Pollution

CEH Core Strategic Programme 7

Microbial biodegradation of xenobiotics in rhizosphere soils

Chlorobenzenes (CBs) are widely used as solvents, degreasers, deodorants and are also produced as intermediates in the synthesis of dyes and pesticides. Their extensive use in the past few decades has resulted in large quantities being released into the environment, both by accident and through routine disposal in waste treatment facilities. These releases have resulted in pollution of groundwaters and soil, fuelling concerns about the fate and persistence of these compounds in the environment. Removal of CBs from the environment can only occur via enzymatically catalysed reactions. Therefore, investigations have focused on microbial populations capable of degrading (mineralise and remove) the compound. In collaboration with ITE Monks Wood, we have investigated the nature of the initial impact of CBs on the indigenous microbial community within the recipient habitat and recorded how this response is influenced by different exposure conditions. Several factors are likely to influence the response of a soil microbial community to pollution events, not least its concentration and the mode of entry into the habitat. We have monitored the impact of introducing 1,2-dichlorobenzene (1,2-DCB) either as single or multiple doses on its rate of mineralisation and its direct effect on the diversity and biomass (Fig. 25) of the soil microbial community. After 22 weeks of exposure the rate of 1,2-DCB mineralisation and the cumulative amount mineralised was significantly lower in soils exposed to multiple doses. The impact of 1,2-DCB application on taxa composition of the culturable bacteria was determined by fatty acid methyl ester (FAME) analysis of isolates. Compared to the control soil samples, soils exposed to a single dose of 1,2-DCB had a lower percentage of Gram-positive genera (*Arthrobacater* and *Micrococcus*) and increased presence of pseudomonads. Conversely multiple doses of 1,2-DCB significantly reduced the population density of pseudomonads to only 2% of the total compared with 46% and 71% of the untreated control and single-input treatments. These data underline the nascent ability of the natural microflora to transform and remove introduced xenobiotics.

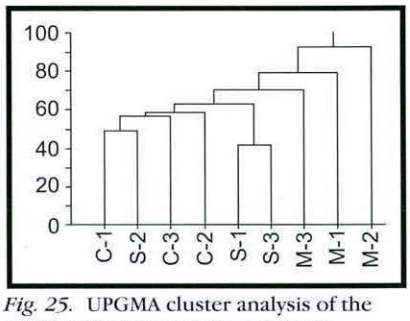


Fig. 25. UPGMA cluster analysis of the relationship between the metabolic potential of total extracted microflora profiles as determined by BIOLOG-GN microtitre plate assay. Soil treated with 100µg of 1,2 DCB delivered as a single dose (S) or as 10 equal 10µg doses applied weekly (M) were compared against untreated soil (C) after 22 weeks of exposure

Fig. 26. Littlemore Brook sampling site

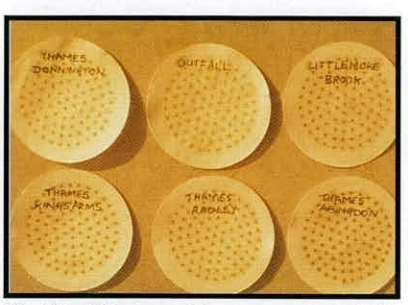


Fig. 27. Filters used for measurement of suspended sediment in river water

Modelling the transport and fate of viruses in the aquatic environment

Human enteric viruses occur as contaminants in many aquatic environments. Accurate assessment of the potential hazard they pose requires a detailed knowledge of their persistence and fate. Models to predict the transport and survival of *E. coli* in rivers have previously been developed and validated but insufficient data are available for viruses. Standard bacterial indicators of faecal pollution may not be accurate forecasters for enteric viruses since they have markedly different physicochemical and biological properties from bacteria which influence their interactions with the environment. Previously used methods for viral detection are time-consuming and relatively insensitive. As a consequence, there has been little systematic work to track their movement through the aquatic environment. The ability to predict viral loads in river water has important implications for end-users, such as water companies, and those involved in water sports. In collaboration with the Institute of Hydrology, we are monitoring a sewage outfall and river water at strategic points both upstream and downstream, for the presence of human enteric viruses (Fig. 26). Water samples are initially concentrated by membrane filtration then concentrated and purified. The detection method used for enteroviruses (group A rotaviruses, human caliciviruses, hepatitis A and polioviruses) was reverse-transcription polymerse chain reaction RT-PCR, together with nested PCR for adenoviruses. These data are being assessed in the context of the physical and chemical properties of the receiving water, its flow dynamics and sedimentological characteristics (Fig. 27), to discover the key processes governing virus survival and transport. Such data will be used to highlight differences in temporal and spatial behaviour between the indicator bacteria and viruses (Fig. 28).

