



**UNIVERSITY OF  
STIRLING**

THE INSTITUTE OF AQUACULTURE

# Annual Report 2002-2003



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## Highlights ▶ ▶ ▶

- ▶ Three new lecturers appointed funded by University Research Development Fund
- ▶ Wellcome Trust-funded confocal microscope commissioned
- ▶ Institute will receive £2.3 million SRIF funding for laboratory and equipment refurbishment
- ▶ New laboratory building commissioned at Buckieburn Freshwater Unit
- ▶ Increased undergraduate teaching income for 2003-2004
- ▶ Gene expression study on the salmon louse completed
- ▶ New vaccines against pasteurellosis and bacterial kidney disease under field trials
- ▶ Effects of photoperiod on growth and maturation in cod now being trialed commercially
- ▶ Major BBSRC-funded collaborative research programme on molecular analysis of commercial traits in Atlantic salmon underway.
- ▶ A new EU-funded project is investigating the use of shrimp processing wastes in fish diets
- ▶ Institute research has led to the development of new commercial diets for marine fish
- ▶ Research is demonstrating the scope for more efficient use of environmental capacity through improved feeding systems and diet formulation
- ▶ A range of research programmes are developing international strategies for tilapia development
- ▶ Stirling Aquaculture has developed a series of training courses for industry with BBSRC support



## Introduction ► ► ►

Whilst the Institute has continued to develop strongly in 2002-2003 the year was overshadowed by the untimely death of Professor Niall Bromage. Niall was a scientist who conducted his research to the highest professional standards and this was reflected in his international stature. Niall will also be warmly remembered as a supervisor and mentor to numerous research students, many of whom have gone on to successful research careers of their own. The contribution which Niall made to the academic and social life of the Institute was immense and he is sorely missed by his friends and colleagues. Niall played a very significant part in the development of the Buckieburn Facility as an experimental site and it is therefore entirely fitting that the new laboratory building at Buckieburn has been named the "Niall Bromage Freshwater Research Facility" and has been officially opened by Niall's wife Anne.

This year has seen a number of staff changes within the Institute. Dr Donald Baird, Senior Lecturer in Ecotoxicology, has left to take up a chair at the University of New Brunswick, Canada, and Mr Stuart Millar, Chief Technician, has joined an industrial laboratory near Edinburgh. Both Donald and Stuart made major contributions to the Institute and we wish them every success in the future.

Appointments have now been made to the three new lectureships funded through the University Research Development Fund. These have been awarded to Dr David Penman (Genetics), Dr Andy Shinn (Parasitology) and Dr Dave Little (Aquaculture Development). All three of these appointees have excellent track records in teaching and research within the Institute and will provide for an element of succession within their respective research groups. I am also very pleased to report the award of a personal chair in Aquatic Immunology to Sandra Adams.

The Institute has been awarded some £2.3 million under the forthcoming SHEFC Science Research Infrastructure Fund (SRIF) scheme.

This is a unique opportunity to make substantial improvements to our research facilities and equipment which is unlikely to be repeated in the foreseeable future. We hope to rationalise our warm water aquaria and to substantially increase laboratory space available for molecular and biochemical research, areas we see as expanding in our research programme in the future. Other laboratory improvements, as well as refurbishment of the basic fabric of the buildings, will also be carried out. The SRIF funding will also be used to replace our existing obsolete electron microscopes and to improve our capability in basic proteomic research. The latter will have significant implications in terms of maintenance and staffing but we feel that to maintain our position it is essential that we are able to demonstrate capability in this rapidly developing field. Work on the SRIF-funded refurbishments will take place over 2004-2006 and will undoubtedly cause a good deal of disruption, but will result in substantial improvements to our research capability.

There are a number of developments in higher education and research funding which will certainly have an important impact upon the Institute, although it is perhaps too early to predict their effects with any clarity. We have continued to develop a forward

strategy despite these uncertainties and will be guided partly by the results of a recently completed Departmental Review of the Institute carried out by a panel consisting of senior University academics together with external academic advisors. We are confident that these changes will provide the Institute with the staff and facilities to build on its current position as the major Centre of Excellence in Aquaculture.

RANDOLPH RICHARDS  
Director

## Teaching ► ► ►

### Postgraduate

The MSc Aquaculture and MSc Aquatic Veterinary Studies/Aquatic Pathobiology courses were run in 2002-2003. The former had 20 students, including 7 from non-EU countries, whilst the latter courses had 17 participants, a high number although only one student was non-EU. Distinctions in the MSc Aquatic Veterinary Studies/Aquatic Pathobiology courses were awarded to Johanna Smith, Richard Lloyd and James Bailey. Congratulations are due to Charlie Price from the MSc Aquaculture course and Richard Lloyd from the MSc Aquatic Veterinary Studies course who were awarded the Royal Highland and Agricultural Society of Scotland Silver Medal. Drew Oliver and Scott Dowd from the MSc Aquaculture course were jointly awarded the External Examiner's Prize.

The MSc in Sustainable Development has commenced in 2003 but start of the M Res in Aquatic Science has been postponed until 2004.



Mrs Anne Bromage opening the Buckieburn Freshwater Research Facility

Fourteen new postgraduate research students started their studies in 2002-2003, including eight from non-EU countries. This is a very similar number to the intake for the previous year. Twenty one research students graduated in 2002-2003.

The Institute was awarded one NERC studentship in 2002-2003 which has been taken up by Adam Brookner who will study with Drs Shinn and Bron. Sean Kennedy will take up a BBSRC CASE award with supervision by Dr Tocher and Professor Adams. CASE funding will be provided by BIOMAR Ltd.

### Undergraduate

There was only one BSc Hons Aquaculture student in 2002-2003, who graduated with a 2(i), and three BSc Freshwater Science students, two of whom obtained a 2(i) and one a 2(ii). Overall numbers of undergraduate full time equivalents (FTEs) fell compared to 2001-2002 and numbers of Honours year students will again be low in 2002-2003. Honours year courses have been rationalized to take account of this. However, overall undergraduate FTEs will be healthier in 2003-2004, mostly due to increased numbers taking our second year course, and to a lesser extent third year courses. Whilst it is unlikely that student numbers for the BSc Aquaculture and BSc Freshwater Science will increase greatly we hope that the greater numbers of FTEs derived from second and third year teaching will continue, thus providing an improved and stable source of income.

### Other teaching

The annual fish disease course was held in January with 18 participants.

Additional BBSRC supported courses for industry were run in 2002-2003 as detailed elsewhere in this report (p 40). SCOTPIL courses for Home Office personal licence holders were again run and a number of student groups from UK and European higher education establishments visited the Institute and Howietoun

## Fish Health

### Parasitology

Sea lice continue to represent one of the major research areas in Parasitology. Dr James Bron, in collaboration with Professor Alan Teale, Professor Christina Sommerville of IOA and Professor Huw Rees of the University of Liverpool and with the support of Scottish Quality Salmon, has successfully completed a 3 year BBSRC funded project investigating the triggering of moulting and metamorphosis in copepodid larvae of the salmon louse. This project has employed DNA microarray technology and is the first such project to have been carried out at the University of Stirling. Use of microarray and a range of allied molecular techniques has successfully demonstrated the regulation of key genes involved in the process of parasite transformation following host attachment. This is the first time that this has been achieved for an aquatic parasitic arthropod and similarly, is the first time that moulting and metamorphosis have been studied at this level for any copepod species, free-living or parasitic. This research has established a time-course for key gene regulation events and allowed identification of critical components of the parasite response to the salmon host, thereby contributing substantially to knowledge of the biology of this important pathogen. Key genes identified for the first time by this research may provide

targets for the future development of highly parasite-specific medicines and vaccines. The data resource provided by this project, as well as the development of specific tools and expertise, will also greatly facilitate the further study of this critical process.

Janet Stone and Sally Boyd, in collaboration with Professor Sommerville, continue their research into the development of sea lice treatments at the Marine Environmental Research Station at Machrihanish. Despite the release of these efficacious compounds onto the market, the search continues for alternatives to provide a choice of treatments for different farming settings and to allow for the rotation of treatment methods to help reduce the potential development of resistance.

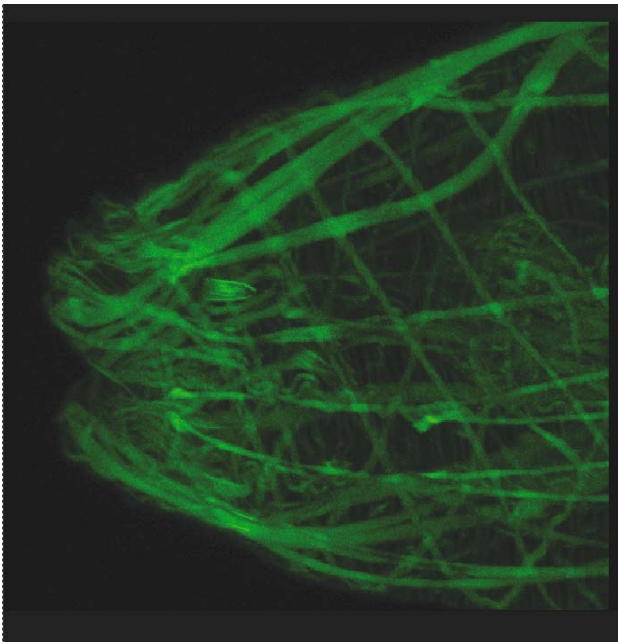
Keith Walton has completed the first year of his University of Stirling and industry funded PhD study investigating the host-parasite interaction between Atlantic salmon and the salmon louse using a variety of *in vivo* and novel *in vitro* techniques. The study continues under the supervision of Professor Sommerville, Dr Wootten and Dr Ian Bricknell of the FRS Marine Laboratory in Aberdeen.

Data collection for Nick Taylor's PhD project on the epidemiology and ecology of freshwater fish louse *Argulus* spp. infections in UK stillwater trout fisheries is now complete. The project now enters its final phase under the supervision of Professor Sommerville and Dr Wootten and sponsored by the Environment Agency and a number of trout industry bodies. A number of risk factors associated with problem infections of *Argulus* spp. have been identified and their interaction with the parasite's population ecology assessed. The data from this work will be used to predict susceptible sites, and to develop effective management practices that will be used by fishery owners to reduce the impact of this parasite.

Andy Tildesley has joined the Parasitology team to conduct a one-year preliminary research project aimed at understanding the population ecology of the parasitic stages of the 'gill maggot', *Ergasilus sieboldi* in a stillwater trout fishery. In recent years this pathogen has emerged to cause significant economic losses in a number of UK sport fisheries. The project will be extended and developed into a two-year industry funded MSc by research.

Dr Andy Shinn, working in collaboration with Professor Sommerville and Dr Jim Kay from the University of Glasgow, has successfully completed a DEFRA funded project to develop a semi-automatic diagnostic system for the rapid identification of the notifiable pathogen *Gyrodactylus salaris*, from closely related species parasitising British salmonids.

Two systems have been developed, one based on point-to-point morphometric measurements that characterise the attachment hooks of *Gyrodactylus*, and a second based on shape descriptors extracted from the outline of the hooks. The systems, which are based on a serial combination of image analysis and methods of statistical classification, have been installed for use by the CEFAS Fish Health Inspectorate in the Weymouth Laboratory. These systems will assist inspectors in the differential diagnosis of species that parasitise salmonids and aid in the early detection of *G. salaris*.



■ Confocal microscope image of the anterior end of the parasitic worm *Gyrodactylus* demonstrating muscle actin and the flame cells of the excretory system.

Astrid Holzer, supported by the Austrian Academy of Sciences, has now completed the experimental work of her PhD study, which has focused on the myxozoan parasite *Sphaerospora truttae*. Her studies on the molecular relationships between *S. truttae* and myxozoans co-occurring within the same habitat have elucidated three new myxozoan life cycles. DNA sequencing studies have determined the phylogenetic positioning of the species found within the study environment.

Ichthyophthiriasis or whitespot disease, which costs the British trout industry close to £2 million p.a., remains an important area of research. A BTA funded study by Dr Shinn and Dr Wootten has already identified an efficacious in-feed chemotherapeutant against whitespot. The work continues with a recent DEFRA-BTA award and Nick Taylor will join the project to develop effective management strategies to control this pathogenic ciliate protozoan.

The Parasitology Laboratory has had another busy year with six scientists visiting to undertake collaborative projects under the European Commission's Access to Research Infrastructures Action of the Improving Human Potential Programme. This brings the total number of scientists visiting the Parasitology Laboratory under the EC programme to 22. Dr Edit Eszterbauer from the Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest made a return visit to continue her *in situ* hybridisation studies on fish-parasitic myxosporeans. Ivan Fiala from the Institute of Parasitology, Czech Academy of Sciences made his first visit to the Institute in June this year together with returning colleague, Roman Kuchta to study cestodes and myxozoans from marine and migratory fish from the North Atlantic. The Director of the Institute of Parasitology, Professor Tomas Scholz, also made two visits this year to investigate the morphological and genetic variability, geographical distribution and biology of Scottish populations of pseudophyllidean tapeworms. Dr Bahram Dezfuli and Ms Luisa Giari from the University of Ferrara, Italy also made visits under the EC Programme to undertake an immunohistochemical investigation of neuromodulators in the intestine of farmed and wild fish parasitised by specific protozoan and metazoan parasites.

## Vaccines and immunology

The Aquatic Vaccine Unit, now an active partner in the newly formed Scottish Fish Immunology Research Centre (SFIRC), continues to provide a platform for research in immunodiagnosics and vaccine development. Professor Sandra Adams heads the unit and the core staff currently comprises post-doctoral scientists Drs Kim Thompson and David Morris, and three technicians, Mrs Hilary McEwan, Mr Niall Auchinachie and Dr Rowena Hoare. Mrs Karen Snedden, who oversees the monoclonal antibody facility, is seconded as the project scientist for Aquatic Diagnostics Ltd, a spin-out company from the Institute of Aquaculture.

Proliferative kidney disease (PKD), caused by an unusual myxozoan parasite *Tetracapsuloides bryosalmonae*, continues to impose a severe restriction on rainbow trout farming in the UK. Immunological studies/vaccine development and the identification of novel chemotherapeutants for this disease are areas currently being addressed. Dr Morris is working on a 3-year DEFRA-funded project involving collaboration with Professor Secombes at Aberdeen University, the Moredun Research Institute in Edinburgh and Dr Steve Feist at CEFAS, Weymouth and is investigating the humoral immune response of rainbow trout to *T. bryosalmonae*. In addition, this work has identified an antigen that is common to a wide range of pathogenic myxosporean species.

Most of the other research in the Aquatic Vaccine Unit involves bacterial pathogens. The results from a recent project on pasteurellosis vaccine development, funded by the EC AIR programme, were presented at a workshop held by the project partners (University of Patras, Greece; University of Udine, Italy; Schering Plough Aquaculture and Selonda Aquaculture) in Udine this year. The vaccine developed on this project is currently being field tested, as is a vaccine against bacterial kidney disease (BKD) recently developed on a Link Aquaculture grant.

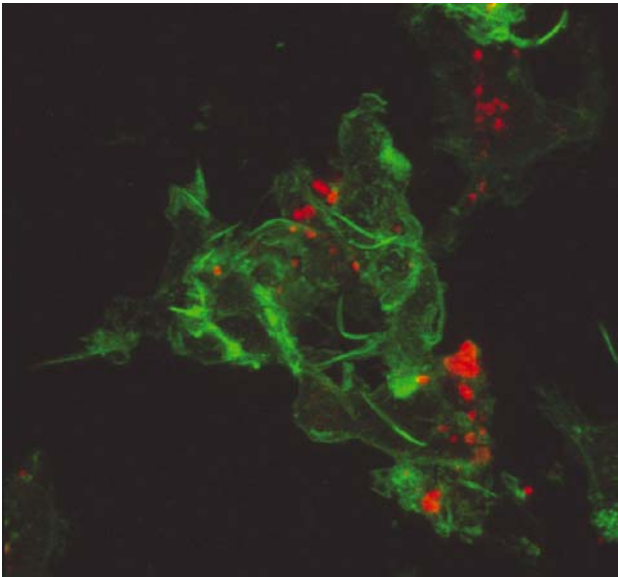
DNA-based diagnostic tests have now been developed in the Aquatic Vaccine Unit for the detection of a variety of fish pathogens, including *Flavobacterium psychrophilum*, *Piscirickettsia salmonis*, *Renibacterium salmoninarum*, *Tetracapsuloides bryosalmonae*, ISAV, IPNV and *Mycobacterium* spp. These PCR tests are being used in parallel with established antibody-based tests to detect pathogens in fish and the environment. *In situ* hybridisation methods have also been established for the detection of pathogen DNA in fixed tissue sections. The project on real-time PCR for the detection of mycobacteria, funded by an innovation grant from the University SURE Team to Professor Adams and Dr Thompson, is now completed.

