



UNIVERSITY OF
STIRLING

The Institute of Aquaculture
Annual Report 2003-04



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Cover Image: Cercariae of *Cryptocotyle lingua*, a digenean parasite of cod. Confocal image by Dr J Bron.



- ▶ Two new lecturers appointed in molecular ecotoxicology and reproduction
- ▶ Departmental superintendent appointed to manage and develop technical services
- ▶ Distance learning MSc to be developed with Bangladesh Agricultural University
- ▶ Management of DFID Aquaculture and Fish Genetics research programme to be extended to 2006
- ▶ SRIF-funded laboratory and equipment refurbishment in progress
- ▶ Stirling Civic Awards to staff for research on rice-fish culture
- ▶ Darwin Initiative award for conservation of endangered Mexican fish species
- ▶ Substantial DEFRA funding received for research into welfare of farmed fish
- ▶ Major NERC funding for study on diagnostic molecular responses to pollutants in marine fish
- ▶ Expansion of research on cod at Machrihanish and hatchery development for other marine species
- ▶ Increased involvement in collaborative research on the role of unsaturated fatty acids (HUFA) in human neurodevelopmental disorders
- ▶ Research on photoperiod regulation of growth and maturation of cod has led to the development of management strategies in commercial farms
- ▶ A LINK aquaculture programme on selective breeding of rainbow trout has contributed to breeding programmes by a number of commercial farms
- ▶ Management strategies have been developed for crustacean parasites of stillwater trout fisheries



The Institute has continued to make good progress in 2003-2004 with significant developments in many areas. The Report of the Departmental Review carried out by senior University and external academic staff was very positive and endorsed our strategy, particularly in research. We intend to continue on a broad research front to maintain our unique reputation as a centre for all aspects of sustainable aquaculture. Our international development work has always been vital to our success and in this respect I am very glad to be able to report that Professor Muir will continue to manage the DFID Aquaculture and Fish Genetics Research Programme for a further year until 2006. The Institute receives a good deal of favourable publicity for its overseas development work and we were delighted that David Little and Anton Immink received Stirling Civic Awards for their DFID-funded work on rice-fish culture.

The SRIF funded refurbishments are currently underway and work in the tropical aquarium and some other laboratories has been completed. Work is now in progress on new molecular biology laboratories and in the new year will commence in the biochemistry laboratories, the final area involved. New equipment will start to arrive shortly as refurbishments in the relevant laboratories are completed. Taken together both laboratory and equipment refurbishment will substantially increase our capability in many research areas. A number of relevant new grant applications have been submitted and substantial new research council funding for genomic and proteomic work has already been received. There has been a welcome upsurge in successful grant applications in 2003-2004, particularly to research councils, although EUFP6 funding has been disappointing thus far.

There have been significant new staff appointments in 2003-2004. Dr Mike Leaver and Dr Herve Migaud have been appointed to lectureships in Molecular Ecotoxicology and Reproduction, respectively. These appointments will enable us to continue to develop these important and well funded research areas, as well as exploiting new equipment and facilities. We now have a strong core of younger lecturing staff which will ensure an element of succession in most current areas of research and teaching.

Mrs Cathryn Dickson has been appointed Departmental Superintendent with overall responsibility for technical services throughout the Institute. This is the first time for many years that we have made such an appointment and Cathryn will help to improve management and development of our extremely valuable and dedicated technical staff.

The Institute's MSc courses continue to attract good numbers of students but at both MSc and BSc levels it has proved difficult to substantially increase student numbers. The Commonwealth Scholarship Commission funding received by Dave Little and Andy Shinn to develop a distance-learning MSc in Aquatic Resources Development, in conjunction with Bangladesh Agricultural University, Mymensingh (BAU), is thus particularly welcome. This model of course development may well be applicable in other situations and could represent a major opportunity to grow student numbers. It is very appropriate that this agreement with BAU should have taken place on the 25th anniversary of what has been a very fruitful collaboration between our two institutions. It is also fitting that the Institute should have been represented at the

anniversary celebrations in Mymensingh by Professor Christina Sommerville in her position as Dean of the Faculty of Natural Sciences, since she was the very first member of Institute staff to visit BAU.

The Institute will seek to continue to develop its research, teaching and commercial activities to fully exploit its resources and to take advantage of all available funding, while keeping our goal of remaining a pre-eminent centre for aquaculture research.



In 2003-2004 three students graduated with honours degrees in Aquaculture, one with a first, one an upper second and one a lower second class honours degree. We were particularly pleased to be able to award a first class honours degree to Craig MacIntyre who is continuing with postgraduate research in the department. Two students graduated with honours degrees in freshwater science, one with a first and one with a lower second class honours degree. Again, we would particularly like to congratulate Jafet Andersson (from Sweden) on his first class honours degree.

Overall numbers of undergraduate FTEs (full time equivalents) in 2003-2004 were, as expected, double those of the previous year. At least in part, this was due to reorganisation of the core science programmes offered by the now combined School of Biological and Environmental Sciences that provide a slightly reduced range of options in line with considerable staffing reductions. We would expect that the total FTE income from undergraduate teaching will remain at or around the 2003-2004 level in subsequent years. There is little or no opportunity to expand undergraduate teaching as the university operates to a quota set by SHEFC where only part-time or non-EU students fall outside imposed limits. Our target remains a total of 25-30 aquatic science students in year 4 (split between Marine Biology, Aquaculture and Freshwater Science) that would ensure a reasonably healthy overall undergraduate FTE income to the department.

The MSc Aquaculture course was run in 2003-2004 with 15 students, including five from non-EU countries. The Royal Highland and Agricultural Society of Scotland Silver Medal was awarded to Hugh Mortimer and the External Examiner's Prize to Eric Leclercq. Distinctions were awarded to Hugh Mortimer and Brian Chua.