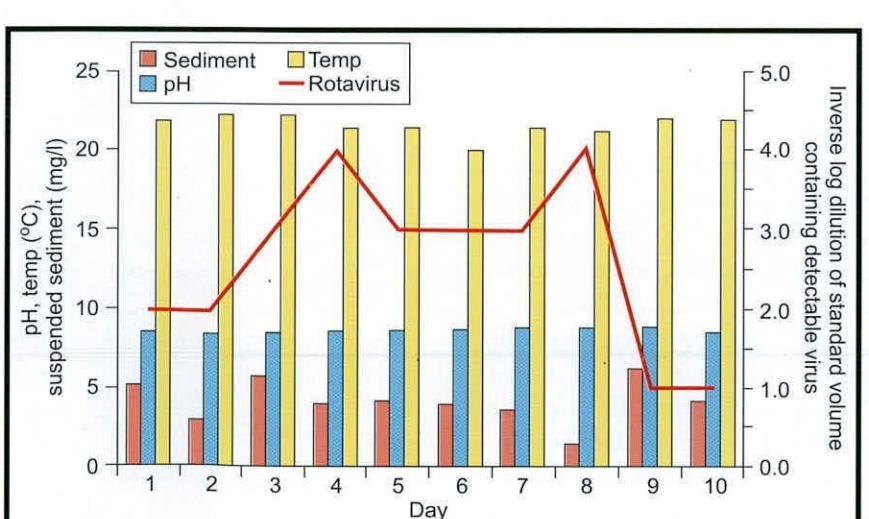


Fig. 28. Variation in rotavirus concentration at a single site on the River Thames, together with variation in water temperature, pH and suspended sediment, over a ten-day period

Global change

CEH Core Strategic Programme 9

Microbial basis of methane oxidation in soils

The impact of global change on the activities and diversity of soil is being investigated in two studies in IVEM.

- In collaboration with ITE Merlewood and IFE Windermere, we are investigating the microbial basis of methane oxidation. Specifically, using a site in mid-Wales (Fig. 29) we are examining the microbiological basis of methane production in soils, to determine why some are net producers whilst others are consumers of methane. This is being investigated using a combination of fatty acid profiling and molecular methods to characterise microbial populations involved in methane metabolism.

- The impact of altering CO_2 levels on the diversity of heterotrophic soil bacteria is being investigated in a controlled environment facility, the Ecotron, at the NERC Centre for Population Biology. In collaboration with the University of Liverpool, a range of culture and molecular methods is being used to assess soil microbial diversity response to increasing levels of CO_2 within the Ecotron chambers. Amongst the techniques being used to examine soil community shifts in response to altering ambient conditions is the BIOLOG system, a rapid and effective method of assessing the ability of whole soil communities to metabolise 95 different sole carbon sources, contained on a microtitre plate (Fig. 30).

Fig.29. Site in South Wales

Fig. 30. Vicki Goddard, student, examining a root

Integrating generic science – biotechnology

CEH Core Strategic Programme 10

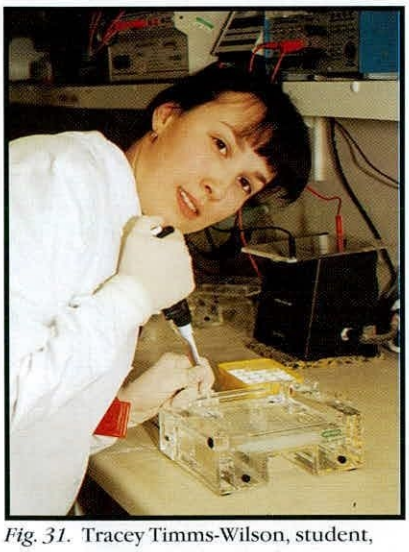


Fig. 31. Tracey Timms-Wilson, student, loading a DNA submarine gel

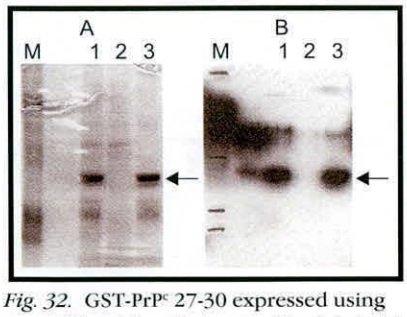


Fig. 32. GST-PrP^c 27-30 expressed using recombinant baculoviruses. Track 1 - total infected cell lysate; track 2 - the insoluble fraction; track 3 - the soluble fraction. M - molecular weight markers. Panel A is the stained gel and panel B a western blot developed with anti-PrP peptide sera

BIOMATE

Biosensors for multi-determining and assessment of toxic environments is an exciting new collaborative project supported by the LINK-Biological Treatment of Soil and Water programmes that combines the expertise of scientists from commercial, academic and government institutions (Fig. 31). The objectives of the venture are to develop biosensors for industrial and environmental applications which are able to monitor pollution events or changes in prescribed processes in real time.