The equipment purchased for the SFIRC (Bioplex and fluorescent activated cell sorter) has now been installed and will enable the development of novel multiplex assays for fish and the detection of different cell populations in fish. Bioplex assays are being developed/optimized for use in fish to detect immune responses, for the multiplex detection of pathogens (eg *Vibrio* species) and for viral epitope mapping (nodavirus). Simple assays for the detection of IgM are initially being used as model systems. As soon as anti-cytokine antibodies are available then multiplex assays will be set up to detect fish cytokines and these methods standardized with the Aberdeen laboratories.

The image analysis equipment purchased last year is being used in conjunction with 2D electrophoresis to facilitate vaccine development. This technology allows single proteins

to be fractionated from complex mixtures and subsequently analysed. The analysis can use MALDI-TOF fingerprinting or *de novo* sequencing. We are also currently analysing the protein components of fish sera to identify humoral factors that are up or down regulated during an immune response following infection.

Two PhD students successfully completed their theses this year; Dr Lorna Marshall on the effect of organic chromium on the immune response in fish, and Dr Sema Akinlar Yuksel on piscirickettsiosis. Two other PhD students are currently completing their theses; Ms Rubina Sharif on fish interferon and Mr Charlie Morris on PKD. Other students currently working on research projects in the laboratory include Mr Fuad Matori from Malaysia who is working on the characterisation of *Vibrio* species, Mr Charles McGurk from Scotland examining control strategies for PKD, Ms Una McCarthy from Ireland researching the detection and control of *Piscirickettsia salmonis*, Ms Farah Manji from Uganda working on rainbow trout fry syndrome and Ms Sandra Laffon Leal from Mexico investigating the response of fish to mycobacteria.



Confocal microscope image of salmonid cells infected with *Piscirickettsia* (red) showing the relationship with the actin cytoskeleton (green).

Visiting scientists to the Aquatic Vaccine Unit this year included Drs Galena and Zsigmond Jeney, from HAKI, Szarvas, Hungary who were funded by the EC Improving Access to Infrastructures Programme to work on sturgeon and carp immunology. A new British Council grant with partners at HAKI is funding research on the immune response of different carp lines. Two researchers from China also visited the laboratory. Dr Jinyu Shen from the Institute of Freshwater Fisheries, Shanghai worked on *Aeromonas hydrophila* vaccine development, while Dr Houbu Wu worked on the characterisation of aquatic *Vibrio* species. The Vaccine Unit was also host to Mr Tomasso Petoichi, from the University of Perugia, Italy who examined sera composition of rainbow trout with clinical PKD using 2D electrophoresis. During July/August this year, the Aquatic Vaccine Unit was host to two Nuffield bursary students from Dollar Academy who examined the parasites of invertebrates from Airthrey Loch under the supervision of Dr Morris.

Commercial activities include feed and vaccine trials in the Aquatic Research Facility, serology services in the Aquatic Vaccine Unit laboratory, and the licensing of antibody producing hybridoma cell lines to Aquatic Diagnostics Ltd.

The latter company was set up in December 2001 as a spin-out company from the University, following a SMART award to Professor Adams. The company markets monoclonal antibody probes to detect fish pathogens and immune responses in different species of fish. The antibodies were developed in the Aquatic Vaccine Unit over the last 15 years and the hybridoma cell lines producing the antibodies have been licensed to Aquatic Diagnostics Ltd with royalties paid to the University. Dr Thompson has been seconded part-time as Technical Director for the company. The Aquatic Vaccine Unit is also sub-contractor in a SMART award to 'Aqualife', in collaboration with the Department of Computing Science and Mathematics at Stirling, to develop an automated vaccination machine for salmon and cod.

## Virology

Infectious salmon anaemia virus (ISAV) and fish nodaviruses have continued to represent the main research areas of the virology laboratory under the direction of Dr Starkey and Professor Richards and with the support of staff from the Aquatic Vaccine Unit.

Research aimed at the development of real-time diagnostic procedures for ISAV has continued. This is a three-year study funded by the European Commission involving partners from Scotland, Norway and Canada. Infectious salmon anaemia has caused serious economic losses to the salmon farming industries of several countries including Scotland, Norway, Canada and the United States. Sensitive diagnostic procedures for ISAV are urgently required to identify and contain future outbreaks. Molecular diagnostic procedures developed in the virology laboratory permit the detection of specific virus targets during the process of nucleic acid amplification, without the need for time consuming analyses of reaction products inherent in conventional PCR methods. Real time RT-PCR and nucleic acid sequence based amplification (NASBA) procedures have now been developed for ISAV and in preliminary experiments both procedures have been found to be approximately 1000 fold more sensitive than the conventional RT-PCR methods currently used for ISAV detection. More rigorous analysis of the real-time methods involving analysis of diverse ISAV isolates from the UK, Norway, Canada and the Faroe Islands is in progress. Dr Kim Thompson has developed a bank of monoclonal antibodies against the virus using conventional methods, phage display and DNA vaccination.

An understanding of virus determinants recognised by protective immune responses is of crucial importance for the development of viral vaccines and immunodiagnostic reagents.

Miss Janina Costa, a PhD student funded by the Portuguese government is identifying B-cell epitopes on fish nodaviruses. A panel of murine monoclonal antibodies (Mabs) has been produced and categorised with respect to immunoglobulin class and neutralising activity. Determination of the regions of the nodavirus capsid recognised by these Mabs using phage display, and pepscan mapping using the recently developed luminex technology is ongoing.

A new PhD student, Gavin McKinley, has recently joined the virology laboratory to study the role of innate immunity in infectious pancreatic necrosis virus (IPNV) infection of salmonids. This project is run in collaboration with the FRS



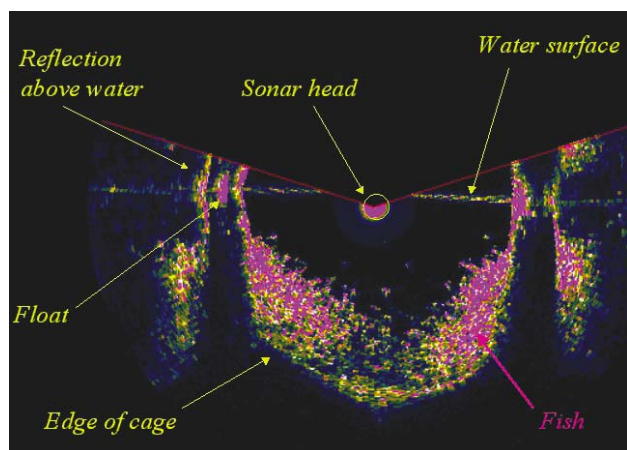
Marine Laboratory, Aberdeen. IPNV is currently the most serious viral disease affecting the UK aquaculture industry and associated losses incurred by UK fish farms have been estimated to be greater than five million pounds annually. The incidence of the disease is rapidly increasing. The aims of this project are to investigate the role of the interferon system in acute and persistent IPNV infection. The results of the project will facilitate the design of IPNV vaccines. Initial work is aimed at developing quantitative RT-PCR based assays for IPNV and fish interferon response genes.

A three-year project aimed at the development of vaccines for use in aquaculture has been completed during the previous year. This work was funded by Novartis plc, and is part of a collaborative project with researchers based at the University of Porto and the University of New Brunswick, Canada. Inactivated whole virus and recombinant protein-based vaccine preparations were produced at Stirling. Initial efficacy testing of these candidate vaccines has now been completed. The second phase of this project has now commenced, and is aimed at developing vaccines against a wider range of viruses affecting the aquaculture industry.

In the near future, the virology laboratory is to host a NATO Fellowship Funded project aimed at the isolation and characterisation of viruses associated with diseases of farmed tuna. Sampling of tuna farmed in Mediterranean waters was completed in 2003. Cell culture and molecular characterisation of samples will be performed in 2004.

### Epidemiology and welfare

During the last year the Institute has continued to be active in this area with a number of ongoing projects, publications and applications for funding.



■ An image of the space occupied within a marine Atlantic salmon production cage using scanning hydroacoustics. From LINK Aquaculture project SAL 18.

The DEFRA-BTA funded project carried out by Dr Jimmy Turnbull and Ben North investigating the effects of stocking density on the welfare of farmed fish in collaboration with CEFAS Weymouth is now in its third year. An initial trial investigating the effects of different stocking densities of rainbow trout in tanks with identical flow rates assessed fish welfare through the use of the following putative welfare indicators: haematocrit, cortisol, lysozyme activity, plasma glucose and measurements of fin erosion; overall results showed there to be no significant differences in growth and most other parameters over the 9-month period under the different stocking densities, provided that good water quality was maintained. Work is ongoing with a follow-up experiment that is investigating the effects of different rates of water

exchange on groups of rainbow trout stocked at the same density. A questionnaire survey of British rainbow trout farms was carried out earlier in the project and is now being backed up with farm visits and analyses of farm data where groups of fish have been followed in a series of different culture systems and at a range of stocking densities. A novel image analysis system has also been developed to measure fin shape and area under a variety of culture conditions. Collectively these data would suggest that providing certain water quality parameters are maintained then fish welfare will also be safeguarded.

Dr Turnbull, Dr Bron and Mr North have also been involved in a number of other activities relating to fish welfare including a DEFRA funded workshop, the RSPCA Freedom Foods steering group and a positioning paper on fish welfare for the Fisheries Society of the British Isles.

The second project to receive further funding is in collaboration with the Environment Agency on the epidemiology and pathology of category II non-native parasites in British freshwater fisheries. This project is attempting to develop a rational strategy to quantify the risks from these parasites and therefore the need for continued control. The first year's results were well received and the project has now received sufficient funding to allow Mr Williams to use the study towards a PhD.

Two DFID funded projects have completed their first phase during this year. The first, "The Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia", has accumulated a considerable body of information on the provision and uptake of aquatic animal health expertise in Asia and specifically the potential role of epidemiology. The project, led jointly by Dr Turnbull and Dr Chinabut of the Aquatic Animal Health Research Institute, Bangkok has also involved participants from Mangalore, India, Ho Chi Minh City and Can Tho, Vietnam and the Universities of Sussex and Liverpool in the UK. The first phase concluded with a meeting held in Stirling during August 2003 and attended by collaborators from all the participating institutes.

Dr Crumlish of Stirling and Ms Dung of Can Tho University, Vietnam, jointly lead a second project examining the causes, effects and control of a disease of farmed pangasius catfish in the Mekong Delta. This project, also involving Professor Young of the Department of Marketing and Dr Smyth of SAC Edinburgh is nearing completion and it is anticipated that the findings will be disseminated to farmers and other authorities in the Mekong Delta within the next six months.

### Diagnostics

A large number of submissions continue to be received from a range of countries. The development of new aquaculture species has led to a number of interesting cases. Vibriosis leading to dermal ulcerations led to continuing problems in cod. The serotypes of the bacterium involved are unusual, which may have implications for vaccine development. A further unusual condition observed in cod has been a pseudomonad ophthalmitis and cellulitis. In halibut a reovirus – like hepatitis has been an ongoing problem.

A number of notable conditions have also been observed in salmonids, including glaucoma in Atlantic salmon associated with low temperatures and mortalities associated with an interstitial nephritis caused by pre-spore myxosporean stages in brown trout. Filamentous bacterial enteritis continues to cause difficulties in rainbow trout.



## Reproduction and Genetics ▶ ▶ ▶

2003 has been a difficult year for this research group with the sudden death of Professor Niall Bromage in May. There have been many tributes paid to Niall by his friends and colleagues worldwide. As a group we hope to remember him by maintaining the quality and diversity of work on the reproductive biology and endocrinology of fish. At the time of his death Niall was still very active with many research students and projects, as well as maintaining his close links to the aquaculture industry.

Staff and postgraduate students in Reproduction and Genetics have continued to focus their attentions on the management of broodstock and the production of fish as a controllable and sustainable resource. This involves studies of the photoperiodic control of maturation, puberty and growth, the perception and sensitivity of fish to light, the mechanisms involved in sex differentiation and gender control and the development and management of programmes of selective breeding. All of these areas combine the use of the newer techniques of molecular biology with whole animal studies of physiology and function, in particular where they are relevant to commercial culture. A major thrust in recent years has been the application of this expertise to questions raised by the ever-expanding interests in new aquaculture species and species diversification, particularly with regard to marine fish.

### Marine fish and maturation

Professor Bromage, Dr Porter and Andrew Davie, a NERC funded PhD student, completed two separate grants in 2003, from the British Marine Finfish Association (BMFA) and Crown Estate Commission (CEC), for work on the photoperiodic control of growth and maturation in the Atlantic cod and Atlantic halibut. The project on cod was supporting a long-term photoperiod study based at the Machrihanish Marine Environmental Research Laboratory, which ended in July 2003. Preliminary analysis of the results is very promising. Due to the success of the Machrihanish Marine Farm cod hatchery in 2003, Andrew Davie has been able to test research conclusions from the initial cod photoperiod studies at a number of commercial facilities across the UK.

The team is also investigating the effects of spectral composition and light intensity on melatonin production in cod and other species; melatonin levels change in response to light and dark and hence are able to provide a most useful and practicable tool for the assessment of how fish see light. This work is carried out in collaboration with the Institute of Marine Research (IMR) in Bergen, Norway. There have also been study visits to Stirling by the Bergen group with the support of the EU Improving Access to Research Infrastructures programme.

Andrew Davie, Dr Porter & Professor Bromage continued their long running collaborations with the Seafish Industry Authority Ardtoe marine farm unit in conjunction with Dr Carlos Mazorra. A one year investigation into seasonal melatonin profiles in Atlantic halibut and haddock was completed in Spring 2003. Due to the preliminary results, a two year investigation of photoperiod manipulation of growth and maturation in haddock was initiated in January 2003 and included the newly appointed Dr Herve Migaud. It is hoped to test the conclusions of this trial on a commercial scale in Canada, starting in 2004.

In October 2002, a 3-year project funded by the EC 5th Framework Programme started and Dr Herve Migaud was appointed as a postdoctoral research fellow. This project aimed to investigate the photoperiod control of puberty in farmed fish and to develop new techniques and research into underlying physiological mechanisms. This involves collaboration with colleagues in France, Spain, Norway and Holland. It also involves support from the British Trout Association (BTA) and work with a number of salmon farms. The overall objective is to develop improved photoperiod protocols for delaying first sexual maturation (puberty) in commercially farmed European fish species by improved knowledge of the mechanisms of activation of the brain-pituitary-gonad (BPG) axis during puberty in fish, improved understanding of the importance of differences in light intensity and spectral quality in affecting the BPG axis and the initiation/postponement of puberty, by assessment of pineal melatonin production *in vitro* and *in vivo* in salmon and sea bass and improved understanding of the interactions between photoperiod protocols, genetic background and adiposity in arresting/promoting puberty in these species. In Stirling, we are investigating sensitivity of the pineal melatonin production to light of different intensity and spectral composition *in vitro* and *in vivo* in order to determine the minimum amount and spectral composition of artificial light to be superimposed on the ambient light for delaying puberty in Atlantic salmon and sea bass.

