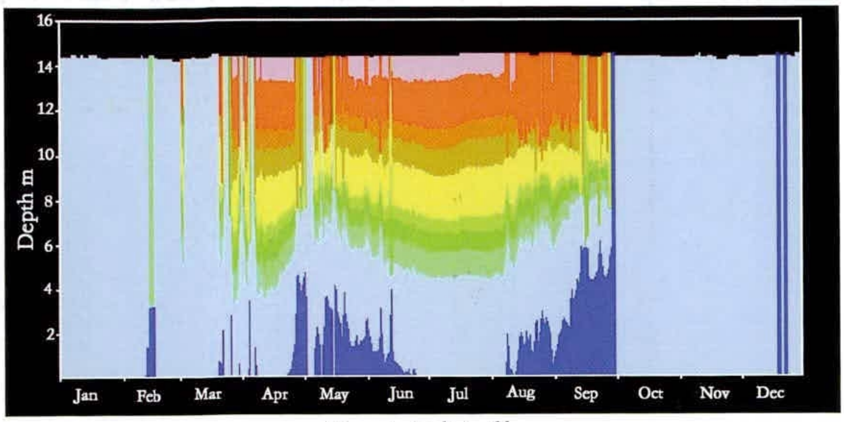

Figure 5. Light profile.

The model is set up to test ecosystem theory.

the model to test the theory of intermediate disturbance by determining when and by how much the environmental conditions should be altered to prevent exclusion and promote the development of alternative species. Indeed, germane to the whole issue of biodiversity and its maintenance, the model is set up to determine what it is necessary to impose in order to maintain maximum species richness.

It may seem obvious that this is how the model should be applied to the theories on which its applications were based. It may not follow that the model behaves as we anticipate. What is necessary is a systematic "screening" of the reaction of each alga to a series of single variables (temperature, daylength, nutrient supply, dependent grazer) in order to test the sensitivity of the model and to verify its ability to distinguish the maximum performance of each species under each variable. When that has been done, the mutual impacts on species allegedly competing with each other to achieve optimal biomass and so influence the ways in which communities are assembled may be systematically investigated.

It is one of the ironies of the modern research climate that the scientifically desirable should have to play a conspicuously second fiddle to the commercially expedient. To have been able to bring this instrument to the podium at all has been possible through funds released under the NERC Special Topic "Testable models of aquatic ecosystems". This support is gratefully acknowledged.

Population regulation in resident trout

The sustainable management of natural resources is an important aspect of the research programme of CEH. Freshwater fish are naturally renewable resources that often provide valuable fisheries. It is therefore important to discover how persistent populations are regulated because such information is essential for those responsible for the management of a fishery. For many fish species, especially those in the salmon family, density-dependent survival in the early juvenile stages has been shown to be the chief mechanism for population regulation.

In marked contrast to the situation in other salmonid populations, a resident trout population (*Salmo trutta* L.) in a Lake District stream showed simple proportionate survival in the early life-stages with no evidence for density-dependent regulation (Figure 6). However, this persistent population fluctuated within narrow limits. Mature adults, especially during spawning, were the only possible life-stage left in which regulation might occur. Further analysis of the population data showed that this did, indeed, occur. The number of spawning females produced in each year-class was strongly density dependent on the initial number of females that laid eggs at the start of the year-class (Figure 7a). Similarly, total egg production in each year-class was density dependent on initial egg density (Figure 7b). Both relationships were well described by the Ricker and Beverton-Holt stock-recruitment models and the goodness-of-fit was similar for both models. This study is probably the first to provide clear evidence for fish population regulation in the adult, rather than the juvenile, stage. Such regulation may be

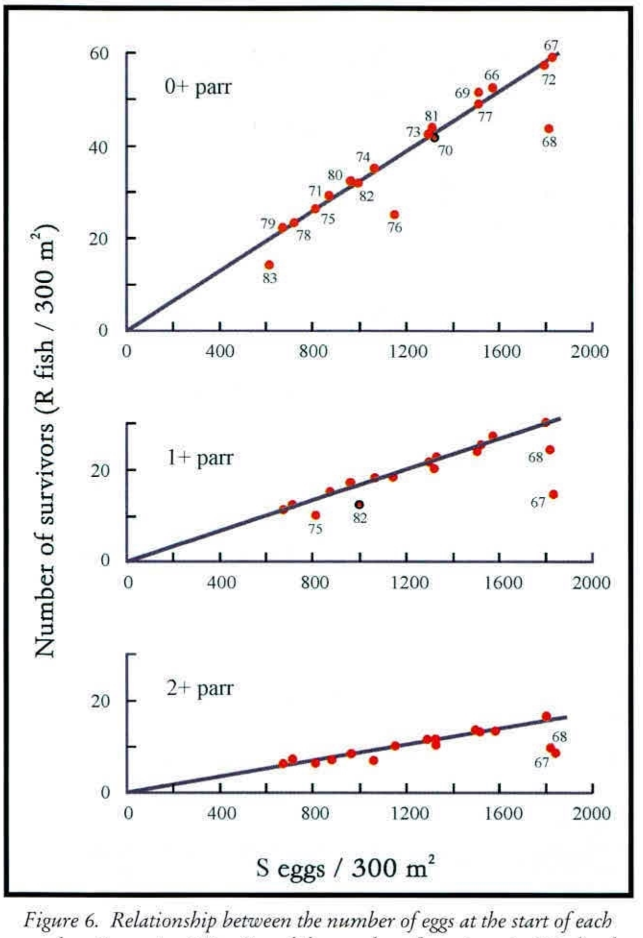

Figure 6. Relationship between the number of eggs at the start of each year-class (S eggs per 300 m²) and the number of survivors in October for different life-stages: 0+ parr, 1+ parr, 2+ parr including some mature male parr. The following year-classes affected by a spate or drought were excluded from the analyses: 1968, 1976, 1983 for 0+ parr; 1967, 1968, 1975, 1982 for 1+ parr; 1967, 1968 for 2+ parr. Intercepts for all three regression lines were not significantly different from zero and their regression coefficients provided estimates of constant proportionate survival at 3.25% for 0+ parr, 1.65% for 1+ parr, 0.83% for 2+ parr (the year-class is given for each value of 0+ parr and for values of 1+ and 2+ parr affected by a spate or drought).

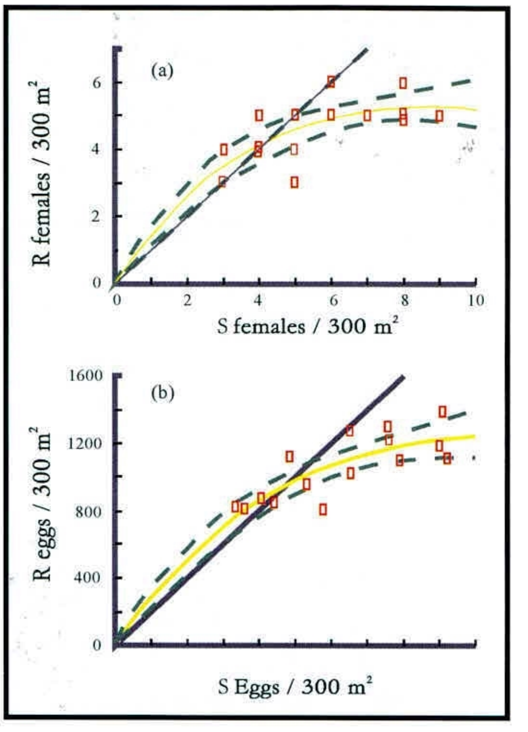

Figure 7. (a) Relationship between the number of spawning females produced in each year-class (R females per 300 m²) and the initial number of females that laid eggs at the start of the year-class (S females per 300 m²). (b) Relationship between total egg production in each year-class (R eggs per 300 m²) and the initial egg density at the start of the year-class (S eggs per 300 m²). For both figures, the stock recruitment curve is given by solid line with broken lines indicating 95% C.L. (line of equality also shown as straight line).

more important in low density populations of trout than in high density populations of anadromous salmonids.