Prion diseases

Diseases now grouped as the transmissible spongiform encephalopathies (TSEs) have been known for some time. The most well characterised of the group is scrapie, a neurodegenerative disease of sheep that has been studied experimentally for many years. The diseases have become prominent in the public perception in the UK in recent years due to the sudden onset and rise of bovine spongiform encephalopathy (BSE) in domestic cattle. The TSEs have evoked considerable scientific interest as the infectious agent is considered to be a wholly proteinaceous molecule, the prion, a normal constituent of neuronal cells that is highly conserved between species. The presence of the normal prion form, as well as the introduction of the disease form, is essential for new disease transmission. Wildtype and disease forms of prion share the same primary sequence suggesting that disease must associate with an altered tertiary structure of the protein. Drawing on our experience in the use of recombinant viral technologies, IVEM is developing methods for the efficient production of prion proteins as a prerequisite to understanding the structure of the molecule. An effective source of the protein would also allow the isolation of ligands for the molecule and might spawn improved diagnostic procedures for the detection of prions in environmental samples. Initial developments seem encouraging as good levels of expression have been achieved for the Syrian hamster prion protein based on its fusion to a simple carrier protein (GST) using vectors specifically designed at the Institute and the construction of recombinant baculoviruses (Fig. 32).

Institute overview

Highlights of the year

Oxford Centre for Environmental Biotechnology

IVEM has recently joined with the Departments of Engineering and Plant Sciences at the University of Oxford to establish the Oxford Centre for Environmental Biotechnology to make a significant contribution to the remediation and prevention of environmental contamination. The expertise of the Centre includes engineering (chemical engineering, membrane separation and bioreactors), plant sciences and, within IVEM, microbial ecology, microbial diversity, molecular biology and biosensors. The multidisciplinary nature of the Centre has enabled a broad programme of integrated research to be established aimed at developing cost-effective solutions to resolving serious environmental problems associated with pollution.

The Centre has four main aims:

- to develop processes to remediate contaminated soil and water;
- to develop processes for the treatment of liquid effluent;
- to develop more effective impact and recovery assessment methods;
- to develop new processes for clean synthesis.

The Centre was formally established in October 1997, with Professor Christopher Knowles as the Director. Professor Knowles, who is a NERC Fellow, will be based in both IVEM with his research group, and in the Department of Engineering.

Oxford VACs Ltd

IVEM's first spin-off company, Oxford VACs Ltd, was born on 10th April 1997. It was created for the commercial development of a novel family of anti-inflammatory proteins discovered in the saliva of ticks by Dr Guido Paesen, working in Professor Pat Nuttall's research team. These proteins block the action of histamine molecules by a totally different mode of action to anti-histamines. They have potential applications in the treatment of certain allergic conditions, such as asthma. The Company, a 50-50 venture with VACs of Life plc, aims to exploit the properties of arthropod saliva. Financial backing from VACs of Life will enable the tick proteins to be tested in clinical trials as novel biopharmaceuticals.

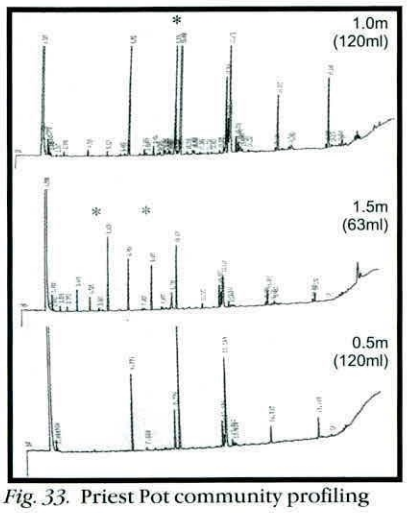


Fig. 33. Priest Pot community profiling with FAME

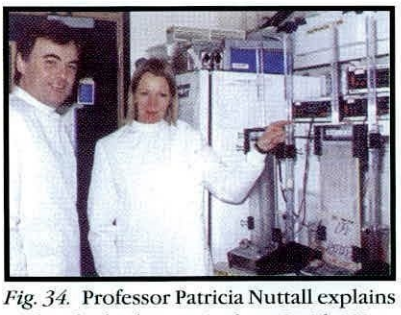


Fig. 34. Professor Patricia Nuttall explains molecular biology to Andrew Smith MP