### Photoperiod and growth

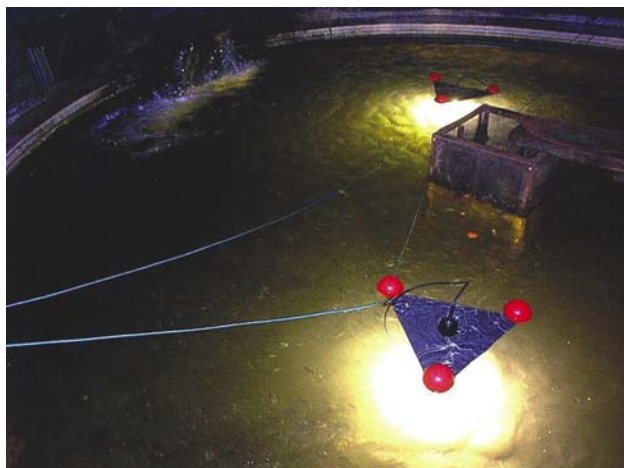
Funded by grants from NERC (ROPA Award), the BTA and a Broodstock Research Club to Professor Bromage, John Taylor is finishing his project on the influence and interactions of photoperiod, growth factors and energetic status on growth and reproduction of rainbow trout. Findings from these studies have led to the application and investigation of constant light photoperiod regimes under a variety of commercial fish farming systems. These techniques are providing a simple but effective means of improving farm production with significant improvements observed in specific growth rate and feed conversion. Currently, further work is being undertaken in order to examine new lighting technologies in an attempt to reduce numbers of light units required, as well as improve feeding regimes to further benefit from increased production. A recent innovation has been the establishment of a new assay for one of the growth factors involved in the transmission of information on day length to the growth axis of fish. This is a most important development for it should enable us to understand how photoperiod and growth interact in the control of maturation.



☐ Floating lights used in trout raceway

Further research is also being undertaken to validate the use of a variety of mammalian assays in studying the role of energetic and appetite hormones in the control of puberty.

Funded jointly by Nutreco Aquaculture Research Centre (Marine Harvest) and the Faculty of Natural Sciences from September 2002, Matthew Sprague is investigating, under the supervision of both Gordon Bell and Herve Migaud, the variability in the growth and feeding responses in salmonid fish to photoperiod manipulations, focusing around the commercial farming difficulties associated with the use of lights on cages. Within the salmon farming industry there remains a high degree of scepticism over the use of lights and their effects on growth and maturation. The overall aims of the research project are to study the effects of light (quality, quantity and photoperiod) on growth and maturation and on the short- and long-term feeding responses. Currently, farmers report a characteristic "growth dip" that often occurs in some, but not all, salmon populations following the onset of lights and investigations are now underway to understand whether husbandry factors or physiological responses are the cause.



● Floating light used in trout tank system

### Photoperiod and maturation

Iain Berrill has recently been awarded his PhD on the environmental influences on growth, precocious maturation and smoltification in Atlantic salmon, supported by a NERC CASE award to Niall Bromage and Mark Porter in collaboration with Marine Harvest Scotland. An initial commercial scale photoperiod project that highlighted the importance of the early developmental period in triggering early maturation, was further investigated through analyses of commercial data. Further trials investigating the effects of feed ration and dietary lipid level were also completed at the Institute of Aquaculture's Buckieburn Freshwater Research Laboratory. This work has provided important information on the multifactorial control of early maturation in salmon.

Antonio Campoiz-Mendoza with the support of the Mexican Government has continued his work on the molecular basis for the photoperiod control of reproduction in tilapia. He has recently successfully sequenced the melatonin receptor gene and is now completing his Ph.D by investigating its change in response to photoperiod stimulation.

### Seasonality and immunology

With the support of a NERC CASE award, supervised by Dr Kim Thompson and initially Dr Mark Porter and Professor Niall Bromage, and currently Dr Herve Migaud in collaboration with

EWOS, Alison Morgan is investigating the effects of seasonality on the immune response in the rainbow trout. Seasonality dominates the life history of fish, it co-ordinates reproductive activity, affects body weight and condition, food intake and locomotor activity and it co-ordinates the immune system. The effects of photoperiod and melatonin administration on the immune system have been studied through both winter and summer trials carried out at the Buckieburn Freshwater Research Laboratory. Results to date suggest that there is a significant correlation between season and the immune system. Melatonin was also found to significantly enhance phagocytosis in June and fish fed an immunostimulant diet had a significantly higher level of survival following challenge with *Vibrio anguillarum*. Recently, results have suggested the immune system of rainbow trout exhibits a circadian rhythm.

The DFID AFGRP-funded project on genetic management and improvement of exotic (i.e. non-indigenous) carp species in Asian aquaculture, awarded to Dr David Penman and Professor Brendan McAndrew with colleagues from UW Swansea, BFRI and DOF Bangladesh, the University of Agricultural Sciences, Bangalore, India and RIA 1, Vietnam has now entered its last year. The major thrust in this period is towards translating the project results into management strategies that can be used to sustain and improve the genetic quality of the stocks of carps, and to disseminate good quality stocks widely in the countries concerned. In India, a selective breeding programme for common carp stocks in Karnataka has been developed and implemented, while in Bangladesh, a strategy to integrate management and dissemination of good quality stocks is being developed, with the first stage already adopted by the government.

Almas Ara Gheyas is in the second year of a PhD, working on applications of molecular genetic markers to management of aquaculture stocks of carps in Bangladesh. Microsatellite DNA markers will be used, for example, as genetic tags for family identification in an experiment to estimate heritability for harvest weight in the silver carp stock held by one of our collaborating institutes in Bangladesh.

### Selective breeding in European aquaculture species

A major area of current and future research initiated principally by Professor McAndrew, is the use of genetic molecular markers in the management of selective breeding programmes for a number of farmed fish, including salmon, trout, sea bass, halibut, sea bream and cod.

The LINK aquaculture project on the selective improvement of rainbow trout has been analysing the levels of molecular variation in UK rainbow trout strains using microsatellite loci.

The project is in collaboration with the BTA and Professor John Woolliams of the Roslin Institute. Two different multiplex systems using different microsatellite loci have been developed and tested. These have been used to genotype all the broodstock involved in a number of breeding programmes in the winter of 2002. The offspring from the broodstock have been ongrown under commercial conditions. Over 600 different families have been constructed in five different rainbow trout strains and more than 6000 5g fingerlings have been PIT tagged and are being genotyped to identify their family assignment. These fish will be analysed for a range of

growth parameters through to processing. The fish have been exposed to a very severe natural PKD challenge during the year and samples collected from mortalities will be genotyped to look for evidence of innate resistance to this parasite in UK trout strains.

Jose Ureta Schmidt is in the third year of a PhD looking at the potential for selective improvement in rainbow trout. Jose has been using genetic fingerprinting to look at growth and disease resistance in specially constructed commercial populations of trout under normal production conditions. This will enable him to assess individual and family values for the traits being studied. Information on these genetic parameters will be made available to the farms so that they can select the best fish for breeding under their given conditions.

Cameron Brown has recently been awarded a PhD for his work on the genetic improvement of the gilthead seabream in Cyprus in collaboration with Alkioni Sea Farms. This protandrous hermaphrodite species presents a number of problems when it comes to designing broodstock management and improvement programmes. Cameron has used microsatellite loci to monitor the numbers of fry produced by differing broodstock populations. This work has shown that less than 30% of the individual broodstock contribute offspring, which has longterm implications for the design of broodstock replacement programmes if inbreeding is to be avoided. The company has been recommended a selective improvement programme using a combination of genetic fingerprinting and PIT tagging that minimises inbreeding and maximises genetic gain in this species.

Anu Frank Lawale is a Teaching Company Scheme associate working with Otterferry Sea Farm in Argyll. Anu has been using a combination of genetic fingerprinting and PIT tags to develop a broodstock replacement programme for Atlantic halibut. Early results suggest that there has been a high level of domestication selection that has reduced the potential number of families available as replacement broodstock. Detailed genetic analysis of growth data produced over three years from PIT tagged halibut is being analysed to look for potential genetic differences in growth rate and age at sexual maturity.

The EC funded BASSMAP project has completed its second year. Dr John Taggart has constructed a bacterial artificial chromosome (BAC) library for seabass which will contain at least six copies of the bass genome divided up into 150 kilobase pair lengths contained in a bacterial vector. To make this library more useful German collaborators took a sample of each of the 69,000 bacterial colonies grown separately and transferred these onto nylon filters. These have now been distributed to the other partners in Belgium, Crete and Portugal. Dr Taggart has now moved to the BBSRC funded TRAITS programme and has been replaced by Dr Helen Whitaker. Helen has begun to identify expressed sequence tags (ESTs) from seabass cDNA libraries. These will be probed to the microarray filters to identify BAC clones containing these genes. These will then be added to the gene map being developed by all partners.

Professor Teale continues to lead the molecular genetic research on fatty acid synthesis, conducted in close collaboration with the Nutrition Group. The most notable advance has been the cloning and functional characterisation by Dr Xiaozhong Zheng and Ms Cathryn Dickson of a functional delta 6 fatty acid desaturase of Atlantic salmon. Having this gene in hand, together with the delta 5 desaturase gene cloned by the group previously, will

enhance studies of variation in fatty acid synthesis in salmon and thus constitutes a critical step towards selection for improved polyunsaturated fatty acid synthesis in this important cultured species. Genomic analyses by Dr Zheng have also established that Atlantic salmon almost certainly have more than two polyunsaturated fatty acid desaturase loci, and work will continue on their characterisation, and also on control of gene expression in response to dietary signals. Work carried out in collaboration with INRA-IFREMER, St-Pee-sur-Nivelle, France led to the cloning and first functional characterisation of a fatty acid desaturase of a marine species, the gilthead sea bream. This achievement was made possible by the visit to the Institute of Iban Seilliez of INRA-IFREMER to work with the group and opens the way to detailed molecular studies of fatty acid synthesis in marine species.

### **Molecular analysis of commercial traits in Atlantic salmon**

A new four-year project known as TRAITS (Transcriptome Analysis of Important Traits in Salmon, [www.abdn.ac.uk/sfirc/salmon](http://www.abdn.ac.uk/sfirc/salmon)), funded under the Exploiting Genomics Programme of the Biotechnology and Biological Sciences Research Council, was initiated in March 2003 with a grant to Professor Alan Teale, Dr Douglas Tocher and Dr John Taggart. The project is being undertaken in partnership with the Universities of Aberdeen and Cardiff, and with strong collaborative ties to ARK-Genomics at the Roslin Institute and the Norwegian Salmon Genome Project, University of Oslo. The objectives are to examine the transcriptomics of fatty acid synthesis, protein metabolism, immune response and smolting in Atlantic salmon with the purpose of developing simple molecular genetic tools with which farmers and biologists can monitor these key physiological functions in farmed and wild salmon populations. Dr Taggart will manage the research in Stirling with the focus on fatty acid synthesis transcriptomics. To-date, efforts have been concentrated on supplementing existing genetic resources with genes that respond to signals triggering trait-associated changes in target organs. In the coming year microarrays will be constructed and used to interrogate samples that are currently being collected from appropriate populations.

Under the supervision of Dr Taggart and Professor Teale on a Natural Environment Research Council Studentship, Karen Frake will make use of molecular approaches to study the genetic contribution of pre-smolt sexually mature Atlantic salmon males in populations breeding in the Dee system of NE Scotland. This work establishes a collaboration with Alan Youngson and Eric Verspoor of the Fisheries Research Services Freshwater Laboratory, Pitlochry, and John Webb of the Atlantic Salmon Trust.

### **Antibiotic resistance in aquaculture**

During the year Dr Kerry Bartie and Ms Cathryn Dickson have continued to lead the molecular genetics efforts of the EC-funded project *Asiaresist* ([www.medinfo.dist.unige.it/asiaresist](http://www.medinfo.dist.unige.it/asiaresist)). This project is examining the extent and nature of resistance to antibiotics in aquaculture environments in South East Asia, and the risk it poses to consumers and farmers. The project is a collaborative effort involving partners in the University of Can Tho in Vietnam, University Putra Malaysia, AAHRI in Thailand, the University of Ghent in Belgium and RILAB in Genoa. The project has now made a large collection of antibiotic resistant bacterial isolates in all three SE Asian countries, and molecular typing in Stirling has been a key component of the identification process. Work has also been initiated on characterisation of the antibiotic

resistance genes carried by selected isolates preparatory to studies of antibiotic resistance transfer between bacteria to be conducted in the coming year.

### Sex control and molecular analysis of tilapia

Dr Simon Harvey left the Institute in April 2003, after working for three years on a BBSRC-funded project on molecular-cytogenetic analysis of sex determination in tilapia, awarded to Dr David Penman and Professor Niall Bromage and Dr Darren Griffin of Brunel University. Ann Gilmour will continue to work on an extension to this project, which will finish in October 2003. This project has significantly advanced our understanding of sex determination and the evolution of sex chromosomes in tilapia. The main sex determining gene in *O. niloticus* is located in the middle of the long arm of chromosome 1, with differences between the developing X and Y chromosomes seen principally as differences in the copy number and distribution of transposable elements. This appears to mediate delayed pairing of the X and Y chromosomes during meiosis. The sex determining gene has now been mapped into the *O. niloticus* linkage map, through collaborative research with Professor Tom Kocher of the University of New Hampshire, giving a series of polymorphic DNA markers linked to this sex determining gene. We are currently physically mapping a variety of DNA sequences onto the *O. niloticus* sex chromosomes and other chromosomes and extending research on sex determination to other tilapia species.

Chuta Boonphakdee is nearing the end of the third year of her PhD, the main focus of which has been the identification and physical mapping of sex-linked and potentially sex-determining genes and DNA sequences in the tilapia genome. It is hoped that such markers can be used, for example, to compare the structure of the sex chromosomes between species within this group.

Jose Cuitlahac Mota Velasco Gallardo is also in his second year, working on physical and genetic mapping of traits of commercial interest in tilapia. Part of his research focuses on physical mapping of markers from different linkage groups (using BAC clones containing these markers as FISH probes), which allows integration of the linkage and physical maps for *O. niloticus*.

F<sub>1</sub> crosses between the fully inbred clonal lines of tilapia that we have developed over the last few years are being evaluated as control lines in a selective breeding programme, in a DFID CRF-funded project with ICLARM and FRI, Malaysia, UW Swansea and CLSU, the Philippines.

Sayema Sayeed completed her Ph.D on the immune responses and disease resistance in clonal lines of Nile tilapia. This work was jointly supervised by Dr Sandra Adams and Dr Kim Thompson of the Aquatic Vaccine Unit together with Dave Penman and Brendan McAndrew and showed clear and repeatable differences in the level of the specific immune response observed between different clonal lines of tilapia. Challenges using *Aeromonas hydrophila* on the differing lines showed that immune response was inversely related to mortality with highly significant differences in survival between the lines. The clonal lines have proved a useful model for assessing the genetic basis of disease resistance and future work is planned.

## Nutrition ► ► ►

### Lipid nutrition

Lipids are the major focus of the Nutrition Group with research continuing into the requirements, metabolism and function of polyunsaturated fatty acids (PUFA) in aquatic organisms. The current situation of, at best, stagnant and, at worst, declining global supply of the principal ingredients of aquaculture diets, fish meal and, particularly, fish oil, dictates that alternatives to these traditional dietary components must be developed for the aquaculture industry to continue to expand. The primary focus of the Nutrition Group is the development of new diets for cultured fish species, including salmonids and marine fish, that are sustainable, environmentally friendly and cost effective but which do not compromise the final product as a high quality food providing high levels of the important and beneficial n-3 highly unsaturated fatty acids (HUFA) for the human consumer. These aims are central to the theme "Fish as Food for Humans" but also relate to the other major themes in the current Institute Strategic Plan including "Aquaculture Development and Sustainability" and "Welfare of Farmed Fish". In consequence, a major part of the research programme has focussed on investigating the effects of substitution of fish oil with vegetable oils in the diets of farmed fish, particularly Atlantic salmon.