River fish habitat – Ignorance is bliss!

With the current interest in developing models such as PHABSIM and HABSCORE for assessing river conditions for fish stocks, there is a desperate need for information on fish habitat preferences. Studies in the past have used electric fishing and visual observation to determine habitat use. These approaches are suspect because of the high risk of disturbing or failing to see fish. This, potentially, leads to measures of 'refuge' habitat use. Little heed has been paid to the possibility of seasonal, daily, or other periodic changes in fish behaviour, leading to the present state of ignorance regarding habitat use.

Recent observations on a common, small, river fish, the dace, have highlighted the deficiencies of the above approaches. Fish bearing tiny radio tags are released into the river and they are tracked, day and night, throughout the year, using a portable receiver.

Existing models of habitat use by river fish largely ignore critical periods of activity from dusk to dawn, and those occurring in deep or turbid water.

Figure 8. Mobile tracking – Radio-tagged dace can be precisely located.

Radio tracking has already revealed many unknown, amazing facts about the life of the dace. In winter when there are floods, the fish often occupy fields or fens some distance from the river bank. It is not unusual to find dace, (tagged and untagged) sheltering under a tussock of grass in the middle of a meadow. The fact that millstreams are preferred over main river sites as spawning areas for this species and its peculiar post-spawning periods of fasting in the lower reaches of tributary streams have also been revealed.

Figure 9. Dace – These abundant little fish flourish in many British rivers.

Perhaps the most astonishing feature of dace behaviour has proved to be its daily shifts of habitat. At dusk the fish leave their day-time habitat and rapidly migrate up to 800 m to distinct night-time spots, returning at dawn to their daylight haunts. Individual fish appear to be able to return to precisely the same spot in the river at both day and night.

Undoubtedly there will be lots of equally exciting discoveries made, when other species are examined. The possibilities of simultaneously following predators and their prey, of examining sex segregation of the same species or of keeping track of long migrations and the strategies for surmounting obstacles, seem to be almost limitless.

Extraordinary migrations associated with spawning and feeding are revealed by tracking and video observation of fish.

Management of Grayling

The European Grayling *Thymallus thymallus* (Figure 10) is a salmonid with a geographical range of central and north-west Europe. In Great Britain its natural distribution is localised, but during the last century it was successfully introduced, presumably for angling, to a large number of rivers, particularly in the south of England. However, in the mid reaches of chalk streams it became prolific and was perceived by anglers to be a competitor for the more popular and valuable brown trout.

Thus, management of the grayling began to include an annual removal programme with angling clubs and fishery managers performing large scale electric fishing and netting exercises. Grayling captured by these mechanisms were usually treated as vermin and culled, although they were occasionally moved to other fisheries. The Institute of Freshwater Ecology has been monitoring these exercises for a numbers of years to assess their effects.

Although removal was designed to depress the populations, it did not seem to be having any effect on the absolute abundance, with the numbers removed remaining constant over time (Figure 11). However there were effects on the sizes and ages of fish remaining in the river. Electric fishing, particularly, was biased to the removal of larger fish and populations which were exposed to these removal exercises, had a predominance of younger fish in comparison to populations without removal (Figure 12).

Figure 10. European grayling Thymallus thymallus.

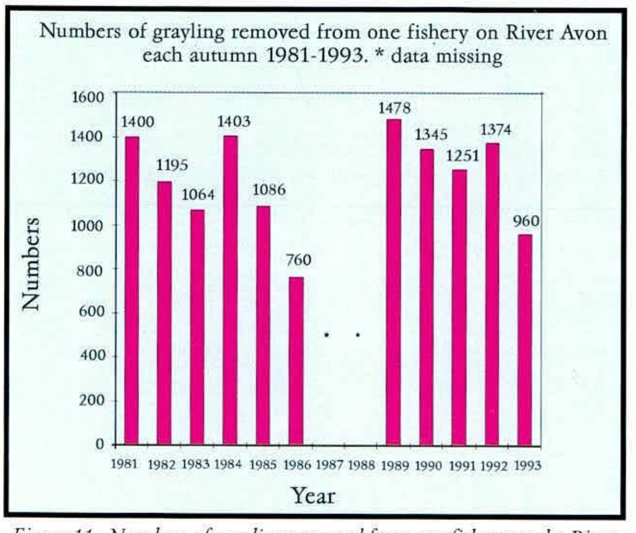

Figure 11. Numbers of grayling removed from one fishery on the River Avon each autumn 1981-1993.
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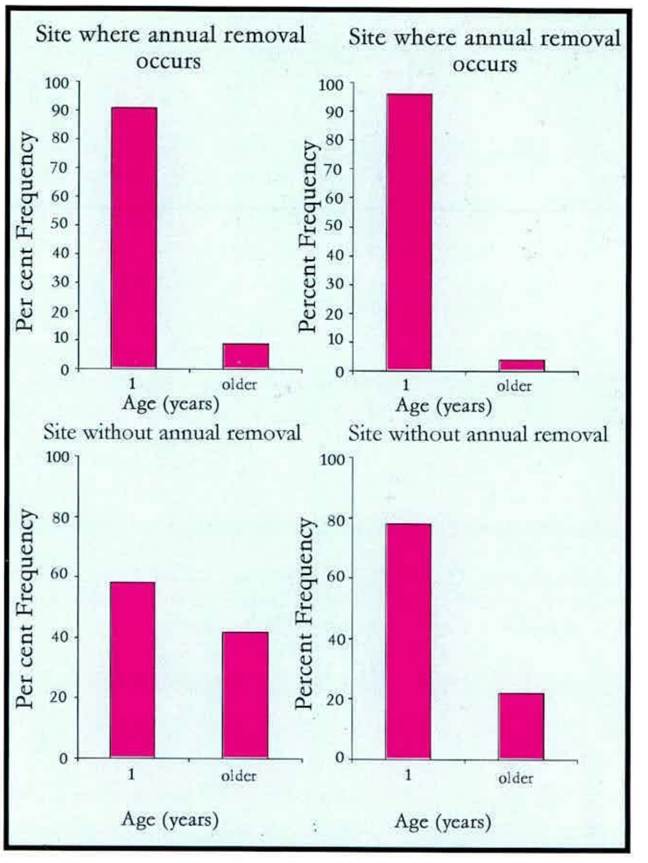

Figure 12. Age structure of grayling populations at two site where annual removal does occur and at two sites where it does not.