Databases and networks

FAME and genetic database of bacteria found in the environment

A unique database of microbial isolates collected from a single field site has been established at IVEM over an eight-year period. The dataset is based on isolate characterisation according to the fatty acid methyl ester content of pure cultures. Analyses are performed by silica column gas chromatography and diagnostic profiles generated according to FAME-fingerprinting using commercial software. The commercial library of over 40,000 isolates has been augmented by >10,000 of our own samples. The most extensive database is for isolates collected from the rhizosphere, phyllosphere and soil from a small field of sugar beet (var. *Amethyst*) established at the University Farm, Wytham. However, smaller datasets have also been established with isolates obtained from collaborators studying different sites. The dataset for the Wytham study has been assembled during long-term monitoring of the site since it was first established from fallow pasture in the spring of 1989. The suitability of the FAME approach was confirmed by a number of molecular approaches evaluating both genotypic and phenotypic characteristics.

The dataset, which documents the natural dynamics and seasonal succession of a microbial community, provides an extremely useful benchmark against which assessments can be made, including evaluation of impact and change in biodiversity as a consequence of climate change.

The FAME dataset (Fig. 33) is further supported by an increasing number of bacterial and algal isolates from Priest Pot (a lake in Cumbria). Bacteria from Priest Pot include many isolates which are identified as *Pseudomonas* spp.

A smaller collection of genetically characterised isolates of *Pseudomonas* and *Erwinia* from the phytosphere and soil is also available. These unique datasets include over 1500 isolates and have been constructed using methods that enable identification at the clonal level and the establishment of phylogenies for bacterial populations.

Further datasets are available that describe the genetic diversity of plasmids associated with phytosphere microbial communities. The focus has been on replicons that carry antibiotics, particularly resistance to mercury. The availability of this plasmid dataset is of value in resolving the extent of plasmid genetic variation, for the development of a scheme for plasmid phylogeny.

Events and staff news

Visit of Andrew Smith MP

Andrew Smith MP (local MP and then Labour Shadow Transport Minister) visited the Institute on 14 February 1997. Mr Smith toured the Institute's facilities and met some of its scientists (Fig. 34). He applauded IVEM's efforts to bring its science into the marketplace, and to explain its research to the public at open meetings about research on genetically engineered viruses.

A public meeting to discuss genetic engineering of baculoviruses

Scientists at IVEM have been conducting field experiments with genetically modified baculoviruses

at the Wytham field station for over ten years. These studies have progressed from the use of simple, genetically marked viruses to those containing an insect-specific toxin gene originally found in a scorpion. The results from these trials have been reported in the scientific literature. The problem with such articles is that they are rarely easily accessible to the general public and tend to be written in highly technical language. Other accounts of the work sometimes appear in the popular press (Fig. 35), but these are presented second-hand with no facility for the reader to ask further questions.

In order to redress this imbalance, over the last few years we have held a number of open meetings where anyone can come and meet the scientists involved with the work using genetically modified baculoviruses. The third such meeting was held in Oxford in May 1997 at a local conference centre and was a total success. In a change to the regular format, instead of presenting our work as structured oral presentations, we used poster displays to describe the ongoing work. (We are grateful for the help provided by Sheila Anderson and colleagues in Policy and Communications at Swindon in the preparation of the boards.) This offered people the chance to talk one-to-one with IVEM staff. The day was organised so that school parties could attend in the afternoon and anyone else in the evening. The afternoon session was reasonably quiet, but in the evening we had some lively debates as the attendance swelled. The topics of discussion were not just confined to our work on genetically engineered baculoviruses, but also involved the wider issues of genetically modified foods. The meeting was held just after the controversy over the importation into Europe of genetically modified crops from the USA. It provided a salutary reminder that it is not possible to control the discussion in such meetings and it is necessary to be an expert in all areas of biology! While many of the opponents of our work with baculoviruses were not necessarily in total agreement with our arguments, there was general appreciation of the opportunity we had presented for people to come and talk with us. Future public meetings will be held to continue this process.