### Atlantic salmon nutrition

The four year EC FP5 project, "Researching Alternatives to Fish Oil in Aquaculture (RAFOA)" is in its third year and progressing well. The first phase involved feeding post-smolts graded levels of linseed oil (LO) until they reached 2 kg, followed by a six month washout period where fish fed all diets were returned to a diet containing fish oil (FO). The fish attained a final weight of ~4.5kg after the washout phase and there were no differences due to previous dietary treatment. The inclusion of LO did not affect growth rate or feed conversion but deposition of fatty acids in the flesh and liver was closely correlated with dietary fatty acids. Flesh n-3HUFA levels were reduced with each addition of LO such that values in salmon fed 100% LO were around 30-40% of values in fish fed FO, whereas flesh C<sub>18</sub> PUFA levels were considerably increased. Flesh lipid content was not affected by dietary treatment but liver lipid was significantly higher in fish fed high LO. Deposition of astaxanthin was not affected by dietary LO level. Hepatic HUFA synthesis activities were increased with inclusion of LO but activities tended to reduce with increasing age/size of fish. Prostaglandin production was reduced in salmon fed higher levels of LO and this was related to reductions in tissue arachidonic acid (ARA). In addition, some parameters of the non-specific immune system were negatively affected by LO inclusion which may be related to ARA levels and prostaglandin production. Samples of fresh and smoked salmon were analysed by trained taste panels and, in addition, by an "electronic nose" which detects differences in volatile taste/aroma components in the flesh. Fish fed LO could be detected by both methods but, in general, the changes were positively received by the consumers. During the washout phase it was easier to "wash in" n-3HUFA than to wash out the C<sub>18</sub> PUFA. However, although there were still differences between the fish fed the different LO diets pre-wash/out the flesh values for n-3HUFA were restored to 80% of the values in fish fed FO throughout.

In the second phase of the RAFOA project, a diet containing a blend of vegetable oils (linseed, rapeseed oil (RO) and palm oils) and FO (75/25 w/w) were fed to replicate groups of



salmon from first feeding through to harvest, followed by wash out. The blend of vegetable oils was chosen to match the ratio of saturated, monounsaturated and polyunsaturated fatty acids found in fish oils. The trial was begun in March 2002 with first feeding fry and no differences in growth were seen up to seawater transfer in April 2003.

- ▶ The combined effects of replacement of FO with vegetable oil (VO) and high energy diets has been studied in a project funded by the NERC LINK Aquaculture programme, "Fish Oil Substitution in Salmonids (FOSIS)". Four diets were fed to salmon fry from late March 2000 following yolk-sac absorption through to harvest at 2kg after which sub-groups from all treatments were fed a high-energy FO-containing commercial diet for a further 6 months. The four diets were low fish oil (LFO), high fish oil (HFO), low vegetable oil (LVO) and high vegetable oil (HVO). The VO was a blend (1:1 w/w) of RO and LO. This project finished in March 2003 and the achievements of this highly successful project are summarised below:
- ▶ Final weights were significantly lower in fish fed the low oil diets, LFO and LVO. SGR values were similar in all groups and appropriate for their developmental stage. FCR values were highest in fish fed LFO and lowest in fish fed HFO. Flesh lipid content varied with respect to dietary lipid and liver lipid was significantly elevated in the VO groups although no pathologies were evident. Flesh n-3HUFA values were reduced to ~30% of the values in fish fed HFO, in fish fed HVO, while values for C<sub>18</sub> PUFA were increased by up to 20-fold in the HVO fish. Flesh astaxanthin was significantly higher in fish fed HFO compared to the other three treatments. Liver fatty acid desaturase activities were increased in fish fed VO although it is likely that the ability to produce EPA, and especially DHA, from 18:3n-3 is negligible, suggesting that these n-3HUFA will still need to be provided in the diet to meet requirements for normal growth and development. Plasma and gill cell prostaglandin production was reduced in fish fed VO and this was correlated with reduced tissue levels of ARA. Although some elements of the non-specific immune system and *in vitro* assays of macrophage activity were depressed in fish fed VO, a challenge trial with *Aeromonas salmonicida* suggested that the ability of fish to respond to a pathogenic challenge was not impaired by feeding VO. Taste panel assessment of fresh and smoked salmon showed that fish fed the HVO diet were preferred for three, and equal to HFO fish in one, of the assessment parameters in fresh fish, while in smoked salmon, HVO fish were preferred in two of the six parameters and were equal with HFO for a further three assessment parameters. Fish from each treatment were fed the HFO diet for 24 weeks to follow restoration of fatty acid compositions in the flesh. After 24 weeks n-3HUFA values were restored in fish fed HVO to 80% of values in fish fed HFO throughout, while C<sub>18</sub> PUFA remained somewhat elevated.
- ▶ Additional funding was obtained from HIE and the Highland Council with matching funding from the five industrial partners in the LINK project, namely BioMar Ltd, Ewos Innovation, Marine Harvest, Skretting and Uniq Foods Ltd, to conduct dioxin and PCB analysis of the flesh samples from the FOSIS trial. Replacement of dietary marine FO with VO reduced flesh dioxin concentrations to 25% of values in fish fed HFO diets. Following the restoration of HFO diets to all treatments for 6 months, flesh dioxin values in fish

previously fed HVO were 37% of those in fish fed HFO throughout. For dioxin-like PCBs the equivalent values were 41% and 62% in HVO fish before and after re-feeding the HFO diet.

The Nutrition Group forms an integral part of a European thematic network, "Fish Oil and Meal Replacement (FORM)" funded by the EC FP5 that will run for four years from January 2003.

The network comprises a cluster of FP5 programmes investigating aspects of fish oil and meal replacement including RAFOA and FPPARS (see below), as well as PEPPA (Perspectives of Plant Protein Use in Aquaculture), PUFAFEED (Single Cell Oil Substitutes) and GUTINTEGRITY (Gut Function in Vegetable Oil Substitution). The Network's remit is to address the following specific issues; (a) alternative feed resources – influences on feed consumption, protein and lipid metabolism, physiological changes and fish quality, (b) feed resources – with special focus on genetically modified ingredients and/or ingredients derived from genetically modified plants or micro-organisms vs genetic modification of the produced species themselves, (c) seafood safety – with special focus on dioxins and other potential hazardous substances and (d) healthy eating – with special focus on product tailoring. The first meeting was held in Santorini, Greece in May 2003. This meeting was attended by representatives from the five projects with additional representatives from EC DG14, the fish feed industry and fish producers, as well as retailers and scientists working on human health and nutrition.

#### Molecular studies

In addition to the dietary trial led projects, the lipid nutrition group continues to investigate the basic mechanisms underpinning the applied studies through biochemical and molecular studies aiming to identify and characterise the factors controlling and regulating lipid and PUFA metabolism in fish. An ultimate aim of this work is to provide the basic information, methodologies and tools necessary to develop a novel approach to the design of new diets. This approach is based on the identification and quantification of traits at a genotypic level that are important in determining significant phenotypic responses to nutritional change.

The expression of fatty acyl desaturase and elongase genes in salmon fed VO diets has been studied through real time quantitative PCR (Q-PCR) and mini arrays. RNA was isolated from livers of fish fed graded levels of LO in the RAFOA dietary trial I, and expression of fatty acid  $\Delta 5$  desaturase and elongase genes were determined by quantitative real time PCR, and the ratio of the copy number of the target gene against that of a commonly used housekeeping gene,  $\beta$ -actin, calculated. The results showed that expression of both the  $\Delta 5$  desaturase and elongase genes in liver was increased in a graded manner by increasing dietary LO. Expression of both genes was positively and negatively correlated with dietary 18:3n-3 and n-3HUFA, respectively. The expression of the genes, particularly the elongase, was correlated with the activity of the HUFA biosynthetic pathway and elongation activity reflected the overall activity of the HUFA biosynthetic pathway to a greater degree than  $\Delta 5$  desaturation activity. The salmon desaturase and elongase genes were also included in a mini-array, prepared by colleagues at the NIFES Institute of Nutrition, Bergen, Norway, of around 70 cDNAs of genes isolated from salmon and known to be involved in lipid metabolism. Liver RNA samples from salmon fed graded

levels of RO in the RAFOA trial run in Norway, or LO in the trial run in Scotland, were screened and the fatty acid  $\Delta 5$  desaturase was identified as the gene showing the highest level of differential expression, with increased expression in fish fed 100% RO or LO compared to fish fed 100% FO. These studies are elucidating the mechanisms that underpin the nutritional regulation of fatty acyl desaturation and elongation in response to feeding VO and will facilitate identification of the molecular differences in the pathway between freshwater/salmonid fish, which can thrive on diets containing VO, and marine species which must have significant levels of dietary FO for optimum growth and survival.

The *in vivo* assay using a deuterated tracer which was developed by Michael Bell to measure the rate of formation of DHA from 18:3n-3 has continued to give valuable information on the metabolism of these fatty acids in fish. Trout reared on VO-based diets lacking long-chain n-3HUFA, conditions under which the biosynthetic pathway is fully induced, showed rapid changes in the ability to synthesise DHA during early development. Biosynthetic ability increased rapidly from first-feeding to peak in fish of 1-2 g weight followed by a rapid, almost exponential, decrease thereafter. Trout reared under this dietary regime were unable to maintain their body concentration of DHA. This finding has considerable implications for attempts to substitute fish oils for vegetable oils in salmonid diets. Specifically, fish reared on VO cannot convert 18:3n-3 to DHA at a sufficient rate to accumulate DHA in neutral lipid and therefore give flesh with the high n-3HUFA content desired by consumers.

In these experiments most of the dietary 18:3n-3 was catabolised, as is the case in many other species including humans. Fish reared on the VO diet were twice as fatty as those reared on an equivalent FO diet. This is an undesirable effect of high VO diets and the mechanism by which this arises is unknown. This observation is very relevant to work within the group which seeks to identify the factors controlling lipid homeostasis. In addition to identifying that much dietary 18:3n-3 was oxidised, the *in vivo* studies also identified intestinal tissue, particularly pyloric caeca, as an important site of HUFA synthesis.

The feeding studies with deuterated 18:3n-3 have also allowed us to determine the deposition of newly synthesised DHA and all the intermediate n-3 PUFA in different tissues and in the different phospholipid classes. Low amounts of intermediate PUFA were found in liver and caeca, two tissues actively involved in DHA synthesis, with most tracer present as DHA from early time points. The pattern of labelled PUFA in blood reflected that in liver and caeca closely. However, central nervous tissue showed an accumulation of pentaene fatty acids (20:5n-3 and 22:5n-3) suggesting that the final steps of DHA synthesis were slow in these tissues. Adipose tissue showed far greater accumulation of initial substrate than the other tissues examined.

Understanding the mechanisms whereby fish regulate lipid homeostasis, that is the regulation of various lipid biosynthetic and catabolic pathways in specific tissues, is particularly relevant to the optimal formulation of aquaculture diets.

Over the last decade it has become apparent from studies on lipid dysregulatory states in human conditions, such as diabetes and cardiovascular disease, that a family of protein transcription factors, the peroxisomal proliferator-activated receptors, or PPARs, are critical to the regulation of mammalian lipid homeostasis. Following research grants from the BBSRC and the EC FP5 (FPPARS), Drs Mike Leaver, Douglas Tocher and Tariq Ezaz have, along with collaborating groups in Greece and Spain, undertaken a functional study of fish PPARs. The results of these studies have shown that plaice, sea bream, sea bass and Atlantic salmon, in common with mammals, possess genes for three PPAR subtypes, designated PPAR $\alpha$ , PPAR $\beta$  and PPAR $\gamma$ . Interestingly, work in Stirling has shown that Atlantic salmon have two PPAR $\beta$  isoforms, the significance of which awaits further studies. The PPARs are expressed in fish in a highly tissue specific manner, with PPAR $\alpha$  predominant in liver and heart, PPAR $\gamma$  predominant in adipose tissue and spleen, and PPAR $\beta$  having a broad and possibly ubiquitous pattern of expression. These patterns of expression are in agreement with the proposed roles for mammalian PPARs. PPAR $\alpha$  is required for the ability of tissues such as heart and liver to adapt to conditions where high levels of  $\beta$ -oxidation are desirable for energy generation. In contrast, PPAR $\gamma$  appears to be necessary for the development of correctly functioning adipose tissue and hence lipid storage. PPAR $\beta$  has an essential, although as yet uncharacterised role in many tissues. A particularly interesting characteristic of PPARs is that they are only activated to functional gene regulatory factors after binding fatty acids and other structurally related compounds. One aim of our work, now nearing completion, was to determine the lipid activation profiles and DNA binding characteristics of fish PPARs. These results indicate that PPAR $\alpha$  from all of the fish species tested has a broader unsaturated fatty acid activation profile, and also binds to a broader range of potential DNA sequences present in the regulatory regions of various genes, than either PPAR $\beta$  or PPAR $\gamma$ . Furthermore, specific fatty acids particularly potent in activating fish PPAR $\alpha$  were identified, and these observations formed the basis of *in vitro* experiments to investigate fatty acid- and PPAR-dependent gene expression profiles in isolated fish cells. Currently, we are undertaking *in vivo* dietary trials using diets formulated on the basis of the molecular genetic data summarised above.

Linked to the work on PPARs, a further project was recently initiated to study the effects of dietary conjugated linoleic acid (CLA) in Atlantic salmon. CLA is an unusual fatty acid that has been shown to have several positive health benefits including decreasing body fat and increasing lean body mass, improving feed efficiency, protection against atherosclerosis, enhancing immune function, and anticarcinogenic properties. Recently, CLA has been shown to be a powerful PPAR agonist suggesting PPARs may be involved in the mechanism of CLA action. Therefore, the specific roles of CLA as a potentially very useful dietary supplement in fish diets, an agonist of PPARs critically important in the regulation of lipid and fatty acid metabolism in fish, and as an immune system effector are being investigated in a BBSRC-funded studentship in collaboration with Professor Sandra Adams of the Aquatic Vaccine Unit of the Institute, with additional support from BioMar Ltd, Grangemouth, Scotland. Mr Sean Kennedy has recently been appointed to the studentship, and a large dietary trial initiated at the Marine Research Station, Machrihanish, investigating the effects of dietary oil content and graded levels of CLA on growth, lipid metabolism and immune function in Atlantic salmon post-smolts in sea water.

## Marine fish nutrition

While salmon nutrition is the primary focus of the Nutrition Group, marine fish nutrition is an important area that will undoubtedly expand in the near future. Two projects currently address the role of ARA in marine flatfish nutrition. The importance of providing optimal broodstock nutrition for obtaining maximum egg and larval quality is well established as is the importance of both EPA and especially DHA in early larval development. However, the importance of the third bioactive essential fatty acid, ARA, has been neglected. In a PhD project undertaken by Emma Alorend and supervised by Dr Gordon Bell and the late Professor Niall Bromage, the importance of ARA in egg and larval quality is being investigated at Otter Ferry Seafish, jointly funded by the British Marine Finfish Association, The Crown Estates Commissioners, Trouw Aquaculture and Otter Ferry Seafish. This project is now in its final year and data collected so far indicates that supplementation of broodstock diets with 0.4% ARA leads to improvements in egg and larval quality. This research has led to the development of a new range of broodstock feeds by Skretting, marketed under the product name Vitalis mix®. However, the importance of ARA in fish nutrition in general has been somewhat neglected. Recent studies have confirmed a role for ARA in the culture of marine fish larvae, especially with respect to early growth rates and stress resistance. Thus, the role of ARA in on-growing commercial diets, containing ARA-rich triglyceride oil produced by fungal fermentation, is being investigated in juvenile turbot by Gordon Bell in a study sponsored by DSM Food Specialties, Delft, The Netherlands. No differences were seen in growth, SGR and FCR in fish fed diets containing 0-0.4% ARA but those fed 0.8% ARA showed reduced weight gain and SGR.