As a result of the evidence on changes to age structure, collected by the Institute of Freshwater Ecology, removal programmes on many rivers have ceased and fishery managers are moving towards a greater appreciation of this species as an angling resource.

Automatic counting of salmon smolts

Within the national decline of Atlantic salmon in the UK, chalk stream populations have been affected particularly badly. For the past 24 years, the IFE has been monitoring the number of adult fish migrating to the spawning grounds on the Frome, a Dorset chalk river. In the last 6 years numbers of salmon annually ascending the river have been in the range 1000-1300 compared with the long term average of 2500. It has become imperative to know whether the problem lies in the marine or freshwater phase of the salmon's life cycle or both.

One way of assessing the success of the breeding population is to count the number of juveniles (smolts) leaving the river for the marine feeding grounds. Traditional counting methods have used traps and nets but due to the delicate nature of smolts, a method to avoid handling or interrupting their natural migration was needed. Trials have been conducted to deflect smolts to a suitable counting site using a combination of air and sound to create an acoustic bubble screen (developed by Fish Guidance Systems Ltd; Figures 13 and 14).

Significant deflections, both in daylight (20-44%) and in darkness (73%) were achieved.

IFE has developed an automatic smolt counter based on the same principle used in counting adult salmon. The accuracy of the counter changes with smolt number and is 97% at low numbers, 86% at medium numbers but at high numbers, when smolts are migrating in shoals, the accuracy falls to 42%. As the timing of these shoals can be predicted, a combination of video

In addition to adult salmon, smolts can also be counted accurately.

Figure 13. Acoustic bubble screen on trial above the Fluvarium.

Figure 14. Acoustic bubble screen diverting smolts into the millstream.

recordings and counter data has proved the most efficient method of accurately estimating smolt numbers.

The annual smolt counts will be related to the adult counts to produce a stock : recruitment relationship for the Frome for spawning target assessment. Results will be integral to the joint study with ITE Furzebrook on parr habitat and the existence of genetic sub-populations.

Treatment of fungal infections of farmed fish

Fungal infections of farmed fish, primarily by fungi of the genus *Saprolegnia*, represent a significant economic and welfare problem, particularly with respect to the husbandry of sexually mature broodstock and fertilised ova of salmonid fish. This state of affairs has been exacerbated by existing and anticipated restrictions on the use of the most effective fungicide hitherto available, malachite green. Malachite green has long been employed as a fungicide and ectoparasiticide but concerns regarding the mutagenicity, carcinogenicity, and teratogenicity of this chemical have led to restrictions on its use.

Although significant effort has been directed towards identifying a safer therapeutant which is as effective as malachite green in combating fungal infections of fish and fish eggs, no suitable candidates have yet been located.

In a collaborative study between IFE Fish Division, CCAP and Grampian Pharmaceuticals Ltd, part-funded by the LINK Aquaculture scheme, a novel fungicide has been identified and evaluated. Bronopol (2-bromo-2-nitropropane-1,3-diol) is a biocide which is widely used as a preservative in medical and pharmaceutical products, cosmetics and shampoos but has not previously been used as a therapeutic agent.

The study comprised two main elements. Although salmonid fish are naturally susceptible to *Saprolegnia* infection at certain stages of their development and most UK waters present a continuous background fungal zoospore challenge, it was decided that a reproducible disease model would provide the most appropriate approach to quantitatively

Fungal infections of farmed fish, primarily by fungi of the genus Saprolegnia, represent a significant economic and welfare problem.

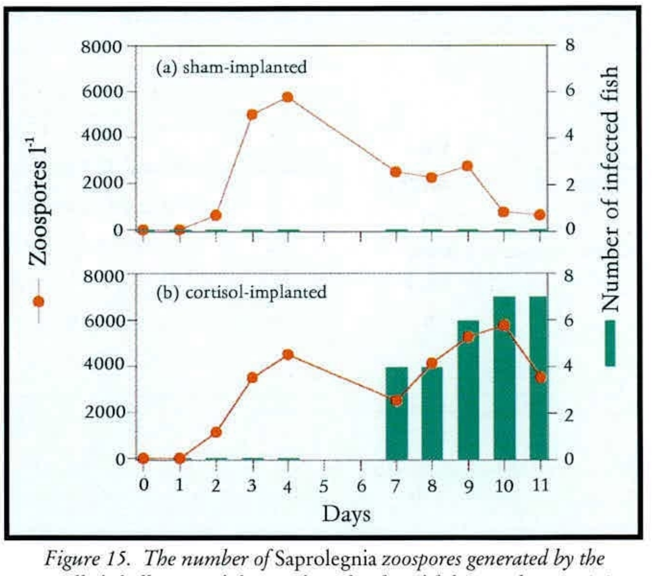

Figure 15. The number of Saprolegnia *zoospores generated by the controlled challenge and the number of infected fish in tanks containing (a) sham-implanted and (b) cortisol-implanted rainbow trout exposed to continuous zoospore challenge for 11 days.*

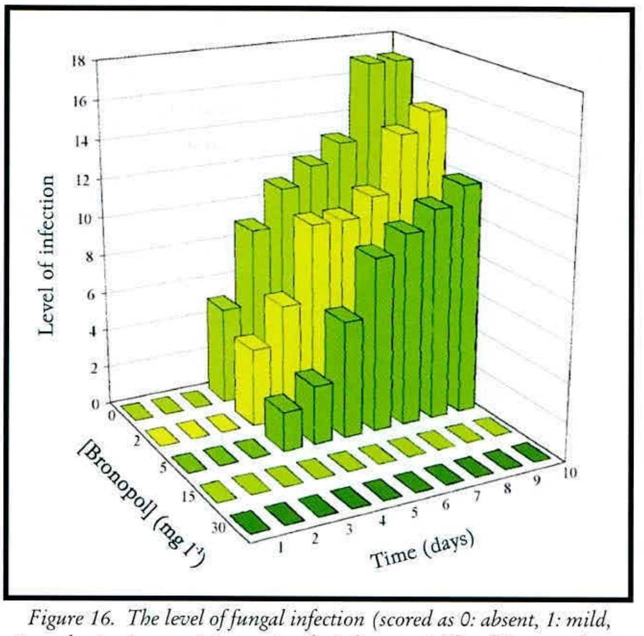

Figure 16. The level of fungal infection (scored as 0: absent, 1: mild, 2: moderate, 3: severe) in groups of rainbow trout (five fish per tank, ten fish per treatment) exposed to a continuous S. parasitica *zoospore challenge and treated daily with bronopol at concentrations of 0, 2, 5, 15 or 30 mg l^{-1} for 60 minutes.*

estimating the efficacy of bronopol in combating *Saprolegnia* infection. The disease model we adopted comprised two elements: (i) a reproducible and controlled *Saprolegnia* zoospore challenge; (ii) a susceptible fish host.