Envirogenomics

Envirogenomics represents the acquisition and exploitation of sequence data from environmentally relevant micro-organisms. This initiative parallels that of Pathogenomics, the genetic information of medically important pathogens. To canvas views on whether or not NERC should play a role in promoting Envirogenomics, a Town Meeting was held at the Royal Society in London on 27 February 1997. The meeting quickly revealed how widely environmental microbiology is pervading NERC science. The genetic resource embraced by NERC's remit is enormous: from micro-organisms in soils, the oceans and extreme environments such as deep ocean vents and polar regions. The meeting deliberately did not address which microbial genome(s) should be sequenced. This turned out to be a stumbling block. A positional paper, based on views expressed at the Royal Society meeting, received a very definite 'thumbs down' from NERC's Terrestrial and Freshwater Science and Technology Board. Our hope now is that industry may take a more enlightened view.

Fig. 35. IVEM scientists in the news

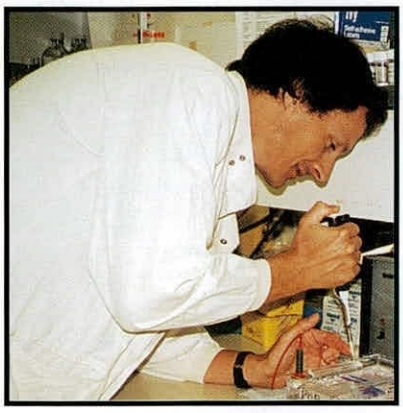


Fig. 36. Professor Robert Possee

Fig.37. Claire Hill, student

Awards

Professor Patricia Nuttall, Ivanovsky Medal for Virology, presented by Professor D.K. Lvov, Russian Academy of Sciences.

Professor Robert Possee: Professor of Molecular Biology, Oxford Brookes University (Fig. 36).

Professor Polly Roy: Professor of Virology, University of Oxford, and Fellow of the Indian Virological Society.

Dr Stephen Sait and Dr Andrew Lilley: NERC Fellowship.

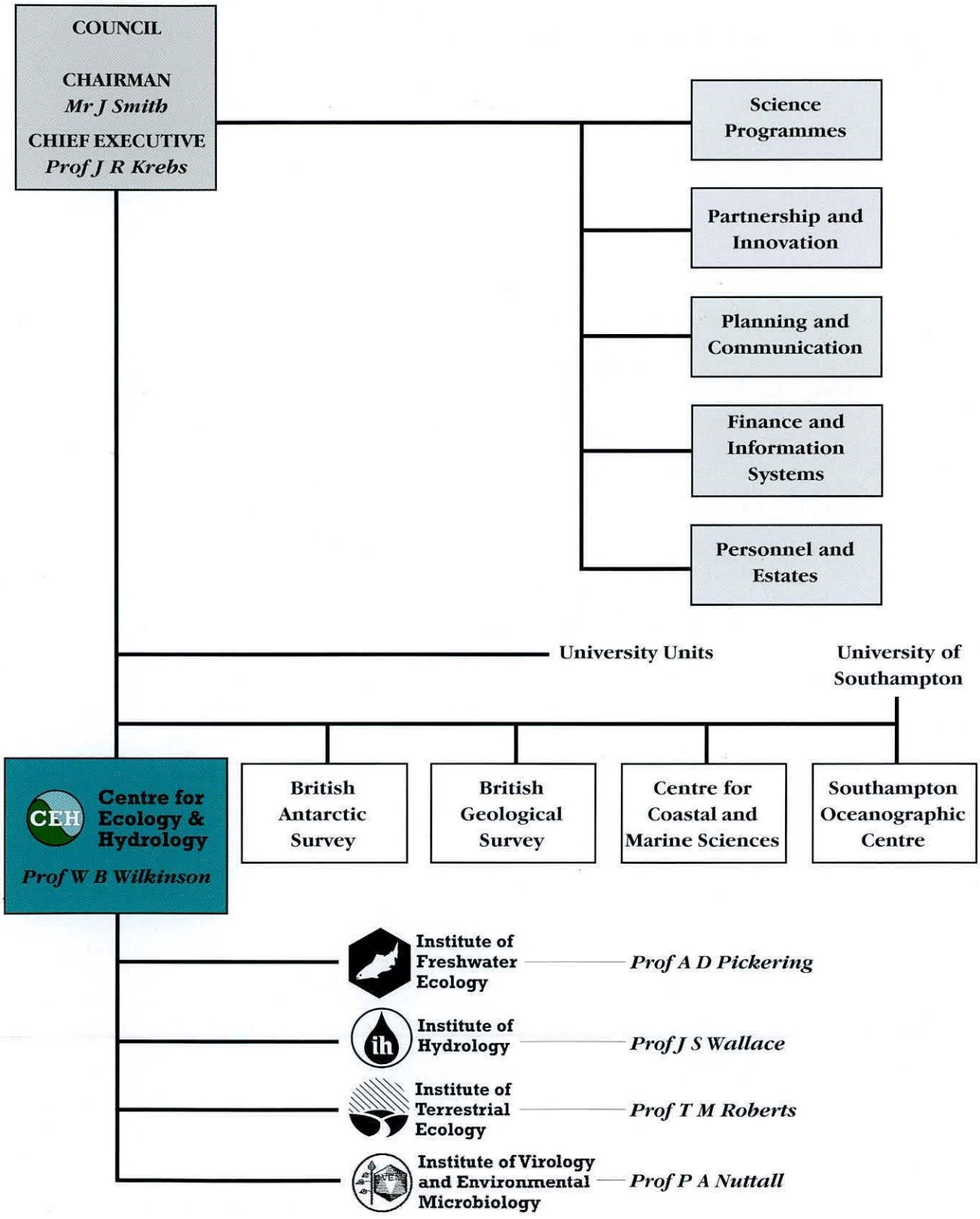
Dr Rosie Hails: Lectureship in Quantitative Methods, St. Anne's College, Oxford, and OECD Fellowship, Princeton University, USA.