## Alternative feed ingredients

Dumping of highly nitrogenous waste from fish processing factories can place an unacceptable burden on water quality. Recently EU restrictions on the dumping of organic waste have been tightened and new methodology to recover the waste from shrimp and prawn processing has been developed. In pilot-scale facilities in Norway and Germany waste material from prawn and shrimp processing has been concentrated into a Protein Omega Concentrate (POC) which is rich in protein, essential fatty acids and carotenoids. An EC-funded CRAFT project, BYPROFEED, will use POC collected and concentrated by different techniques and test its suitability for use as a replacement for fish meals, FO and carotenoids in commercial aquaculture feeds. This two year programme involving Gordon Bell and Fiona McGhee will study the inclusion of POC in feeds for Atlantic salmon parr, Arctic char, rainbow trout, halibut first feeders, and lobster juveniles. The Norwegian product, derived mostly from *Pandalus borealis*, is rich in carotenoids, mainly esters of astaxanthin. The dried POC product contains ~350mg astaxanthin/kg and should be a useful raw material for pigmenting salmonid flesh. This product should be highly suitable for inclusion in organic feed formulations. While the German POC product, derived from *Crangon crangon*, is much lower in carotenoids it should still provide important taste attractant properties. Both POC products are currently being tested on the species mentioned above.

The Nutrition Group are also collaborating with the Institute of Marine Research (IMR), Bergen, Norway, in a project investigating krill as a feed source for farmed fish and funded jointly by IMR and the Norwegian Research Council. The aim of this project is to gain increased knowledge on the suitability of marine plankton as a feed source for Atlantic salmon and

Atlantic cod. Overall the project involves the collection and analysis of krill, diet preparation and feeding studies with Atlantic salmon and Atlantic cod. The Nutrition Group is an analytical partner carrying out lipid analyses of the diets, fish and faeces along with some metabolic studies of lipid fatty acid metabolism focussing on wax ester and fatty alcohol analyses and metabolism.

## Nutritional Analytical Services (NAS)

The Nutrition Group also provides a commercial analytical service that has recently been renamed Nutritional Analytical Services (NAS). The NAS expertise is increasingly in demand for analyses of constituents of fish feed and tissues, mostly fatty acids, vitamins and carotenoid pigments. Mrs Liz MacKinlay and Mrs Irene Younger are employed to support the increasing level of commercial contract analysis being undertaken by the group. The NAS has taken part in the Roche ring test for carotenoid analysis for the past four years. This involves inter-laboratory analysis of samples prepared by Hoffman La Roche and distributed to over 40 participating analytical laboratories worldwide. In 2003 the NAS also took part in the Nutreco ring test which tested our analytical methodology in eight different procedures on eight different sample types between 37 laboratories worldwide. As with the Roche ring test the NAS results compared very well with the other laboratories.

## Diets for new aquaculture species

During the year three of Dr Jauncey's research students successfully defended their PhD theses. Laura Halfyard (Newfoundland) completed her part-time PhD on "Egg quality and first feeding diets in the common wolffish, *Anarhichas lupus*"; Beatriz Basso Mercado (Mexico) completed her studies on growth performance and food utilisation of *Macrobrachium rosenbergii* and finally Noe Sanchez Perez (Mexico) completed his studies on dietary lipid quality in stored diets and effects on feed performance and feeding of juvenile *Penaeus indicus*.

Two new doctoral candidates have embarked on their respective research programmes under Dr Jauncey's supervision with Jutaporn Bundit (Thailand) starting work on feeds and feeding of the marble goby and Mohammad Falamarzi (Qatar) on phytic acid in feeds for tilapia.

## Environment ► ► ►

### Molecular ecotoxicology

Professor Steve George's molecular toxicology group continues to concentrate upon studies which support sustainable use of estuarine and marine ecosystems by investigation of the effects of anthropogenic pollutants upon fish. Whilst there is a strong concentration on basic toxicology and gene responses, a practical output of the work is the identification and development of biological effects measures (biomarker measurements) for determining sublethal effects of selected classes of pollutants. Professor George and other colleagues on the UK National Marine Pollution Monitoring Ecotoxicology group have recently reported to DEFRA (and ICES) results of their analyses on the effects of PAHs, dioxins, heavy metals and endocrine disruptive compounds in flounders in most of the major UK estuaries and on fish around oil and gas installations in the North Sea which were sampled last winter. Comparison with previous surveys shows a gradual improvement in fish health as discharges are more

tightly controlled, however, it has also raised the possibility that flounders in some estuaries may show adaptive responses. Cameron Brown has been investigating genetic diversity between these populations using the microsatellite markers isolated by Tom Dixon during his PhD studies.

The major thrust of the molecular toxicology group is deployment of the latest high throughput technologies in genomics and proteomics to identify toxicologically predictive responses in pollutant-exposed fish.

Following on from our 120 candidate gene DNA chip, Dr Amer Diab with Dr Tim Williams (Birmingham) has recently printed a full DNA microarray of 13000 flounder liver cDNAs from the clones isolated by SSH from pollutant-exposed flounders. This is now being used to identify global changes in gene expression. Dr Vicky Sabine has continued development of quantitative RT-PCR methods for diagnostic marker gene responses and these were successfully applied to the UK field survey as a new generic method of biological effects diagnosis. Miss Audrey Legris used the newly developed procedure to determine effects of copper on sticklebacks in a multigenerational mesocosm experiment carried out at INRA on an EU Improving Access to Research Infrastructures Programme. These studies on copper metabolism in fish are being investigated further by Matteo Minghetti from the University of Bologna as part of the long term collaboration between Professor George and Professor Carpane of the Veterinary School in Bologna under the Improving Access to Research Infrastructures Programme.

Transcriptional changes in gene expression are an early stage effect of many abiotic stimuli, the next event is a change in the protein expression profile. Accordingly we are looking at these changes in our pollutant-exposed flounders by 2D gel electrophoresis in a collaborative study with the Danish Centre for Proteomic Analysis at the University of Southern Denmark in Odense. Currently over 50 changes in protein profiles have been identified in a polluted field population and the proteins are being identified by *de novo* sequencing from the gels.

### Freshwater systems

Research over the past year has been dominated by co-ordination of a UK Environment Agency funded project (LEAFPACS) to develop a monitoring tool for ecological assessment of British lakes and rivers based on aquatic plants, as required by the EU Water Framework Directive. This project involves substantial collaboration with the NERC Centre for Ecology and Hydrology in database construction, as well as the University of Ulster and staff in other parts of the Stirling Faculty in the modelling phase of the work.

Funding from the British Council to Dr Tyler (SBES) and Dr Willby to examine the use of remote sensing systems for large-scale ecological assessment enabled an intensive fieldwork campaign at Lake Balaton, Hungary to study variation in the quality of reed and possible environmental causes of dieback. Support from Scottish Water has also funded research on the biodiversity and environmental response of vegetation in retention basins that are now being widely deployed in central Scotland for management of urban storm-water runoff. Another continuing research theme has been the ecology of large gravel bed rivers, and with funding from Scottish Wildlife Dr Willby has been working with Dr Gilvear (SBES) on environmental controls on plant diversity on gravel islands and backwater channels within a largely natural reach of the River Tummel.

Dr Willby currently co-supervises four PhD students covering projects ranging from the biotic recovery of a newly engineered river diversion and the hydrological impacts of beaver reintroduction to Scotland, to the use of remote sensing for interpreting floodplain wetland vegetation and for understanding patch dynamics and nutrient sources in shallow lakes. These projects benefit from support by the Faculty and a range of other sources including Morrisons Engineering, Scottish Natural Heritage, NERC and Northumbrian Water.

Dr Willby is also now collaborating with Dr Little in the Aquaculture Systems group in relation to aquatic vegetable production in peri-urban systems in SE Asia (EC-funded PAPUSSA project) where there are high risks of heavy metal uptake and transfer.

### Marine systems

Post-graduate research within the marine environment field has been very active during the past twelve months. Dr Telfer's group has been investigating the more efficient use of environmental capacity of marine systems by continuing to work with manufacturers of new feeds and feeding technology on two projects. One, building on previous EU funded work with seabass in the Mediterranean, is investigating the ability of computer controlled feeding systems to improve salmon farming waste management and allow environmental mitigation. This work is being carried out by Richard Corner through a NERC CASE studentship. Results are encouraging and have added significantly to datasets used for dispersion modelling of waste. The second project is researching into the more efficient use of environmental capacity through newly developed fish feed formulation. This project, being carried out by Patrick Reynolds and sponsored by NERC and Biomar UK Ltd, is working on sites in Scotland and Norway. Early results show the potential of different formulation of feeds for effective environmental mitigation, particularly for the control of dissolved nutrients from fish farms. Another PhD nearing completion has been investigating the environmental and social implications for integrated aquaculture in western Canada. This project, being carried out by Stephen Cross, involves the integration between intensive fish farming and shellfish farming by first nation peoples. Information provided by this project will be of particular interest to the Canadian Government in helping to integrate the traditional aquaculture practices of indigenous populations and industrial aquaculture.

Work on the DFID funded project TROPICA, involving the development of simple indicators for carrying capacity of tropical aquaculture, continues with the provision of data from test sites in Bangladesh and Vietnam which is currently being analysed.

### Environmental Services

2002-03 has again been a successful year for Environmental Services. There has been some diversification in the provision of services to the aquaculture industry and contract research, where several new avenues of interest have been opened. Research contracts have been undertaken on topics ranging from investigating the environmental impacts of bubble barriers for protection against jellyfish, to looking at the efficacy and mechanism of novel non-toxic antifoulants for fish cage nets. The nature of the consultancy work of the Environmental Services is changing by providing direct analytical services and advisory services for an aquaculture industry which is employing more environmental managers who deal with the



day-to-day monitoring and regulation. This has resulted in a number of significant contracts from industry for analytical services and advice on environmental management and practice, including a large number of Environmental Impact Assessments for applications for new marine fish farm sites or renewal of leases.

A key report this year is the publication of a study commissioned by the Marine Institute, Ireland as part of their Marine Environment and Health Series publications. This study investigates environmental impacts and capacity of sheltered embayments for aquaculture production, a major issue in environmental management of aquaculture. The publication was launched at a meeting in Donegal, where Dr Telfer gave a presentation of the results to industry, governmental and scientific representatives.

## Aquaculture Systems ▶▶▶

The Aquaculture Systems Group has continued to build on and develop its international reputation in strategic issues of aquaculture development, and has maintained a diverse and active range of activities throughout the year, both in the UK and internationally. Its research programme continues to develop and innovate in a range of themes, in many cases leading international perspectives on the sector and its development. Within the Institute the Group has continued its very significant inputs into teaching and PhD supervision, with continued strength of demand for its multidisciplinary PhD programmes, many run on a 'joint centre' basis, in which fieldwork is carried out in target localities, often overseas. In spite of constraints of staff and resources, through the efforts of key staff, contributions from a range of partners, and the enthusiasm of our active group of postgraduate students, we have been able maintain significant outputs in diverse fields. Key staff issues for the year have been Professor James Muir's continued involvement in strategic issues of international aquaculture and fisheries development, Dr David Little's growing international presence in tropical aquaculture and aquatic systems work, Dr Stuart Bunting's widening inputs into technical design and management of temperate and tropical aquaculture systems, and in peri-urban livelihoods, Dr Janet Brown's further success in the new applied programme in

shellfish culture, Professor Ross' continuing strategic inputs in conjunction with his recently completed role as Dean of the Faculty of Natural Sciences and Dr Krishen Rana's developing involvement in aquaculture systems in Sub-Saharan Africa. Dr Polly Douglas, Penny Beaton, Alison Graham, Catherine Lecouffe and Anton Imminck have also provided important support. Dr Don Macintosh's work in collaboration with the University of Aarhus has continued to develop his interests in tropical coastal systems.

The very active and productive linkage with Professor James Young of the Marketing Department continues to be an important element in the Group's work, and a number of new strategic initiatives are currently under way. The important areas of collaboration with the Environment Group, diminished by the resignation of Dr Malcolm Beveridge last year have now been further affected by the departure of Dr Donald Baird for a Chair in Canada, though in both cases there may be useful possibilities of further inter-institutional collaboration. However, areas of collaboration with Dr Trevor Telfer and Dr Nigel Willby are developing well and becoming increasingly valuable. Further linkages are also developing with the Applied Social Science Department (Dr Samantha Punch) and with the Department of Nursing in areas of social research, and community health and nutrition, respectively.

The DFID Aquaculture and Fish Genetics Research Programme (AFGRP), managed by Professor Muir, continues to provide opportunities for more strategic approaches and international partnerships, developing both context and practical action in aquaculture development, within and outside the University, and between the UK and developing country partners and institutions. The field co-ordinating role of Dr David Little, the programme developed by Dr Bunting in conjunction with Professor Young, Dr Little and Dr Punch and STAQ, and linkages with the Asian Institute of Technology (AIT) and other institutions, have been important in this. The support and involvement of the Environment Group staff, Professor McAndrew and Dr Penman in the Genetics and Reproduction Group and Dr Turnbull, Dr Thompson and Dr Crumlish in the Fish Health Group, have also been very significant.

The Group's themes, developed through Professor Muir's sustainable aquaculture systems work, the tropical aquaculture programme co-ordinated by Dr Little and Dr Bunting, Professor Ross's GISAP (geographical information systems and applied physiology) group, Dr Brown's shellfish group, and the fisheries sector marketing and economics programme run by Professor Young and Professor Muir, continue to shape the Group's strategies and development. Stirling Aquaculture also provides links across these areas. The Group has continued its existing themes:-

### Strategy, planning and development

Following the successful development of the multi-donor/government review of the fishery sector in Bangladesh, covering themes of economic performance, livelihoods, social development and environment, institutional frameworks, policy and legal systems, Professor Muir has provided follow up to the process by which the recommendations of the programme are to be taken up in major public sector and development support investment. Professor Muir has also provided guidance on the development of a major DFID-World Bank support programme in Bangladesh, and led a key review of the performance of the DFID-FAO Sustainable Fisheries Livelihoods Programme, active in 25 countries in W. Africa. He has also carried out a review of the development of the World



Researching farmers' preferences in Cambodia

Fish Centre's strategy for the use of its centre in Abbassa, Egypt for supporting regional aquaculture research. On behalf of DFID, Professor Muir also contributed to the preparation and planning of an innovative international competitive research proposal for donor investment in the agriculture sector.

The GISAP programme continues with a range of activities. Following her successful PhD, Eunice Perez Sanchez (Mexico) has published a number of articles on GIS and social strategies in coastal fisheries/aquaculture in Tabasco State, Mexico, and Luis del Campo, on completion of his PhD has returned to Chile and is developing further approaches for environmental, social and infrastructural strategies for mollusc culture, together with new initiatives on closed cycle systems, in conjunction with the Group. Martin van Brakel's work on a GIS based approach for detailed assessment of interactions between natural resources, poverty profiles and aquaculture potential, based in Vietnam, is proceeding well, with a number of invited papers already presented. Phil Scott has successfully completed his PhD studies on the potential of Sepetiba Bay, Rio de Janeiro for aquaculture.

Jeng Shu-ching successfully completed his PhD studies on innovation and development in the aquaculture sector of Taiwan, and Nelly Isyagi is completing her work on aquaculture development strategies for small-scale farmers in Uganda. Alberto Asiain has made excellent progress on his study on community development processes in promoting rural aquaculture within Mexico, and Adrian Hartley is now in the major field phase of his research on strategic competition issues in the international tilapia market. Together with PhD work by Sergio Zimmerman on tilapia development in Brazil, related work in Bangladesh and India, through the AFGRP, and strategy work by Professor Young and Professor Muir on tilapia production economics and markets, the Group is developing a particularly strong theme in international strategies for tilapia development.

The Group continued inputs into FAO aquaculture strategy issues, with Professor Muir's work on sustainability indicators and on land and water use parameters for aquaculture development. Further inputs on sustainability indicators are now also being promoted via the AFGRP in association with the University of Reading. Following his period as Dean of the Faculty of Natural Sciences, Professor Ross is now resuming his work on strategic development of aquaculture, particularly in Latin America.