A reproducible *Saprolegnia parasitica* zoospore delivery system was developed and demonstrated to be effective in providing a sustained zoospore challenge for up to 10 days. Treatment of rainbow trout with slow-release intraperitoneal implants containing cortisol resulted in chronically elevated blood cortisol levels and rendered the fish susceptible to infection by *S. parasitica* when exposed to the zoospore challenge (Figure 15b). Sham-implanted fish were not susceptible to infection confirming that trout possess a very effective defence system against infection and that immunosuppression is a prerequisite of infection (Figure 15a). Bronopol was effective in protecting predisposed fish from infection by *S. parasitica* when administered as a daily bath/flush treatment at concentrations of 15 mg l^{-1} and greater (Figure 16). Bronopol was also demonstrated to protect fertilised rainbow trout ova from *S. parasitica* challenge when administered as a daily bath/flush treatment at concentrations of between 30 and 100 mg l^{-1}. These data suggest that bronopol is a safe and effective replacement for malachite green and formalin in the prevention of fungal infections in farmed fish.

Fish possess a very effective defence system against pathogens and immunosuppression is a prerequisite of infection.

Biodiversity, the natural biological capital of the Earth, embraces genetic diversity, species diversity and ecosystem diversity. Its preservation and wise utilisation are ultimately linked to the healthy functioning of natural and semi-natural ecosystems, to the quality of life and to wealth creation. Work in this programme area aims to characterise freshwater biodiversity, to understand the population processes responsible for such diversity, to investigate how biodiversity influences ecosystem function and to use this knowledge for the conservation and restoration of aquatic ecosystems.

Programme 5 Biodiversity and Population Processes

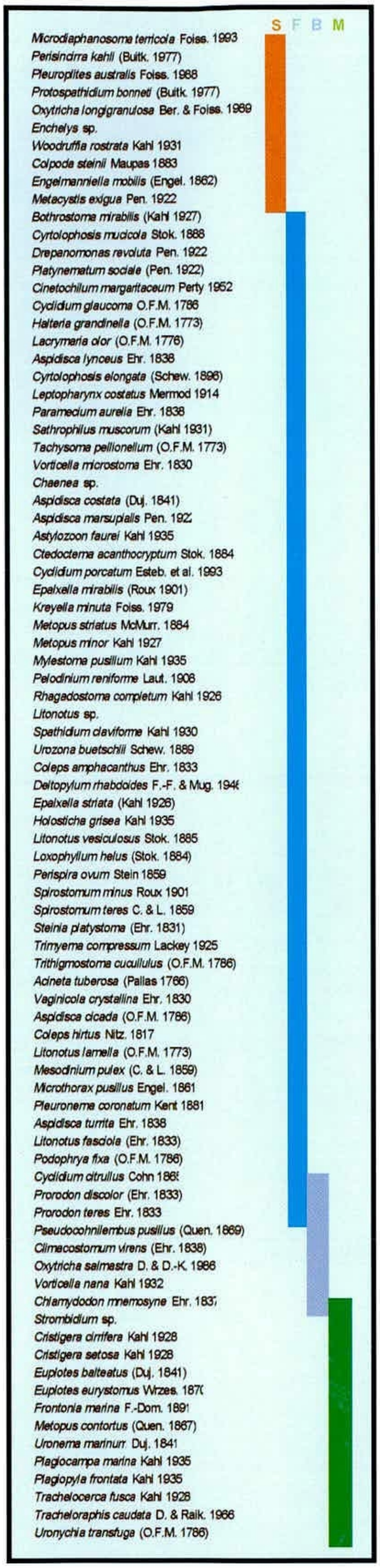

Figure 17. Normal habitat types, and thus likely origins of the 85 ciliate species found in the volcanic crater-lake (Soil [S], Freshwater [F], Brackish water [B], Marine [M]).

Testing the global ubiquity of microbial species

Species of large animals and plants have geographically restricted distributions (i.e. they have biogeography), but it is unclear if this also applies to free-living micro-organisms. We have investigated the ciliated protozoa living in a habitat that is separated from northern Europe by geographical barriers and great distance – the sediment of a Holocene volcanic crater-lake with brackish water, in Australia. Of the 85 ciliate species recorded, none was 'new', and all (apart from one species previously described only from tropical Africa) are known from northern Europe. All species appear to have reached the crater by passive dispersal from other freshwater and marine environments. The significance of this finding lies in the fact that ciliates are among the largest and most fragile of microbes. If ciliate species have global distributions, it is likely that the same is true for the many smaller, more abundant and more easily dispersed microbial species, including bacteria. There is some support for this in the literature, and most species smaller than about 1 mm may have global distributions. This would be consistent with the modest species richness recorded for most microbial groups, and the observation that local species diversity is usually a large proportion of global species richness.

Microbiologists have long been aware of the immense utility of microbes as experimental tools in ecological research, and microbial communities kept in the laboratory have even been used as tools for exploring the 'role' and 'function' of biodiversity in general. But biodiversity at the microbial level has characteristics that are fundamentally different from those of macroscopic animals and plants, and although it may currently be fashionable to do so, it may be difficult to make realistic extrapolations from the attributes of microbial communities, to those of biodiversity in general.

Nitrification in lakewater

The process of ammonia oxidation is vital for the biogeochemical cycle of nitrogen. In a number of collaborative projects with University of Liverpool (funded through NERC non-thematic, thematic (EDGE) and IFE science budget) specific molecular probes have been developed to investigate the diversity of ammonia oxidising bacteria in freshwater. The autotrophic ammonia-oxidising bacteria in Esthwaite, a eutrophic freshwater lake, were studied over a 12-month period. Numbers of ammonia-oxidisers in the lakewater were low throughout the year. However, sediments from both littoral and profundal sites harboured comparatively large populations. Their relation to substrate concentration could differentiate lakewater and sediment populations. Those in sediment or lakewater were selected by high (12.5 mM) and low (0.67 mM) ammonium concentrations respectively. Furthermore, enrichment studies suggested that populations of ammonia oxidisers in the water column were sensitive to high ammonia concentrations. With only one exception, *Nitrosospira* 16S rDNA was amplified by nested PCR from all samples but *Nitrosomonas* (*europaea-eutropha* lineage) was never obtained directly. However, the latter were part of the sediment and water column communities because their 16S rRNA could be detected in enrichment cultures. Nitrosospiras were shown to dominate the sediments although the

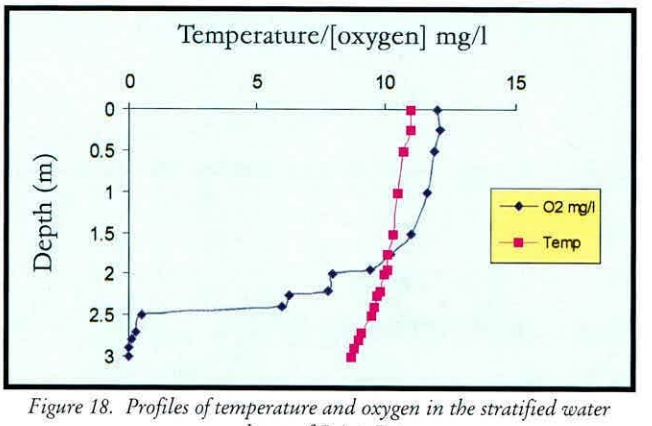

Figure 18. Profiles of temperature and oxygen in the stratified water column of Priest Pot.

patterns of *Nitrosospira* and *Nitrosomonas* detection from enrichment cultures were complex. The enrichment cultures provided evidence for existence of sub-populations distinguishable on the basis of ammonium tolerance that were seasonally distributed between the sediment and water column.