Dr Klaus Kurtenbach: Wellcome Trust Senior Fellowship in Biodiversity.

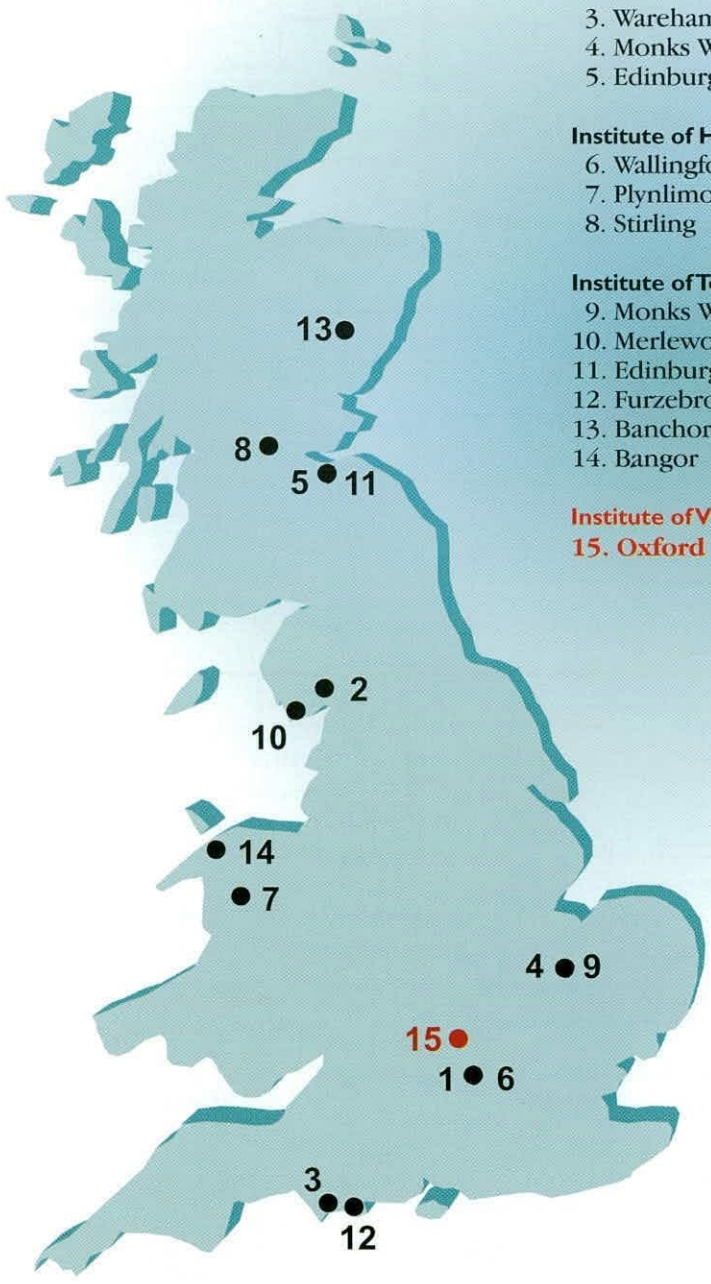
Ms Claire Hill: Best presentation in Oxford University D.Phil. Symposium (Fig. 37).