### Aquaculture production systems

With the DFID AFGRP and Natural Resources Systems Programme, together with EC funding, and with a range of local partnerships and project linkages, Dr David Little, Dr Stuart Bunting and their regional colleagues have contributed in a range of current activities, including:

- ▶ Farmer-managed irrigation systems and aquaculture in water short areas of Karnataka State, India and the dry zone of Sri Lanka. This work, funded by AFGRP, has now been completed by the project's research assistant Francis Murray, in conjunction with CARE Sri Lanka and the Asian Development Bank.
- ▶ Aquaculture integrated within engineer-managed irrigation systems: work with partners in the UK (University of Newcastle) and Sri Lanka (ABC, University of Peradeniya) and India (Tamil Nadu Agricultural University) funded by DFID-KAR is now completed, and the Stirling field research co-ordinator Lindsay Pollock is completing her PhD.

- ▶ Fish seed quality in Asia: with the Asian Institute of Technology and five national institutions funded by DFID-AFGRP; collaborative research has revealed a range of strategic and technical improvement routes, now being actively taken up by public and private sector hatchery operators. Angus MacNiven, a PhD student based at AIT is completing his work.

- ▶ Aquaculture in the peri-urban zone-the Calcutta Wetlands; work by Dr Stuart Bunting, Dr Samantha Punch (Applied Social Sciences) and colleagues, funded by DFID-NRSP, has completed a range of findings and recommendations on livelihood implications of changing resource use in the rapidly expanding city and its adjacent wetlands.

Dr Janet Brown's programme in crustacean production is continuing through a programme of PhD studies, and with STAQ and other field projects.

Professor Ross, Dr Jauncey and Dr Malcolm Beveridge were all involved in project work in Mexico during the year, contributing expertise to project design and objectives.

At Stirling, Dr Martinez Palacios and Dr Myra Toledo Cuevas from Mexico were hosted for a mixture of project design and training missions. The group is now at the stage of pilot scale trials in the field and is working with a community in Ichupio on Lake Patzcuaro to develop a pond trial site which will blend in with the range of family activities which constitute their livelihood. Work is also due to commence with an SME that is interested in diversifying its activities. This combination of small-scale stakeholders and small business involvement has considerable potential for success in the Mexican context.

Work continues with Dr Carlos Martinez's group at the Universidad Michoacana San Nicolas de Hidalgo in Morelia on the endangered whitefish *Chirostoma estor estor*, one of a flock of atherinid species unique to the Mexican altiplano lakes. During the year the group has been successful in obtaining further substantial funding from CONACyT (the Mexican Research Council) and government departments including SAGARPA (Department of Agriculture and Fisheries) and SEMARNAT (Department of Environment), totalling about £1m. The link with the Institute is supported by the British Council.

Dr Little's study, with Dr Kai Lorenzen of Imperial College, London, on the livelihood impacts of self recruiting species in



📷 Carp culture cages in Vietnam

Asia continues to develop well, with a range of studies on the role of these species in three countries of Indochina, eastern India and Bangladesh, and a widening range of impacts in policy development with respect to retaining these species, and encouraging their role, in managed aquatic systems.

Dr Little has continued his involvement with the British Council Link project with Research Institute Aquaculture 1 (RIA 1) in Bac Ninh, near Hanoi, Vietnam focusing on overcoming constraints on the cage culture of grass carp in rivers, and increasing the capacity of RIA1 to appraise developmental constraints and opportunities for poor people in northern Vietnam. He has also taken up a lead role in a major EC programme: "Improved resource use efficiency in Asian integrated pond-dike systems".

Dr Rana continued to expand his research portfolio on developing new species and sustainable aquaculture systems. Two new research programmes were initiated this year. An investigation of the constraints in larval production of common snook, *Centropomus undecimalis*, is in collaboration with Dr Kevan Main of the Mote Institute in Florida and Dr Kim Jauncey. In Oman, research on the reproductive biology and mass seed production and marketing issues of the silver sea bream, *Rhabdosargus sarba*, is underway in collaboration with Dr Goddard of Sultan Qaboos University and Professor James Muir. A research programme has also commenced in South Africa to evaluate the roles of traditional aquaculture in sustainable rural livelihoods; this will complement recent initiatives on developing urban and peri-urban aquaculture in this country.

### System design

Main areas of work in system design continue together with Stirling Aquaculture, providing practical application and development in the commercial context. Luca Montorio, the TCS Fellow working with Alexander Sandison's of Shetland, is now completing a PhD on key issues concerning the improvement of recycle system performance. Dr Bunting's PhD work on the design of integrated aquatic systems has been further developed in collaboration in an EC funded programme (GENESIS) on ecologically improved recycle aquaculture systems. It is also particularly valuable to note that Bart Vlaminc's work on improved offshore cage designs has been further developed, with a range of cages now in use.

## Stirling Aquaculture ► ► ►

### Management & longer-term contracts

The Institute's consultancy and project management arm, Stirling Aquaculture (STAQ), had another busy year, albeit one that was marred by the sad and tragic loss of two long-term colleagues, Bart Vlaminc and Zarko Peric, in a car accident. Bart had worked as a STAQ project manager for over ten years, in Malta, Madeira and Sicily. Zarko was associated over a similar period of time, although only taking on direct assignments for STAQ over the past three years. Their friendship and professional contributions will be sadly missed.

STAQ's management contract of an offshore cage project with Farnoccean International in Libya came to a conclusion in early 2003 with completion of the first production cycle of seabream. The site used both plastic circle and steel Farnoccean 4500 semi-submersible cages. Jonathan Grubb provided overall project

management with other staff including Patrick Morino (site manager and boat specialist), Orazio Baldacchino (dive instructor), Bolla Venkataiah (assistant farm manager), and Louis Baldacchino (consultant and relief farm manager).

STAQ continued to act as the UK National Network Leader for the EC-funded Aquaflow project. This disseminates information about aquaculture research projects to producers and other potential users of technology. The group is also contributing to an EC funded research and demonstration project "GENESIS", which is investigating various approaches to integrated mariculture in the European context. Dr Stuart Bunting is working on bio-economic modelling of the systems, whilst Professor Jimmy Young and other research staff from the Department of Marketing conducted focus groups to assess consumer attitudes to GENESIS systems. Other partners are located in Israel, France, Wales and Scotland.



Trout farming in Papua New Guinea

### Consultancy

Jonathan Grubb made a second visit to Papua New Guinea early in the year, accompanied by Professor Jimmy Young, to carry out further investigations into the trout farming sector in the Highland regions, and its support requirements. Particular emphasis was given to marketing issues and further assessments of technical and financial feasibility. The project was conducted at the request of the late Governor Fr. Louis Ambane, and funded by the Commonwealth Secretariat. Hopefully his initiative will lead to further support interventions based on the development strategy recommended by the team.

A significant review of marine aquaculture technology and likely future developments was carried out for the Royal Commission on Environmental Pollution.

This involved inputs from other colleagues within the Institute of Aquaculture, and from the Department of Marketing, the particular emphasis was on implications for environmental impacts and overall sustainability.

Smaller consultancy projects included advice to a company planning a novel aquaculture system in Scotland, market research on fish feeding systems and advice on fishery matters provided in partnership with Dr Colin Bull of the Forth Fisheries Foundation.

## Aquaculture Technology Centre

The development of the Aquaculture Technology Centre (ATC) as a broader grouping of commercial services within the Institute of Aquaculture was strengthened during the year with a series of three training courses for industry run with financial assistance from BBSRC. The first, in Environmental Monitoring and Management, was held in Stirling over three days in September 2002, and included a day of practical work at a site near Oban. The second, a two day course on aquaculture feed management, was organized in collaboration with partners from the University of Glasgow in November 2002, with inputs from Dr Kim Jauncey and Dr Gordon Bell of the Institute's Nutrition Group. The third course in Information Technology for Aquaculture was run entirely over the Internet, using the University's "Virtual Learning Environment" – WebCT, with inputs from the Department of Management and Organisation and external tutors.

## Staff

There have been a number of staff changes during the year. Polly Douglas left to join a new unit helping to promote innovation and commercialization in the health care sector, and was replaced firstly by Penelope (Penny) Beaton, and then by Catherine Lecouffe. Alison Graham completed her temporary contract and commenced preparations for a PhD. Adam Brooker also completed a temporary contract with STAQ during the year, working especially on the BBSRC short courses.

## External Facilities ► ► ►

The Institute External Facilities of Howietoun, Buckieburn Freshwater Research Unit, Machrihanish Marine Environmental Research Laboratory and Machrihanish Marine Farm Ltd have had another busy and ultimately successful 12 months.

### Howietoun

Smolt production at Howietoun has seen another record year with 630,000 smolts being delivered to mainland on-growing sites. This is a 10% increase on last year's production and deliveries and 28 day survivals have been excellent with none of the IPN losses reported last year. The latter have been traced back to a strain of eggs which appears to be particularly susceptible to IPN; needless to say this strain will be avoided in the future. This spring we used a Norwegian well boat for our smolt deliveries and for the first time cleared all three sites over a forty-eight hour period.

The loss of malachite green for control of fungal infestations has not so far caused serious problems. We have reviewed how we handle fish, the types of vaccine used and have modified some protocols. To some extent the loss of malachite is affecting companies differently; those with a high fungal challenge are suffering more than those with low spore counts. In this respect tank sites fare better than cage sites which are generally more conducive to fungal infestations.

The warm dry summer of 2003 has been very good for fish growth but at the time of writing supplies of water are running short in many areas, including Howietoun and Buckieburn, and causing concerns for smolt producers. There have been welcome signs of a marked improvement in salmon prices since mid summer and because of reduced smolt input in both Scotland and Norway this autumn the outlook for salmon prices over the next 24 months is very good.

Brown trout sales from Howietoun have been good this year with steady demand from our large and loyal customer base, but as in previous years there is heavy competition from restockers south of the border. We lost one of our best customers, the Carron Reservoir, this year to a much cheaper supplier from the south despite having stocked and built up a relationship over the last five years with Scottish Water. However, there appear to have been problems with the fishing and anglers have largely ignored this excellent facility. As a result Scottish Water will not organise fishing on the Carron Reservoir again and are currently looking for a tenant to lease the fishing on a reservoir which for the last few years has been arguably the premier brown trout fishery in Scotland used extensively for national competitions.

The fine weather this summer has generally not been good for angling with most clubs reporting poor fishing conditions from May to September, which will undoubtedly affect their finances for fish purchases next year. On the other hand the excellent summer has allowed Scottish residents and tourists alike to get out into the countryside more than usual and we hope this will attract more locals, and particularly youngsters, back to outdoor pursuits.

The much heralded "Strategic Framework for Scottish Aquaculture" was unveiled at Dunblane in the Spring and although stopping short of providing Scottish aquaculture with the regulatory and government support that land farmers enjoy, it nevertheless laid down a strategy for the future which can be worked on with other stakeholders in the Scottish environment. There can now be no doubt that the Scottish Executive understands the important role that aquaculture plays in keeping isolated rural communities alive and the value of the exports it generates to the Scottish economy. Much still needs to be done, however, to provide a level playing field for Scottish salmon farmers in relation to their competitors, particularly from Norway. The Institute staff will continue to be heavily involved in this process through representation on Government and SQS committees.

### Buckieburn Freshwater Research Unit

Buckieburn has had another successful year with a number of academic and contract research studies in progress. However, work has been hampered by the tragic death of Professor Niall Bromage in May 2003. Niall's group are the largest user of Buckieburn facilities and Niall was largely instrumental in promoting the need for new laboratory and office facilities at Buckieburn. A new building was completed in early 2003 and we are delighted that these facilities will be named the Niall Bromage Freshwater Research Facility. The new facilities were formally opened by Niall's wife Anne and children Iain and Sarah at an opening ceremony on the 26<sup>th</sup> November.

The major trial on stocking density effects on fish welfare funded by DEFRA and BTA has continued to provide useful data and is nearing a successful conclusion. A number of new research proposals are being submitted by the Genetics and Reproduction group at the time of writing which, if they are successful, will be carried out at Buckieburn and which will link in well with contract research trials currently under discussion.

The malachite green depuration trial carried out in conjunction with Machrihanish is now complete and has given astonishing results in that the leucomalachite residues took up to 650 days to reach undetectable levels in the flesh of salmon following five routine treatments prior to smolting. It would appear that the benefits of malachite were far greater than as a fungal



treatment alone, as an increase in proliferative kidney disease (PKD) and myxosporean infections, as well as white spot (*Ichthyophthirius*) are being reported in farmed salmonids following the banning of the use of malachite in June 2001. It now appears that these parasites were being unwittingly controlled by the use of malachite and its derivatives. A novel potential fungicide is being trialed at Buckieburn as a replacement for malachite green. This product, which is widely used in the drinking water industry, would be a cost-effective replacement if found to be therapeutic.

### Machrihanish

Machrihanish has had another productive but not particularly profitable year. The lack of finance within the salmon industry over the last 18 months has caused major funding problems for suppliers of the industry who form the main customer base for Machrihanish. This situation may change in the next few months and we are hopeful of securing more contracts as the industry moves into profitability.

However, this year has seen a large increase in the number of student projects commencing at Machrihanish, primarily based on cod. We now have five PhD projects in progress or about to commence in the areas of parasitology, genetics, nutrition/environment, physiology and reproduction and behaviour. This is partly as a result of the Institute move into "New Species" as part of its overall research strategy but it places Machrihanish squarely as a leading world player in cod research. This, coupled with a grant from AIE and the use of our reserves, will allow us to establish a much needed research hatchery in the lifeboat station. When coupled with the use of live feed from the nearby facilities in MMF this will enable us to apply for research grants to develop new cold water aquaculture species such as hake.

Although the cod industry is currently small it has high potential for development in Scotland due to the ready access to a vast market in the UK and Europe and the ease of transfer of technology to salmon farms wishing to diversify. Andrew Davie's PhD project on maturation control of cod is coming to an end and his results are already being extensively used by the developing industry. We hope to initiate a "Cod Club", similar to the very successful "Smolt Club", pioneered by Niall Bromage and his colleagues, through BMFA and Seafish to allow Andrew's work to continue on BMFA member sites.

The cod crisis in the North Sea has resulted in sale prices for farmed cod being 50% higher than farmed salmon. This coupled with the very high fillet yield and excellent taste and texture of farmed cod makes farming of this species a highly attractive option.

However, the development of the cod industry in Scotland is being hampered by a lack of juveniles.

In addition to holding potential cod broodstock of both farmed and wild origin prior to transfer to Machrihanish Marine Farm's broodstock facilities, Machrihanish has been carrying out a number of trials on cod vibrio vaccines. This is undoubtedly the biggest disease problem so far encountered for farmed cod, in that the licensed vibrio vaccines do not contain the O2B serotype of *Vibrio anguillarum* which is very pathogenic to cod in Norway. It would appear that this serotype is also

prevalent in Scotland and therefore a specific vaccine is urgently required. Under a SMART award to Professor Adams, Imke Wijmemgen from the Netherlands has started a new project on the use of adjuvants in farmed cod vaccines and the end point of this project is the planned development of a Scottish-produced vaccinating machine.

Machrihanish continues to carry out a large amount of work with salmon, most still focusing on sea lice. We continue to work on aspects of "SLICE" and we are also developing a new product for a major pharmaceutical company which gives excellent protection against sea lice using a novel treatment method. The aim is to bring this product to license as quickly as possible to increase the number of options open to sea farmers. We hope to test both of these products in new species so that treatment options will be in hand should crustacean parasitic problems arise in, for example, cod.

A project on marine amoebae under the auspices of the EC Access to Research Infrastructures Programme has been completed at Machrihanish by Mairead Bermingham from University College, Cork and has provided important new information on treatment of this damaging parasite. The project also showed how ubiquitous this parasite is and how difficult it is to identify in the field.