The most recent work focussed on Priest pot, a small, shallow (3.2m) hypereutrophic pond situated to the north of Esthwaite. The sheltered

Tolerance to ammonia distinguishes sub-populations of ammonia-oxidising bacteria.

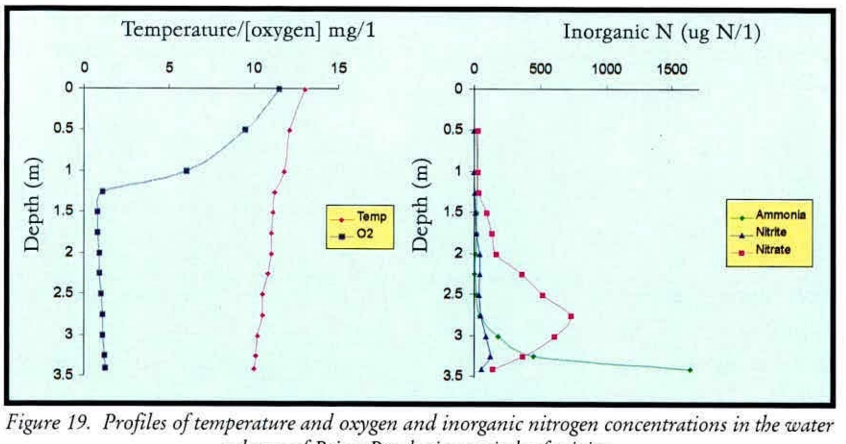

Figure 19. Profiles of temperature and oxygen and inorganic nitrogen concentrations in the water column of Priest Pot during periods of mixing.

location means that the water column is stratified over most of the summer and as the hypolimnion rapidly deoxygenates a steep gradient of oxygen concentration develops in the water column (Figure 18). The boundary layer between aerobic and anaerobic water is only 15cm and the oxycline is a region of intense chemosynthetic activity. The molecular tools developed on Esthwaite combined with high-resolution sampling are being used to investigate ammonia oxidising populations across the oxycline. As stratification breaks down low concentrations of oxygen and high nitrate herald another period of nitrification as ammonia rich deeper water is mixed with the surface layers (Figure 19). Regular sampling can identify these periods and comparative studies will be made.

Concentrations of aquatic free viruses exceeding 10^9 particles/ml have been recorded.

Fluorescence microscopy and the enumeration of aquatic free-viruses

Aquatic viruses occur as attached or freely suspended particles when in their infective form. Water assists their dispersal and enhances their chances of contacting and infecting a host.

As all viruses are obligate parasites (a host is essential for their replication). Aquatic viruses may pose a threat to the life or well-being of all inhabitants and users of a water body whether microbe, plant, animal or man. This is of concern to authorities managing water supplies and aquatic amenities, who may need to monitor the virus content of the freshwaters under their control.

Aquatic free viruses range in size between 25 and 200 nm and can occur in vast concentrations ($>10^9$ virus particles/ml) when conditions are ideal. The size and shape of individual virus particles can be examined using transmission electron microscopy (TEM), as their small size makes them unrecognisable using conventional light microscopy.

Methods of enumerating free virus populations using fluorescence microscopy and TEM have been assessed, compared and developed as the initial stage of a project part-funded by the Foundation for Water Research, and in collaboration with the Institute of Virology and Environmental Microbiology (CEH, NERC), Oxford.

TEM methods, although costly and generally less accessible, enable morphological characterisation and enumeration of viruses. However, simple techniques developed to employ recently available, cyanine-based, nucleic acid-specific fluorochromes, render the viruses brightly visible by fluorescence microscopy. Counts made from colour photo-transparencies taken of fluorescence samples, enable rapid, accurate and low-cost assessments of virus populations to be made (Figure 20). A comparison of methods, tested on a range of freshwaters, showed analytical variance of less than 10% between fluorescence and TEM, and fluorescence microscopy free virus enumeration to be accurate on all but the most humic of waters.

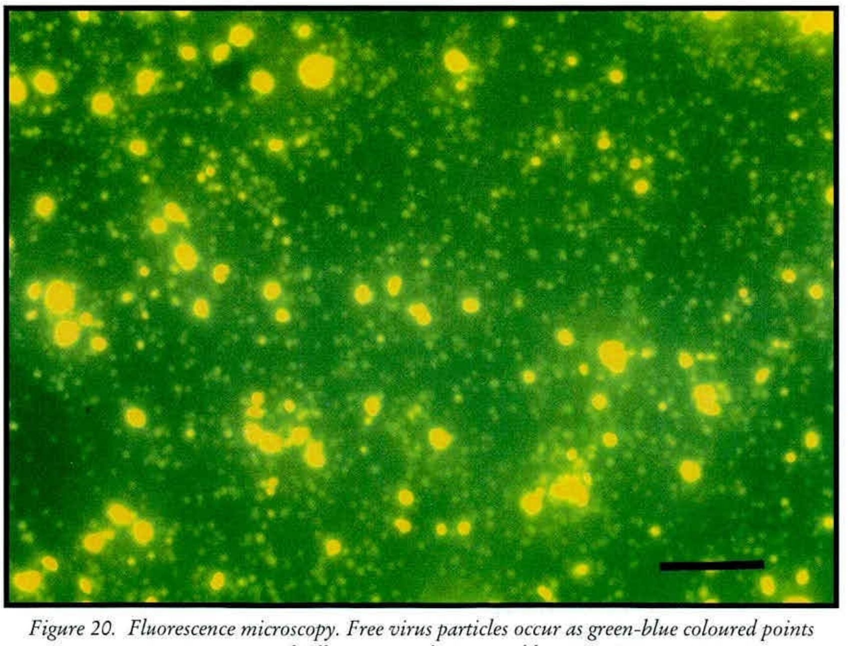

Figure 20. Fluorescence microscopy. Free virus particles occur as green-blue coloured points amongst a scatter of yellow-orange detritus and bacteria. Bar = 5μm.

Freeze-induced injuries in *Vaucheria sessilis*

Cryopreservation (storage at ultra-low temperatures) is widely recognised as the optimal preservation technique for micro-organisms, including microalgae. In theory, assuming the storage temperature has been maintained below –130°C, successfully cryopreserved cultures should remain stable virtually indefinitely. A recent survey, at the Culture Collection of Algae and Protozoa (CCAP) and the National Institute for Environmental Studies (Tsukuba, Japan), of a selection of cryopreserved algae originating from different ecological niches and taxonomic affiliations, demonstrated that post-thaw viability levels were unaffected up to 22 years after storage.

However, to date, conventional cryopreservation has not proven effective for many of the larger, more morphologically complex, or multicellular algae examined. In the filamentous alga *Vaucheria sessilis*, on cooling using a conventional cooling protocol (-1°C min^{-1} ⇨ -35°C ⇨ liquid nitrogen in the presence of cryoprotectant), lack of cellular compartmentalisation and insufficient time for dehydration resulted in the propagation of intracellular ice throughout the thallus (Figure 21). Significant damage was observed at the ultrastructural level with physical disruption of cellular organelles and membranes (Figure 22). Other factors causing both lethal and sublethal injuries include pre-cooling manipulations (e.g. centrifugation), cryoprotectant toxicity and chilling damage. These effects can most readily be detected employing vital staining or measurement of oxygen evolution capacity. In addition, collaborative research with the University of Abertay Dundee has implicated free-radical mediated damage and fluctuations in antioxidant levels in the apparent freeze-recalcitrance of some algae.