APPENDIX I

NERC structure



Location of CEH sites



Centre for Ecology and Hydrology
1. Wallingford

Institute of Freshwater Ecology
2. Windermere
3. Wareham
4. Monks Wood
5. Edinburgh

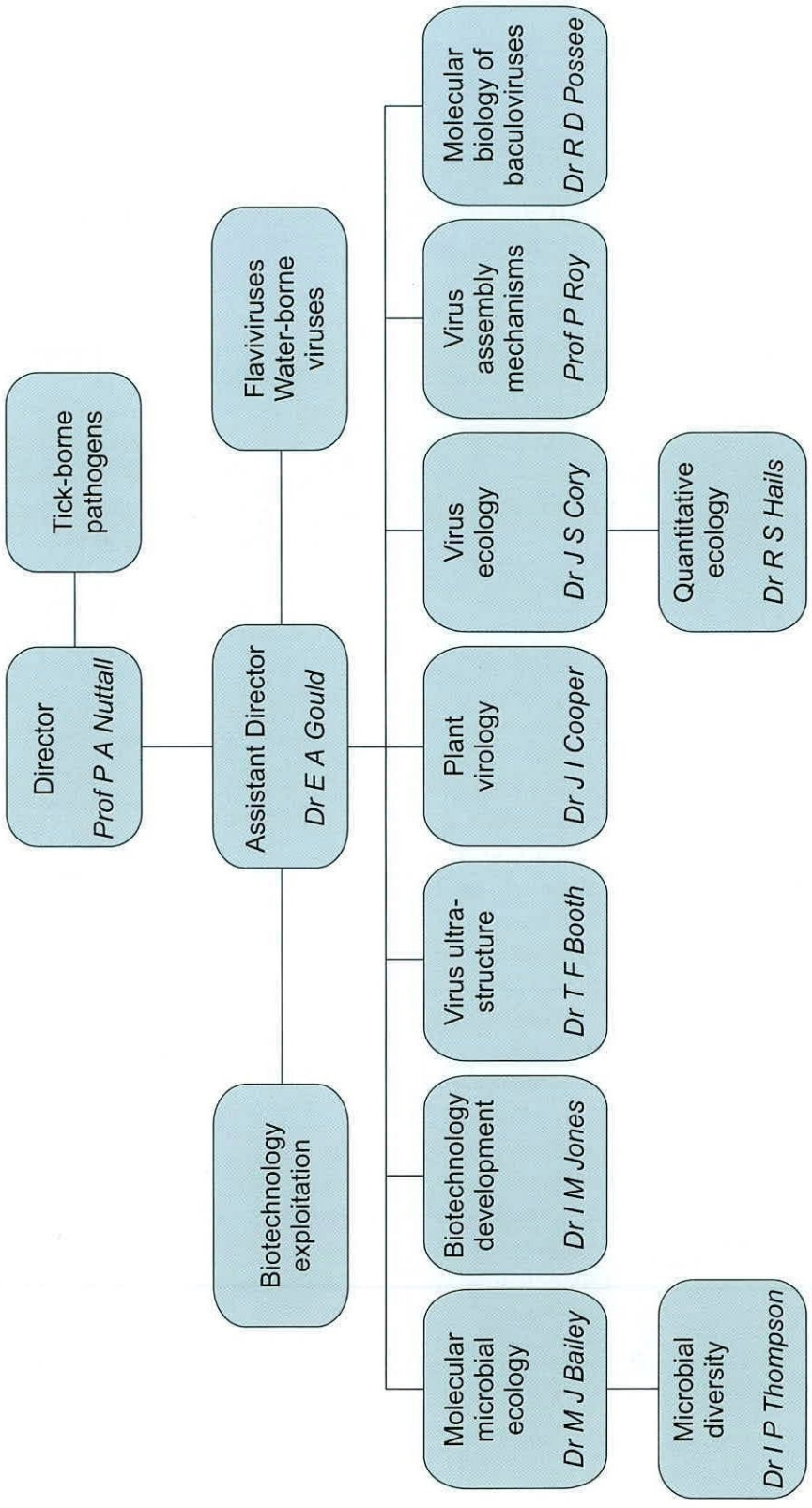
Institute of Hydrology
6. Wallingford
7. Plynlimon
8. Stirling

Institute of Terrestrial Ecology
9. Monks Wood
10. Merlewood
11. Edinburgh
12. Furzebrook
13. Banchory
14. Bangor

Institute of Virology and Environmental Microbiology
15. Oxford

APPENDIX 2

Institute structure (science)