A new and welcome development at Machrihanish has been the holding of the MSc Aquaculture marine field course at the laboratory. The students used the local village hall as a base, in combination with the tank facilities at the laboratory and a field session sampling benthos in Campbeltown Loch. We hope to develop this in the future with more dedicated facilities at the site and also the use of a new seminar room in the lifeboat station, which will be commissioned this winter using funds from AIE and our reserves. This room will double up as a client meeting room and as well as having modern IT and presentation facilities it will have one of the best sea views on the west coast of Scotland.

### Machrihanish Marine Farm Ltd

Construction of the hatchery and phase 2 is now complete resulting in an excellent, well equipped and fully staffed facility. Cooperation with our partner, Lakeland Smolt, through Richard Prickett of Marine Farm Technology continues to be excellent and the technical cooperation between MMF and their counterpart Norwegian hatchery, Grieg Marine, is extensive.

Although the building operation has taken longer than envisaged all of the systems are working well and approximately 150,000 cod juveniles averaging 70 gm will be delivered to Johnson Seafarms in Shetland at the end of November. This will bring a total production of around 200,000 over the last twelve months. This is less than planned but represents around 60% of EU production.

There have been initial problems with farmed cod broodstock. The quality of the juveniles produced at hatch is not as robust as juveniles produced from wild parents and there is a wide size variation. The resultant fry produced have been of good quality but survival has not been as good as expected. To counter this, wild derived and farmed juveniles will be compared in our next production run and additional wild broodstock have been obtained which will form the basis of our broodstock for the future. In time the correct strategy will be to use selected farm broodstock as the basis of a breeding programme, but in these early days it will be necessary to concentrate on wild stock until the teething problems of farmed stock are overcome.

The market for cod juveniles in Scotland, and particularly Shetland, is very strong at the moment, as the market opportunities are enormous for ongrowers. In addition there is considerable interest from Ireland in taking trial batches of fish to ascertain their suitability for Irish conditions. Our production aim for the next 12 months is 1.2 million juveniles which will be delivered to Shetland, Wester Ross and Argyll and, if there is a surplus, to Ireland.

The hatchery was officially opened by the Princess Royal on May 29<sup>th</sup> which allowed us the opportunity to show 70 invited guests around the facility and to promote cod production in Scotland. This opening has paid multiple dividends as, in addition to the extensive publicity we received, there is now a good deal of support from organisations, councillors and officials in our attempts to obtain licenses for cod farming on the west coast of Scotland.



📷 The Princess Royal at the official opening of Machrinhanish Marine Farm Ltd.

## Appendix I ▶ ▶ ▶

### Advisory Committee

Professor C Hallett  
Acting Principal of University and Chair

Professor I D Aitken, OBE  
Scientific Director, Edinburgh Centre for Rural Research

Professor Sir James Armour  
Vice President of Royal Society of Edinburgh

Mr K Clarke  
University Secretary (*ex officio*)

Mr H M Currie  
Managing Director, Lithgows Limited; Chairman CBI Scotland

Mr Mark Davies  
Chairman, British Trout Association (BTA)

Dr Ph. Ferlin  
Director, Institut National de la Recherche Agronomique

Professor R H Richards  
Director of Institute (*ex officio*)

Mr Andy Rosie  
SEPA Aquaculture Specialist

Professor C Sommerville  
Dean of Faculty of Natural Sciences (*ex officio*)

Dr P Sorgeloos  
Artemia Reference Centre, University of Ghent

Dr J Tarbit OBE  
Former Senior Fisheries Adviser, DFID

Professor P N Wilson, CBE  
Professor Emeritus, University of Edinburgh

## Appendix II ▶ ▶ ▶

### Staff List (at 1/10/2003)

Director of Institute  
Randolph H Richards  
RMB Professor of Aquatic Veterinary Studies MA, VetMB, PhD, MRCVS, FIBiol, FRSM, ARAgS

Deputy Director  
Rodney Wootten BSc, BA, PhD

Assistant Director  
James F Muir BSc, PhD  
Professor of Aquaculture Development

Professor of Aquatic Molecular Genetics  
Alan J Teale, BA, MA, VetMB, MSc PhD, MRCVS

Professor of Clinical Fish Pathology and Microbiology  
Hugh Ferguson, BVM&S, PhD, DipACVP, MRCVS, FRCPath

Professor of Aquatic Physiology  
Lindsay G Ross BSc, PhD

Professor of Aquatic Parasitology  
Christina Sommerville BA, PhD

Professor of Aquaculture Genetics  
Brendan J McAndrew BSc, PhD

Professor of Environmental Toxicology  
Stephen G George BSc, PhD, MPhil

Professor of Aquatic Immunology & Diagnostics  
Sandra Adams BSc, PhD

Reader  
Michael V Bell BSc, PhD

Professor Emeritus  
Allen J Matty BSc, PhD, DSc

Professor Emeritus  
Ronald J Roberts BVMS, PhD, FRCPath, FRCVS, FIBiol, FRSE

Professor Emeritus  
John R Sargent BSc, PhD, FIBiol, FRSE

Honorary Professor (Marine Fish)  
John H S Blaxter MA, DSc, D.Univ (Stir.), FIBiol, FRSE

Honorary Professor  
David Mackay Ch.Biol, FIBiol, FIWEM, FIMgt, MIFM

Senior Lecturer  
J Gordon Bell BSc, PhD

Senior Lecturer  
D J Macintosh BSc, PhD

Senior Lecturer  
Douglas R Tocher BSc, PhD

Senior Lecturer  
Kim Jauncey BSc, PhD

Lecturer & Course Director MSc Aquatic Pathobiology/Vet Studies  
Jimmy Turnbull BVMS, MSc, PhD, MRCVS

Lecturer & Course Director MSc Aquaculture  
Trevor C Telfer BSc, PhD, CBiol, MIBiol

Lecturer  
David C Little BSc, MSc, PhD

Lecturer  
Andy Shinn BSc, PhD

#### Lecturer

Nigel Willby BSc, PhD

#### Lecturer

David Penman BSc, PhD

#### Research Lecturer

James E Bron BSc, MSc, PhD

#### Research Lecturer

Janet H Brown BSc, PhD

#### Research Lecturer

Krishen Rana BSc, PhD

#### Research Fellows

Elizabeth Ashton BSc, D.Phil

Kerry Bartie BSc, PhD

Anne Conrad BSc, MSc, PhD

Margaret Crumlish BSc, MSc, PhD

Amer Diab BSc, MSc, PhD

Herve Migaud BSc, MA, PhD

Michael Leaver BSc, PhD

David Morris BSc, PhD

Victoria Sabine BSc, MSc

William Starkey BSc, PhD

Janet Stone MSc, MIBiol, FIMLS

John Taggart BSc, PhD

Kim D Thompson BSc, MSc, PhD

Helen Whitaker BSc, PhD

Xiaozhong Zheng BSc, PhD

#### Project Officers

Garnet Hooper BSc, MSc, PhD

Caroline Wall MSc

#### Research Assistants

Stuart Bunting BSc, MSc, PhD

Anton Immink BSc, MSc

Ben North BSc, MSc

Graham Taylor BSc, MSc

Lindsay Pollock BSc

Catherine Lecouffe MSc (p/time)

#### Teaching Company Associate

Anu Frank-Lawale, BSc, MSc

#### Veterinary Clinical Officer

Richard Collins, BVM&S, MSc, MRCVS

#### Honorary Lecturers

John Wickens BSc

F J Wrona BSc, PhD

#### Chief Technicians

Linton Brown (EM)

James Dick (Nutrition)

Cathryn Dickson BSc, MSc (Genetics)

Ann Gilmour (Genetics)

Brian Howie (Engineering)

Stuart Miller (Disease)

Stephen Powell BSc (GLP/Genetics)

Keith Ranson BSc (Fish Husbandry)

Elizabeth Stenhouse (Purchasing/Stores)

William Struthers (Environment)

William Thomson (Electronics)

#### Senior Technicians

Gillian Dreczkowski BSc (Bacteriology)

Iain Elliot (Teaching)

Debbie Faichney BSc (Histopathology)

Charlie Harrower (Purchasing/Stores)

Jim Henderson BSc, PhD (Nutrition)

Jacquie Ireland BSc (Virology)

Liz Mackinlay BSc, MPhil (Nutrition)

Fiona McGhee BSc (Nutrition)

Hilary McEwan (Vaccine Unit)

Fiona Muir BSc (Virology)

Irene Younger (Nutrition)

#### Technicians

Robert Aitken BSc (Fish Husbandry)

Niall Auchinachie BSc, MSc

(Fish Husbandry)

Denny Conway BSc (Parasitology)

William Hamilton (Fish Husbandry)

Anne Hammond BSc (Environment)

Jane Lewis (Purchasing/Stores)

Maureen Menzies (Histopathology)

Kirsty Pollock (Environment)

Nora Pollock BA (Environment)

Alan Porter (Nutrition)

Stephen Powell BSc, MSc (Genetics)

Karen Sneddon BSc, MSc (Vaccine Unit)

#### Departmental Assistant

Beatrice Campbell BA

#### Departmental Secretary

Hazel Telfer

#### Secretary

Lynn More

#### Journals Secretary

Christine Kerr

#### Financial Coordinator

Melanie Cruickshank BSc

#### Stirling Aquaculture (Consultancy Group)

#### Manager

John Bostock BSc, MSc

#### Project Assistant (Information and Dissemination) p/time

Catherine Lecouffe MSc

#### Associate Senior Consultants

Jonathan Grubb MSc

David Currie BSc MSc

Trevor Meyer BSc MSc

David Scott MSc

#### **External Facilities**

#### External Facilities Director

D A Robertson BSc, PhD

#### Howietoun Fish Farm

#### General Manager

Robert J A Murray BSc

#### Fishery Manager

Iain J Semple BSc

#### Hatchery Manager

James Rae

Stockworkers  
Colin Forrest  
Stuart Galloway  
Robert Redfern

Secretary  
Grace Smith

Cleaner  
Rhona Smith

Buckieburn  
Site Manager  
Alastair McPhee BSc

Senior Stockworker  
John Gardiner

Dunblane  
Foreman  
Kevin Smith

Machrihanish Marine Environmental  
Research Laboratory  
Manager  
William Roy BSc, PhD

Site Engineer  
Simon Barnett

Foreman  
Chessor Matthew BSc

Technician  
Sally Boyd BSc

Contract Research  
Jocelyn Richard BSc, PhD

Research Fellow  
Janet Stone MSc, MIBiol, FIMLS

Stockworker  
Keith Mathieson

Cleaner  
Elizabeth Irwin

## Appendix III ► ► ►

### PhD Students 2002/2003

Miss K. Frake, B.Sc., M.Sc., UK, (NERC)

Mr I. Al Mohsen, B.Sc., M.Sc., Saudi Arabia, (Saudi Arabian Government)

Miss E. Alorend, B.Sc., M.Sc., France, (BMFA, NUTRECO)

Mr A. Asiain Hoyos, B.Sc., M.Sc., Mexico, (CONACyT)

Mrs B. Basso Mercado, M.Sc., Mexico, (CONACyT)

Mr A. Bell, B.Sc., M.Sc., UK, (LINK Aqua)

Mr I. K. Berrill, B.Sc., M.Sc., UK, (NERC CASE)

Mrs C. Boonphakdee, B.Sc., M.Sc., Thailand, (Government Scholarship)

Mr J. Botero, M.Sc., Colombia, (Colombian Government/INPA)

Ms J. Bundit, B.Sc., M.Sc., Thailand, (Royal Thai Government)

Mr C. Brown, B.Sc., M.Sc., UK, (Alkioni Fish Farms)

Mr A. Campos Mendoza, B.Sc. (Hons), Mexico, (CONACyT)

Miss P. Carter, B.Sc., UK, (BBSRC CASE)

Mr J. D. Chara, B.Sc., M.Sc., Colombia, (COLCIENCIAS)

Mr P. Christofilogiannis, B.Sc., M.Sc., Greece, (Greek Government)

Mr R. Corner, B.Sc., UK, (NERC CASE)

Miss J. Costa, B.Sc., Portugal, (Foundation of Science & Technology, Portugal)

Mrs C. Crichton, B.Sc., Canada, (EC Grant)

Mr S. Cross, B.Sc., M.Sc., Canada, (Private)

Mr A. Davie, B.Sc., M.Sc., UK, (NERC)

Mr L. M. Del Campo Barquin, B.Sc, Chile, (Private)

Mr T.J. Dixon B.Sc., UK, (NERC)

Mr A. Faruk-Ul-Islam, B. Sc., M.Sc, Bangladesh, (DIFD-AFGRP and SUFER)

Mr G. Faruque, B.Sc., M.Sc., Bangladesh, (British Council)

Miss M. Felsing, B.Sc., M.Sc., Denmark, (Faculty of Natural Sciences Studentship)

Mr J. Fonseca Madrigal, B.Sc., BTech MLS, Mexico, (CONACyT)

Mr A. Frank Lawale, B.Sc., M.Sc, UK, (TCS Research Grant/Staff Development)

Miss W. Futter, B.Sc., M.Sc., UK, (BBSRC CASE)

Mrs A. A. Gheyas, B.Sc., M.Sc., Bangladesh, (Commonwealth Scholarship)

Mr A. Gomna, B. Ed., M. Tech, Nigeria, (Government Scholarship)

Miss J. E. Good, B.Sc., UK, (EWOS)

Miss D.A. Guerrero Tortolero, M.Sc., Mexico, (CONACyT)

Mr A. Hajizadeh Kapateh, B. Sc., M.Sc, Iran, (Iranian Government)

Ms L.C. Halfyard, B.Sc., M.Sc., B.Ed., Canada, (Private)

Mr A. Hartley Alcocer, B.Sc., M.Sc., Mexico, (CONACyT)

Miss N. Hastings, B.Sc., UK, (NERC)

Miss A. Holzer, B.Sc., M.Sc., Austria, (Austrian Academy of Sciences)

Mr F. Ibrahim, B.Sc, Oman, (Private Funding)

Mr A. Immink, B.Sc., M.Sc, UK, (Private)

Ms A.N. Isyagi, M.Sc., B.V.M., Uganda, (World Bank Lake Victoria Project)

Mr S.C. Jeng, B.Sc., M.Sc, Taiwan, (Taiwan Government)

Mr V. Karalazos, Ptychio, M.Sc., Greece, (Greek State Government)

Mr I. Karapanagiotidis, Ptychio, M.Sc., Greece, (Greek State Government)

Mr M. Karim, B.Sc., M.Sc., Bangladesh, (World Bank)

Ms S. M. Laffon Leal, B.Sc., M.Sc., Mexico, (CONACyT)

Mr A. Macniven, B.Sc., M.Sc., UK, (DFID)

Miss F. Manji, M.Sc., Kenya, (Private)

Miss L. Marshall, B.Sc., M.Sc, UK, (AVL and Biomar)

Mr C.C. Martinez Chavez, B.Sc, Mexico, (CONACyT)

Mr M. F. Matori, D.V.M., Malaysia, (University Putra, Malaysia)