It is anticipated that elucidation of the key sites of injury will assist in the improvement of existing cryopreservation methodologies and the development of alternative approaches. Areas that present significant challenges include the cryopreservation of large/complex unicellular and multicellular algae. Ultimately the challenge is to develop approaches that are robust, reliable and enable high levels of post-thaw viability to be obtained.

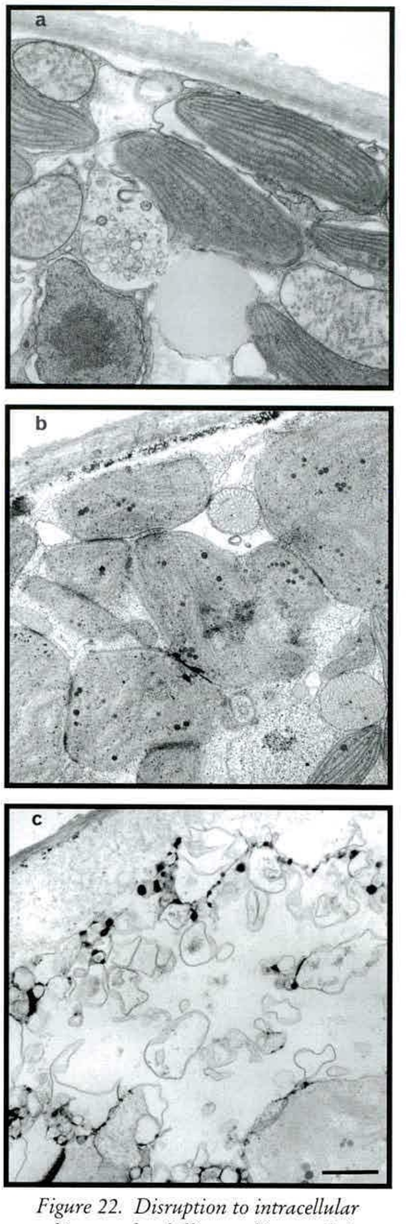

Figure 22. Disruption to intracellular architecture by chilling and intracellular freezing. (a) control; non-frozen filament, (b) –10°C (supercooled); showing accumulation of lipid deposits, (c) –60°C; gross disruption of cell architecture. Scale bar = 1.0 µm.

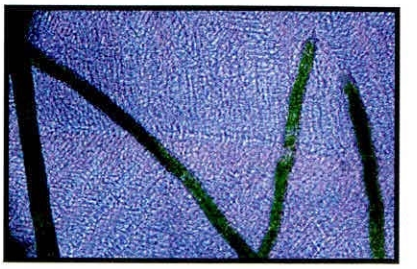

Figure 21. Intracellular ice "running along" a filament of Vaucheria sessilis.

CCAP cultures have remained stable after 20 years storage at –196°C.

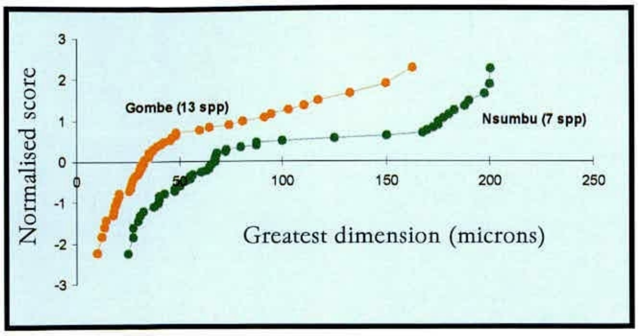

Figure 23. Phytoplankton size distributions and species diversity at 2 relatively unimpacted areas of Lake Tanganyika (Gombe, Tanzania and Nsumbu, Zambia): arrays of 50 randomly chosen organisms ≥ 10 μm from material collected in June 1998 with a 30 μm mesh net towed for 5 minutes in the upper 2m.

A comparison of phytoplankton biodiversity in two incomparable lakes (Leven, Scotland and Tanganyika, East Africa)

An opportunity has arisen to compare in a far more quantitative and repeatable fashion than hitherto, the planktonic algal assemblages of Lake Tanganyika and the very different 13.3km² Loch Leven in Scotland (Table 1). The great age, size and spatial habitat variation of the African lake has resulted in an assemblage of many thousands of aquatic species in total, although possibly only 100 truly planktonic algae (rather than benthic forms). This is significantly less than the approximately 300 species recorded over 30 years more or less uninterrupted weekly/fortnightly sampling of the Scottish lake – where very marked temporal variation in environmental conditions results from a highly capricious oceanic weather system impinging on a shallow basin of water.

June 1998 samples revealed no consistent relationships between overall nutrient status and the numbers of phytoplankton species in arrays of 50 specimens, the ranges in organism size, or the nature of the size frequency distributions (Figures 23 and 24 using normalised scores). Thus, a sample from one remote site (Gombe, Tanzania) revealed 13 species, while only 7 types were recorded from the

The Institute is training African nationals in limnological monitoring of Lake Tanganyika, with major reference to aspects of pollution and its effects on biodiversity.

Table 1. Lake Tanganyika and Loch Leven compared with reference to features affecting phytoplankton species diversity.

Lake Tanganyika	some attributes relevant to phytoplankton diversity	Loch Leven
rift valley formation	origin	glacial scouring
3 to 9 S	latitutude (°)	56 N
20, but isolated for only 1.8	age (millions of years)	0.016
580	mean depth (m)	3.9
650	length (km)	6
upper mixolimnion *ca* 200 m	thermal stratification	intermittent
700-900	conductivity (μS cm^{-1} at 25°C)	250
25-30	surface water temperature (°C)	0-25
ca 9.0	pH	7.5-10.5
<2 but greater after seasonal nutrient upwelling	soluble reative phosphorus (μg P l^{-1})	<1 to 150
ca 2 but greater after seasonal nutrient upwelling	dissolved silica (mg SiO2 l^{-1})	<0.05 to 13
ca 2-5 but greater after seasonal nutrient upwelling	chlorophyll *a* (μg l^{-1})	<1 to 250

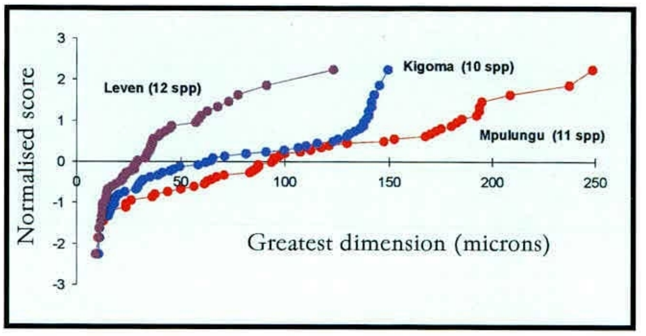

Figure 24. As Figure 23 for Loch Leven and two relatively impacted areas of Lake Tanganyika (Kigoma, Tanzania and Mpulungu, Zambia).

similar area of Nsumbu (Zambia). Apart from the low number of species at Nsumbu, diversity differs little between sites ranging from Gombe (poorest in nutrients), through to the mildly impacted Mpulungu (Zambia), the more populated Kigoma (Tanganyika) and the eutrophic Leven.