APPENDIX 3

IVEM organisation

January – December 1997

Director
Patricia A Nuttall, MA PhD

Assistant Director
Ernest A Gould, PhD

Administration and technical support
Gavin Bird
Head of Administration
Peter Selwood
Administrator
Bridget Lewis
Head of Computing Support
Colin Cox
Computing Support Assistant
Jo Maun
Director's Secretary
Stephanie Price
Assistant Director's Secretary
Pat Newton
Receptionist
Ann Sloper
Receptionist
Graham Metcalfe
Support services
Sheila Morton
Support services
Gill Pinniger
Support services
Rex Bateman
Support services
William Lewis
Stores
Chris Hatton
Photographer
Chris Wilson
Librarian
Ray Broadbent
Engineer
Mac Mackenzie
Engineer
Tony Primarolo
Engineer
Tim Carty
Insectary management
Marcelle Burden
Media preparation
Peter Knight
Media preparation
Carole Broadbent
Biomedical services
Pauline Henbest
Biomedical services

Molecular microbial ecology
Mark J Bailey
Group Leader
Andy Lilley
Gene mobilisation and plasmid transfer
Andy Whiteley
Biosensors
Kirsten Lawlor
Molecular signalling
Sian Evans
Plasmids and survival factors
Tracey Timms-Wilson
Bio-control agents
Han Zhang
Environmental plasmids
Richard Ellis
Biocontrol agents
Eleni Bantinaki
Gene mobilisation and plasmid transfer
Siouxsie Wiles
Biosensors

Microbial diversity
Ian Thompson
Project Leader
Vicki Goddard
Biocontrol agents

Virus ultrastructures
Tim F Booth
Project Leader
Emma Nason
Structural analysis
Claire Hill
Structural analysis

Plant virology
J Ian Cooper
Project Leader
Mary-Lou Edwards
Plant viruses
Delia McCall
Plant propagation
Shi Jiao
Plant biotechnology

Virus ecology
Jenny S Cory
Project Leader
Rosie Hails
Quantitative ecology
Bernadette Green
Risk assessment
Kate Wilson
Insect pathogen ecology
Petrina Smith
Insect virus infections
John Burden
Baculovirus control agents
Steve Sait
Ecology and risk assessment
Andy Reeson
Larval polyphenism

Flaviviruses, water-borne viruses and biotechnology exploitation
Ernest Gould
Project Leader
Linda Jones
Cell mediated immunity

Identification of flavivirus receptors (Wellcome Trust) (E A Gould)
Orthomyxovirus–cell interactions (EC) (P A Nuttall)
Tick-borne virus transmission and replication (P A Nuttall)
Development of VLP candidate vaccines (EEC) (P Roy)

Issue 6.4.3 Pathogen–vector interactions
The role of seabirds in the epizootiology of Lyme disease (P A Nuttall)

Issue 6.4.4 Pathogen–host–vector interactions
Development of a novel and rapid protocol for producing and characterising infectious cDNA clones (BBSRC) (E A Gould)
Wildlife management and zoonotic diseased pheasants (Oxford University) (P A Nuttall)
Bio-regulators (VACs of Life) (P A Nuttall)

Issue 6.5.2 Development of novel methods for detecting viruses in water
Development of gene probe techniques for the detection of viral pathogens relevant to fisheries research (MAFF) (E A Gould)

Programme 7 Pollution

Project 7.5 Organic pollutants
Issue 7.5.3 Biological degradation and transformation
Molecular genetics and process level events (M J Bailey)
Modelling viruses in the aquatic environment (E A Gould)

Programme 9 Global change

Project 9.1 Greenhouse gas budgets and cycles
Issue 9.1.7 Soil sinks of methane
Microbial basis of methane oxidation in soils (I P Thompson)

Programme 10 Integrating generic science

Project 10.4 Biotechnology development
Issue 10.4.1 Virus replication
Bio-sensors for multi-determining and assessment of toxic environments (BIOMATE) (DTI) (M J Bailey)
Virus replication (IM Jones)
A system for eukaryotic virus display: a coupled genotype and phenotype system (RAB Japan) (I M Jones)
Use of Sindbis virus vector (Glaxo) (E A Gould)
Issue 10.4.2 Structure/function relationships of viral proteins
Role of furin in HIV gp160 (MRC) (I M Jones)
Structure and function of prion protein PrPc 27-30 (MRC) (I M Jones)
Trojan Horse methodologies for the production of retroviral vectors (Oxford BioMedica plc) (I M Jones)
Characterisation and cloning of HepC receptor (Glaxo) (I M Jones)

Programme 12 Non-core consultancies
Biotechnology (E A Gould)

APPENDIX 5

IVEM publications 1996

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