Mr N. J. Miller, B.Sc, UK, (Private)  
 Ms U. McCarthy, B. Sc., M.Sc, UK, (University Studentship/AVL)  
 Mr G. McKinley, B.Sc., M.Sc, UK, (University Studentship/  
 Novartis)  
 Mr C. McGurk, BVMS., M.Sc, UK, (NERC)  
 Mr G. Milwain, B. Sc., M.Sc, UK, (DFID)  
 Mr L. Montorio, B.Sc. Italy, (Private, Alex Sandison & Sons Ltd,  
 University of Stirling)  
 Mr E. Morales, B. Sc, Philippines, (DFID/ARP)  
 Miss A.L. Morgan, B.Sc., M.Sc., UK, (NERC CASE)  
 Mr D.C. Morris, B.Sc., UK, (NERC)  
 Mr J.G. Mota-Velasco, B.Sc., M.Sc, Mexico, (CONACyT)  
 Mr F. Murray, B.Sc., M.Sc., UK, (DFID)  
 Ms N. Musa, B. Sc., M.Sc, Malaysia, (Malaysian Government)  
 Mr J.P. Neary, B.Sc., M.Sc., Canada, (University Studentship)  
 Mr B.P. North, B.Sc., UK, (MAFF/Private)  
 Miss L. Pollock, B. Sc, UK, (DFID )  
 Mr R.A. Retamales, M.Sc, Chile, (ORS)  
 Mr P. Reynolds, B.Sc., M.Sc, UK, (NERC CASE)  
 Mr D. Riddell, B.Sc., M.Sc, UK, (NERC CASE)  
 Mr N. Sanchez-Perez, B.Sc., M.Sc., Mexico, (CONACyT)  
 Mr K. Satapornvanit, B.Sc., M.Sc, Thailand, (INCODEV-MAMAS)  
 Mrs S. Sayeed, B. Sc., M.Sc, Bangladesh, (Commonwealth  
 Scholarship)  
 Mr P.C. Scott, B.Sc., M.Sc, Brazil, (Brazilian Government)  
 Miss R. Sharif, B.Sc., M.Sc, UK, (EWOS)  
 Miss N. Sheriff, M.Phil, B.Sc, UK, (University Studentship)  
 Mr M. Sprague, B.Sc, UK, ,(NERC CASE)  
 Ms W. Taparhudee, B.Sc., M.Sc, Thailand, (Royal Thai  
 Government)  
 Mr J.F. Taylor, B.Sc., M.Sc, UK, (Commercial Fish Farm)  
 Mr N. Taylor, B.Sc, UK, (Environment Agency & BTA)  
 Mr D. Turongruang, M. A, Thailand, (EC-INCODEV )  
 Mr J.P. Ureta Schmidt, B.Sc, Chile, (Private)  
 Mr M. L. Van Brakel, M.Sc, Netherlands, (University/DFID)  
 Mr G. Von Hoegen, B. Sc., M.Sc, Germany (Private)  
 Mr K. Walton, B.Sc, UK, (University Studentship, Novartis)  
 Mr M.T. Wayland, B.Sc, UK, (NERC CASE)  
 Mr C. Williams, B.Sc., M.Sc, UK, (Environment Agency)  
 Mr P.M. Williams, B. Sc, UK, (NERC)  
 Mr C. Yanes-Roca, B. Sc, Spain, (Private)  
 Ms S. Yuksel, B.Sc, Turkey, (Turkish Government and  
 Aquaculture Vaccines)  
 Mr S. Zimmerman, B.Sc., M.Sc, Brazil, (GenoMar ASA)

### MSc Aquaculture 2002/2003

Mr L. Av Reyni, Faroe Islands, (Faroes Government)  
 Mr P. Buray, France, (Private)  
 Miss S. Campbell, UK, (Private)  
 Mr G. Dickson, UK, (SAAS)  
 Mr S. Dowd, USA, (Private)  
 Mr R. Gislason, Iceland, (Icelandic Government Student Loan  
 Fund)  
 Mr M. E. Gongora, Belize, (Caricom)  
 Miss M.C.G. Herlin, France, (Private)  
 Mr A. Kakos, Greece, (Private)  
 Mr I. Kanellopoylos, Greece, (Private)  
 Mr N. McDonald, Ireland, (Private)  
 Mr R. A. McMullan, UK, (SAAS)  
 Mr A. Millan Baglina, Chile, (Private)  
 Mr P. Minimulu, Papua New Guinea, (British Council)  
 Mr R. W. Newton, UK, (BBSRC)  
 Mr R. L.A. Oliver, UK, (SAAS)  
 Mr C.E. Price, UK, (BBSRC)  
 Mr M. Schram, France, (Private)

Miss J. E. Smith, UK, (BBSRC)  
 Mr Y.W. Tan, Singapore, (Ministry of National Development,  
 Singapore)

### MSc Aquatic Pathobiology/Aquatic Veterinary Studies 2002/2003

Mr S. Alonso, Spain, (Private)  
 Miss M. Andres, Spain, (Private)  
 Mr V. Andres, Spain, (Private)  
 Mr J. E. Baily, UK, (Private)  
 Mr D. Beltran-Alcrudo, Spain, (Private)  
 Mr J. Castaneira, Spain, (Private)  
 Mr L. Christofilopoulos, Greece, (Private)  
 Miss H. Chubb, UK, (Fishmongers Guild)  
 Miss J. M. Covello, Canada, (Private)  
 Mr M.S. Haberfield, UK, (Private)  
 Mr E. Leguay, France, (Directorate General of Research and  
 Education)  
 Mr W. Leschen, UK, (Private)  
 Mr. R. G. Lloyd, UK, (Private)  
 Mr S. Menanteau, France, (Private)  
 Mr B. Ross, UK, (Private)  
 Mr G. Sirbopoulos, Greece, (Private)  
 Miss J.L. Smith, UK, (Direction Generale d'Enseignement et  
 de Recherche, Ministry of Agriculture)

### MSc by Research

Mr R. Serwata, Diploma in Animal Technology, UK, (Private)

## Appendix IV ► ► ►

### PhD Theses 2002-2003

- Basso Mercado B. Protein digestibility and proteinase inhibitors: tannic acid and gossypol effects on growth and performance of *Macrobrachium rosenbergii* (De Man, 1879).
- Berrill I. Environmental influences on growth, maturation and smoltification in Atlantic salmon parr, *Salmo salar*.
- Carter P. The impact of low concentrations of cadmium on host-monogenean interactions.
- Chara J. D. Interactions between biodiversity and land use in low-order stream catchments of the Colombian Andes.
- Christofilogiannis P. Use of antibiotics in Greek mariculture.
- Crichton C. Responses of the freshwater snail, *Lymnaea peregra*, to pollutants as an indicator of ecological water quality.
- Dixon T. J. Molecular genetic studies of pollutant response in the European flounder, *Platichthys flesus* (L.).
- Felsing M. An experimental approach to determining the fate of mariculture waste.
- Futter W. Perception of light by rainbow trout (*Oncorhynchus mykiss* W.) and its role in development and maturation.
- Halfyard L.C. A biochemical study of egg quality and first feeding diets in common wolffish (*Anarhichas lupus*) aquaculture.
- Hastings N. Teleost fatty acyl desaturase genes: a comparative study.
- Marshall L. Dietary immunomodulation in salmonids with particular reference to organic chromium.
- Sanchez-Perez N. Dietary lipid quality in stored diets and effects on feed performance and feeding of juvenile *Penaeus indicus*.
- Sayed S. A comparison of immune responses and disease resistance in clonal lines of Nile tilapia *Oreochromis niloticus* L.
- Yuksel S. Studies on the characterisation and detection of *Piscirickettsia salmonis*.
- Del Campo Barquin L. M. A bio-socio-economic simulation model for management of the red sea urchin fishery in Chile.
- Jeng S. C. Technological and economic adaptations in aquaculture development in Taiwan
- Wayland M.T. Studies on the biosystematics of species of the genus *Echinorhynchus* (Acanthocephala).
- Taparhudee W. A technical, economic, and environmental assessment of intensive and closed cycle shrimp culture systems in Thailand.
- Retamales R. A. A study of semi-intensive shrimp culture in Ecuador in relation to physical, chemical and biological conditions in the production ponds during El Nino and La Nina events (1996 to 1999).
- Riddell D. Behavioural responses of an experimental aquatic food web.

### MSc Aquaculture Theses 2002-2003

- Av Reyni L. The use of pure oxygen in the pre-growing unit at a sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) hatchery in Portugal.
- Baglina A. M. The efficacy of alternative compounds for the treatment of *Ichthyophthirius multifiliis* (Fouquet, 1870) infecting rainbow trout *Oncorhynchus mykiss* (Walbaum).
- Buray P. R. The effect of dietary arachidonic acid on milk quality in the Atlantic halibut (*Hippoglossus hippoglossus*).
- Campbell S. n-3 Fatty acid requirement of *Macrobrachium rosenbergii*.

- Dickson G. Comparison of reproductive and hatchery performance of *Oreochromis niloticus* strains under commercial seed production conditions.
- Dowd S. Observations on the cardinal tetra (*Paracheirodon axelrodi*) ornamental fishery with an emphasis on assessments of stress.
- Gislason H. R. The role of antioxidants in fish feed pigmentation.
- Gongora M. E. Resource management in Belize: the diversification of aquaculture using geographical information systems.
- Herlin M. A nitrogen budget for farmed Atlantic cod (*Gadus morhua* L.).
- Kakos A. I. A quantitative assessment of the efficacy of Hyperkote™ for foulant removal from fish cage farming.
- Kanellopoulos I. Effectiveness of safety legislation in fish-farming.
- McDonald N. Investigations on feed related ammonia concentration in commercially reared Atlantic halibut *Hippoglossus hippoglossus* (L.).
- McMullan R. A. An examination of rainbow trout *Oncorhynchus mykiss* (Walbaum) egg incubation success in the wild in Scotland.
- Minimilü P. Broodstock management of brown trout (*Salmo trutta*) using microsatellite and passive integrated transponder (PIT) tag systems.
- Newton R. W. Determinants of egg quality and the significance of blastomere morphology for assessment of egg quality in cod *Gadus morhua*.
- Oliver R. L. A. Investigation into the growth performance of juvenile Nile tilapia, *Oreochromis niloticus* L. fed on mixed feeding schedules, with particular attention to the compensatory response.
- Price C. A participatory observation study on the use of pesticides in integrated farming systems of central Thailand.
- Schram M. A qualitative study of the attachment mechanisms of fouling organisms onto fish-cage nets coated with Hyperkote™, a novel fouling-release coating.
- Smith J. E. The effects of fallowing on the biodiversity of sediments under Atlantic salmon marine cages.
- Tan Y. W. Investigation of particulate waste dispersion from a salmon farm and comparison with results predicted by spreadsheet and GIS waste dispersion models.

### MSc Aquatic Pathobiology/Veterinary Studies Theses 2002-2003

- Alonso S. An investigation of a skin disease in sea bass (*Dicentrarchus labrax*) in a commercial Portuguese farm.
- Andres M. Effects of *Hafnia alvei* on sea bream (*Sparus aurata*) during the course of an experimental infection.
- Andres V. Towards the control of *Saprolegnia* in summer smolts of Atlantic salmon (*Salmo salar* L.).
- Baily J. E. A histopathology based investigation of chronic erosive dermatopathy in Murray cod, *Maccullochella peelii peelii*.
- Beltran-Alcrudo D. Effect of the organophosphate methyl parathion on the immune response of white shrimp (*Litopenaeus vannamei*, Boone, 1931).
- Castaneira J. Evaluation of the life cycle and pathology of the myxosporean *Chloromyxum* sp. in salmonids.
- Christofilopoulos L. Investigating Atlantic salmon (*Salmo salar* L.) escapes from Scottish marine farm sites.
- Chubb H. M. A. Aspects of the cod (*Gadus morhua*, L.) immune response to an experimental infection with *Lernaecocera branchialis* (L.).
- Covello J. M. Molecular weight characterisation of Atlantic cod (*Gadus morhua* L.) IgM, and the specific antibody response elicited by immunisation with various antigens.

- Haberfield M. S. Use of ELISA to detect pre-exposure to fish pathogens.
- Leguay E. Pathology of *Argulus foliaceus* (Linnaeus) on *Oncorhynchus mykiss* (Walbaum).
- Leschen W. A. Development of a case control study (for the identification of risk factors) for infectious pancreatic necrosis (IPN) in Scottish salmon farms.
- Lloyd R. G. The use of polymerase chain reaction technology to detect aquatic *Mycobacterium* spp.
- Menanteau S. Prevalence of lysogeny among diverse strains of luminous vibrios.
- Ross W. B. A study of possible factors associated with mortality in migrating Atlantic salmon (*Salmo salar* L.) in a fish pass in north-west Ireland.
- Sirbopoulos G. The effect of dietary palm oil on immune function parameters in rainbow trout (*Oncorhynchus mykiss*).
- Smith J. L. The pathology of the crustacean parasite, *Lernaeocera branchialis* (Linnaeus, 1769) on its fish hosts.

## Appendix V

### Publications

- Adams, C., Braithwaite, V., Huntingford, F., Kadri, S., Pottinger, T. and Turnbull, J.F. (2002) Fish Welfare. Briefing Paper 2, Fisheries Society of the British Isles, Granta Information Systems. <http://www.leicester.ac.uk/biology/fsbi/welfare.pdf>
- Bakopoulos, V., Pearson, M., Volpatti, D., Gousmani, L., Adams A., Galeotti, M., Richards, R.H. and Dimitriadis, G.J. (2002) Investigation of media formulations promoting *Photobacterium damsela* subsp. *piscicida* antigen synthesis recognized by sea bass serum raised after experimental infection. *Journal of Fish Diseases* **25**, 1-13.
- Bakopoulos, V., Volpatti, D., Gousmani, L., Galeotti, M., Adams, A. and Dimitriadis, G.J. (2003) Vaccination trials of sea bass, *Dicentrarchus labrax* (L.) against *Photobacterium damsela* subsp. *piscicida*, using novel vaccine mixtures. *Journal of Fish Diseases* **26**, 77-90.
- Basavaraju, Y., Penman, D. J. and Mair, G. C. (2003) Stock evaluation and development of a breeding programme for common carp (*Cyprinus carpio*) in Karnataka, India: progress of a research project. *NAGA, The WorldFish Center Quarterly* **26**, 30-33.
- Bell, J.G. (2003) Omega-3 deficiency among patients with autism. *Legemidler & Samfunn (Medicine and Society)* **24/4**, 28-31.
- Bell, J.G., McGhee, F., Campbell, P.J. and Sargent, J.R. (2003) Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil "washout". *Aquaculture* **218**, 491-499.
- Bell, J.G. and Sargent, J.R. (2003) Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture* **218**, 491-499.
- Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R. and Crampton, V.O. (2003) Substitution of marine fish oil with linseed and rapeseed oils in diets for Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition: restoration of fatty acid composition following "washout" with fish oil. *Journal of Nutrition* **133**, 2793-2801.
- Bell, M.V., Dick, J.R. and Porter, A.E.A. (2003) Pyloric ceca are a major site of 22:6n-3 synthesis in rainbow trout (*Oncorhynchus mykiss*). *Lipids* **38**, 39-44.
- Bell, M.V., Dick, J.R. and Porter, A.E.A. (2003) Tissue deposition of n-3 FA pathway intermediates in the synthesis of DHA in rainbow trout (*Oncorhynchus mykiss*). *Lipids* **38**, 925-931.
- Berrill, I. K., Porter, M. J. R., Smart, A., Mitchell, D. and Bromage, N. R. (2003) Photoperiodic effects on precocious maturation, growth and smoltification in Atlantic salmon, *Salmo salar*. *Aquaculture* **222**, 239-250.
- Bostock, J.C., Telfer, T.C., McAndrew, B., Penman, D., Young, J. and Muir, J. (2003) *The potential impact of technological innovation on the aquaculture industry*. A Report to the Royal Commission on Environmental Pollution, London.
- Brown, J.H., McCauley, S., Ross, B., Taylor, A. C. and Huntingford, F. (2003) A test of two methods for marking larvae and post larvae of the giant freshwater prawn, *Macrobrachium rosenbergii*. *Aquaculture Research* **33**, 49-54
- Brown, J.H., Ross, B., McCauley, S., Dance, S., Taylor, A.C. and Huntingford, F. (2003) Resting metabolic rate and social status in juvenile giant freshwater prawns, *Macrobrachium rosenbergii*. *Marine and Freshwater Behaviour and Physiology* **36**, 31-40.
- Campos-Ramos, R., Harvey, S. C., McAndrew, B. J. and Penman, D. J. (2003) An investigation of sex determination in the Mozambique tilapia, *Oreochromis mossambicus*, using

- synaptonemal complex analysis, FISH, sex reversal and gynogenesis. *Aquaculture* **221**, 125-140.
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