At each site except Gombe where *Sphaerocystis schroeteri, Chroococcus limneticus* and a lanceolate green alga resembling *Closteriopsis acicularis* accounted for more than 50% of the specimens, only 2 species comprised >25 specimens: *Anabaena flos-aquae fa aptekariana* and stellate colonies of *Synedra actinastroides* at Nsumbu and Mpulungu, the same *Synedra* species along with an *Aphanocapsa* at Kigoma, and *Gomphosphaeria* nr. *lacustris* accompanied by *Aphanocapsa* at Leven. The single array for Leven alone features 12 species, while a total of only 21 different taxa were recorded from the 4 African sites.

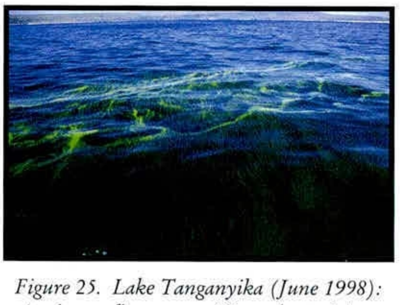

Figure 25. Lake Tanganyika (June 1998): Anabaena flos-aquae *(Cyanobacteria) at Mpulungu, Zambia following seasonal winds and up-welling of nutrient-rich deep water.*

Species introductions and the conservation of vendace in Bassenthwaite Lake and Derwentwater

Recent fish introductions to Bassenthwaite Lake (roach *Rutilus rutilus*, ruffe *Gymnocephalus cernuus*, and dace *Leuciscus leuciscus*) and Derwentwater (roach and dace) (Figure 26) and their implications for local populations of the rare fish vendace *Coregonus albula* have been investigated.

Changes during the 1990s

At Bassenthwaite Lake (Figure 27), the roach population has declined, the ruffe has increased, and the dace was first recorded in late 1996. Juvenile roach are now scarce and restricted to inshore areas, while juvenile ruffe are found in all parts with the exception of the offshore surface waters. The diets of juvenile roach were dominated by *Daphnia*, but this cladoceran only featured in the diet of juvenile ruffe from the offshore and not inshore areas. At times, the winter diet of ruffe included vendace eggs.

At Derwentwater (Figure 28), the introduced roach population has increased dramatically, ruffe has remained absent, and the dace was first recorded in late 1997. Juvenile vendace occupy the surface layer of the offshore waters, while juvenile roach are restricted to shallow inshore areas. The diets of juvenile vendace and roach are dominated by *Daphnia*, but

A total of five species introductions has altered the two fish communities.

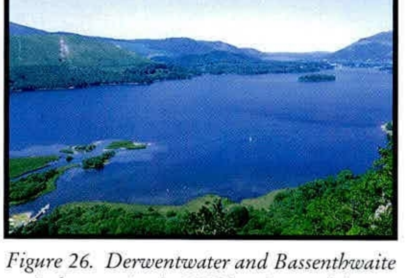

Figure 26. Derwentwater and Bassenthwaite Lake contain the U.K.'s only surviving natural vendace populations.

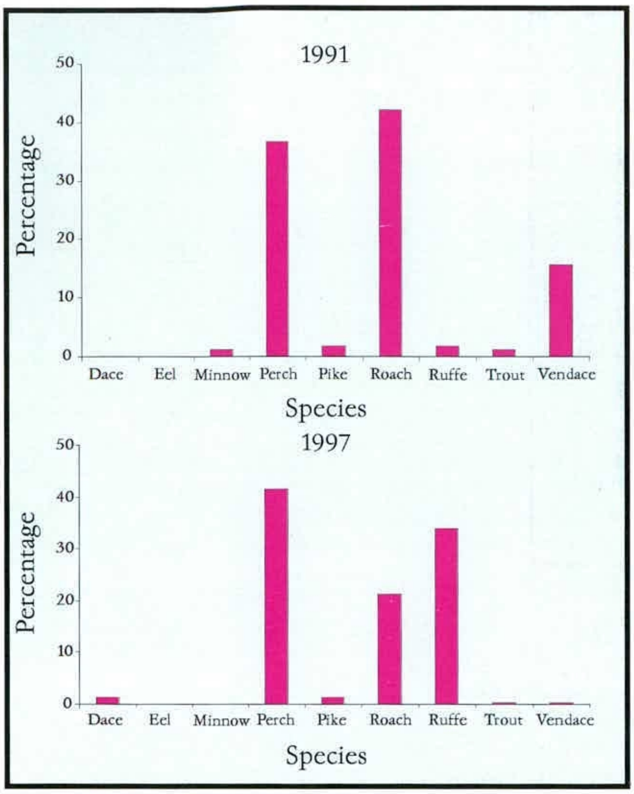

Figure 27. Fish community composition (by numbers) of Bassenthwaite Lake in 1991 and 1997.

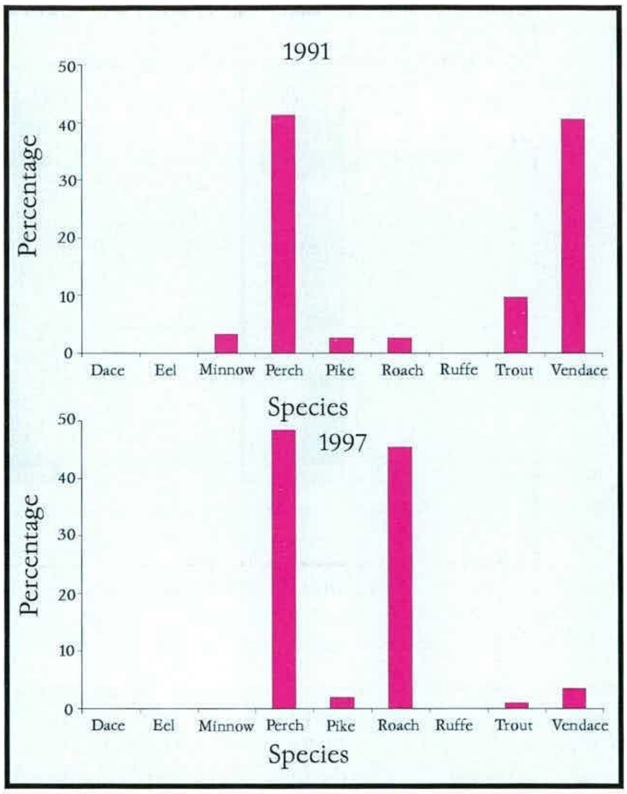

Figure 28. Fish community composition (by numbers) of Derwentwater in 1991 and 1997. In addition, a single dace has been recorded in very late 1997.

competition is unlikely because of their spatial segregation.

Conclusions

The roach populations of Bassenthwaite Lake and Derwentwater are currently not a threat to the vendace populations, although concern remains over the impact of the ruffe at Bassenthwaite Lake through both egg predation and competition for zooplankton. However, introductions of other fish species are not the only threats faced by the vendace populations. The summer vertical distributions of adult vendace are intermittently restricted by low dissolved oxygen levels at depth in both lakes and, in addition, siltation of spawning grounds associated with elevated levels of nutrients and algae appears to be a major problem at Bassenthwaite Lake.

Introductions of other fish species are not the only threats.

Reintroduction of Vendace to Scotland

The vendace *Coregonus albula* (Figure 29) is currently Britain's rarest freshwater fish, now found only in Bassenthwaite Lake and Derwentwater in Cumbria. Another two populations in Castle Loch and Mill Loch in Dumfriesshire became extinct earlier this century. As part of its Species Recovery Programme, Scottish Natural Heritage commissioned the IFE and the Fish Conservation Centre to establish new vendance populations in southwest Scotland from the Cumbria stocks. A three-year programme is in progress.

Figure 29. Vendace Coregonus albula *from Bassenthwaite Lake.*

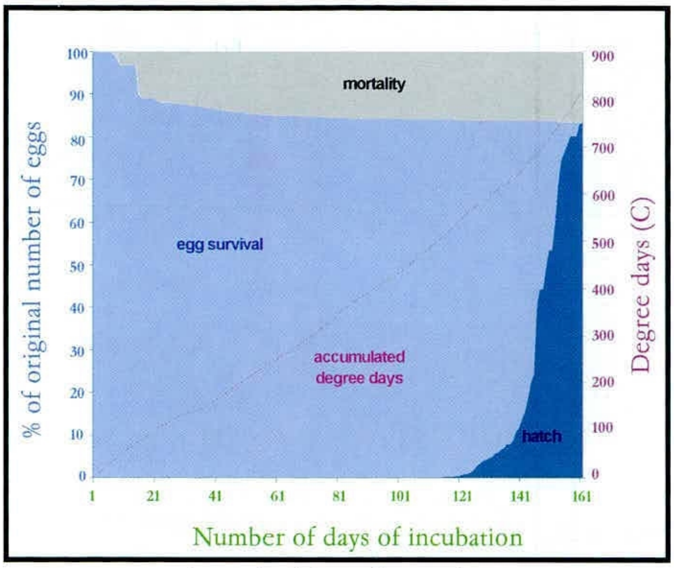

Figure 30. Incubation details for vendace eggs from Derwentwater.

Introduction site selection

The original Scottish sites were not immediately recoverable, so the first requirement was to identify lochs in the area suitable for vendance introduction. 110 lochs were examined for relevant characteristics such as bathymetry, pollution risk, existing fish, usage and security, and ultimately two were chosen, Loch Skeen and Daer Reservoir. To maintain the integrity of the Cumbria stocks and to provide individual safeguard populations, Bassenthwaite and Derwentwater vendace are separately designated to Loch Skeen and Daer Reservoir respectively.

Translocation

Vendace spawn in December and are collected then by netting. For genetic diversity, fish are taken at different times and places. Ripe adults are stripped of eggs and milt and fertilised eggs are taken immediately to hatcheries in Scotland. Vendace eggs have a lengthy incubation time, it takes about 140 days for 50% to hatch. In spring, fry are moved to the new sites within a few days of hatching. To date 17500 vendace fry have gone to Loch Skeen and 13000 to Daer Reservoir. In future, these sites will be investigated to check that breeding populations have been established.

In addition to achieving an important conservation objective, this (and similar projects for Arctic charr *Salvelinus alpinus* and powan *Coregonus lavaretus*) provide valuable ecological and biological information. For example, Figure 30 shows mortality, incubation and hatching rates with accumulated degree-days for a batch of Derwentwater vendace eggs.

Vendace are rare and vulnerable in Britain – it is important to increase their distribution.

RIVPACS International Workshop

In September 1997, over 60 scientists from 20 countries around the world were invited to a three-day workshop held at Jesus College, Oxford. The meeting was sponsored by the Environment Agency, the Institute of Freshwater Ecology and the Australian National River Health Programme.

The workshop took place almost 20 years after the formation of a small team of scientists at the IFE River Laboratory, whose research lead to the development of RIVPACS (River InVertebrate Prediction And Classification System). This software package is used within the Environment Agency, the Scottish Environmental

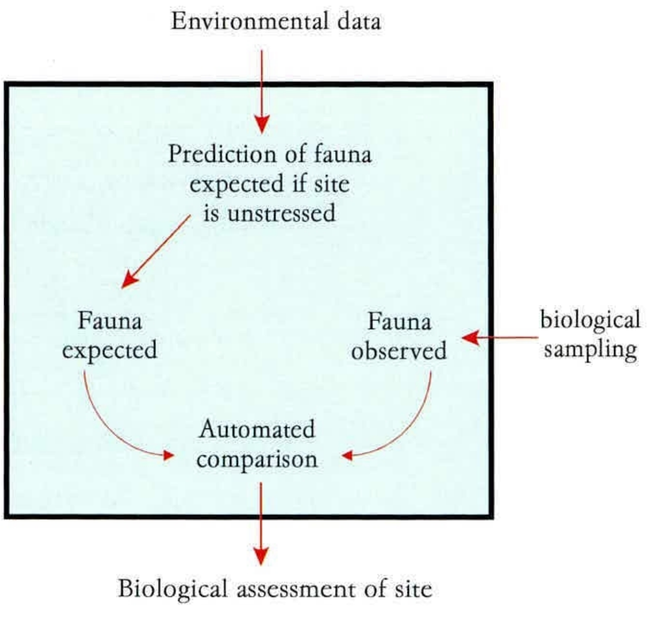

Figure 31. Procedure for a RIVPACS prediction.

Over 60 scientists from 20 countries attended the RIVPACS International Workshop.

Protection Agency and in Northern Ireland for prediction of the macroinvertebrate fauna to be expected at a running-water site in the absence of environmental stress. By comparing the fauna observed at a site with the prediction from RIVPACS, the biological quality of the site can be determined.

Over the past ten years, the RIVPACS approach has attracted widespread interest abroad. After introductory talks on recent developments in RIVPACS within the UK, the workshop continued with a series of presentations based on similar techniques for determining sediment quality in the Great Lakes of North America, for assessment of the health of rivers throughout Australia and for monitoring the quality of the Fraser River in British Columbia, Canada. The formal presentations also included contributions from a number of other scientists who have explored similar or alternative approaches for the biological assessment of river quality.

Many participants offered posters and software demonstrations that generated lively discussions, as did a series of five workshop discussion groups, designed to raise controversial issues and consider future opportunities. The workshop included a Banquet and finished with a boat trip on the River Thames, after which participants returned home to write their manuscripts. A book, based on 19 workshop presentations and the five workshop discussion sessions is to be published by the Freshwater Biological Association in 1999.

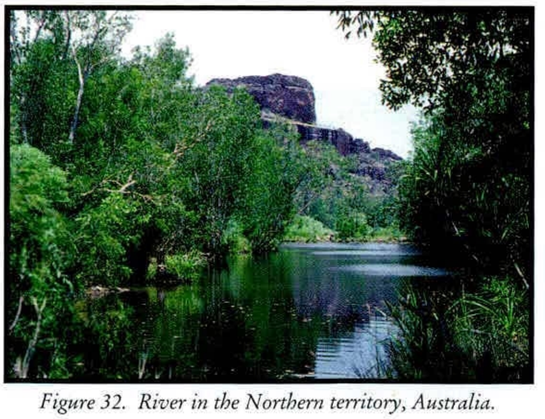

Figure 32. River in the Northern territory, Australia.