The MSc in Sustainable Development was run for the first time with three students and it is hoped numbers will now build up to viable levels.

Fifteen new research students commenced their studies in 2003-2004, including seven from non-EU countries. This is a very similar number to the past few years. The cessation of University studentships in future years is disappointing since the Institute had been very successful in obtaining matching funds from outside bodies to leverage extra, high class EU students from the available University funding. Only seven postgraduate students graduated in 2003-2004, a smaller number than usual especially when compared with the very large number graduating last year. We would anticipate increased numbers to graduate next year.

Dr David Little and Dr Andy Shinn have received a substantial grant of £197,719 from the Commonwealth Scholarship Commission to develop a new MSc programme by distance delivery. The MSc in Aquatic Resource Development (ARD) is being co-developed by staff at the Institute of Aquaculture, and by Bangladesh Agricultural University (BAU), Mymensingh. The modular programme has been developed to empower individuals who wish to advance their education and develop the skills necessary to run and manage aquatic systems without having to leave their employment and lose income earning potential. The development and realisation of the WebCT-based distance learning course will develop their understanding and enjoyment of the field of aquatic resource development. The course will provide participants with a range of skills and abilities, supported by a strong theoretical framework, capable of being used by them to develop, run and manage aquatic systems. The course also fills a vital demand identified by DFID Bangladesh and other development agencies to ensure education is relevant to development demands. The new course will be launched in March 2005.

The annual fish disease course was held in January with 23 participants. SCOTPIL courses for Home Office personal licence holders were again run and several student groups from UK and European higher education establishments visited the Institute and Howietoun.



Parasitic Crustacea remain the major research focus of the Parasitology Group with three major projects exploring the control and transcriptomics of salmon lice, *Lepeophtheirus salmonis*, and another investigating the gadoid parasite, *Lernaeocera branchialis*. In addition to these marine species, three other projects looking at the freshwater crustacean genera, *Argulus* and *Ergasilus* are on-going. In the UK, these latter parasites constitute a problem in stillwater trout fisheries.

Dr James Bron, in collaboration with Professor Alan Teale of IOA and colleagues from the University of Liverpool has continued work on data arising from the BBSRC-funded microarray project into gene expression of infecting salmon louse copepodid larvae. This work includes the sequencing and identification by homology of unknown transcripts from the array and the analysis of patterns of gene expression over time. A PhD study by Keith Walton in collaboration with FRS Marine Laboratory, Aberdeen and funded by Novartis Animal Health, uses established *in vitro* and *in vivo* techniques to isolate proteins secreted by sea lice into their environment and to study their effects on the immune system of Atlantic salmon.

Janet Stone at the Marine Environmental Research Station at Machrihanish continues to explore novel compounds which may be developed as treatments for the control of sea lice. New compounds have been screened in the last two years and some exciting new developments are taking place which should result in greater improvements in fish welfare. Field trials are now being planned as part of the next

stage of development. While previous work has contributed to greatly improved treatments and control strategies, further development of new control methods is vital to ensure there are a range of different treatments available to suit all situations and to reduce the likelihood of lice developing resistance to existing treatments.

Nick Taylor's PhD on the epidemiology and population ecology of *Argulus* spp. infections in UK stillwater trout fisheries has been completed. The project has successfully determined the nature, extent and distribution of problem infections and identified several risk factors associated with them. Novel quantitative techniques have been successfully applied to elucidate important information on the population ecology of the parasite and have determined key times at which control interventions should be targeted. The project has led to the development of a series of management strategies to control and minimise the impact of the parasite, and this information has been disseminated to the industry through the Environment Agency and trout associations that provided funding for the project.

Andy Tildesley continues his work on the population dynamics of the copepod gill parasite *Ergasilus sieboldi*, a pathogen that has recently caused significant economic losses in a number of UK stillwater sport fisheries. Mr Tildesley's preliminary investigation carried out in 2003 for Anglian Water has now



Sea lice, *Lepeophtheirus salmonis*, remain a major pathogen in Atlantic salmon farming and their biology and control are an important research area within the Institute.

been expanded into a full time MPhil project to investigate the free-living stages of the parasite alongside the adult stages. The study which is sponsored by Anglian Water and based at Rutland Water employs cage trials as well as various capture methods and laboratory-based research to understand the life-cycle of the parasite. One aim of the project is to develop a management strategy for fishery managers in order to reduce any economic loss associated with this parasitic infection.

Adam Brooker is now in the second year of his NERC-funded PhD programme of research which focuses on the biology and behaviour of *Lernaeocera branchialis*, a large parasitic copepod that attaches to the gills of cod and other gadoids and anchors itself within the heart of its host. Infections in juveniles commonly lead to poor growth rate, reduced swimming performance, anaemia and, if several copepods are present, death of the host. Mr Brooker's research, under the supervision of Dr Shinn and Dr Bron, continues to investigate naupliar-copepodid development and the mechanisms of host finding by the copepodid and the pre-metamorphosed adult female stage.

Other areas of research focus are on the Myxozoa, Protozoa and the Monogenea. Astrid Holzer has completed her PhD thesis and started a new project which continues her research on myxozoan parasites. Using predominantly molecular tools, the project investigates the seasonality of myxozoan infections in a local brown trout farm. Five species of myxozoans have been detected and their migration and localisation in the host throughout the year has been studied using PCR and *in situ* hybridisation.

The ciliate protozoan, *Ichthyophthirius multifiliis*, or white spot disease, now represents the major parasite problem for the UK trout farming industry, accounting for an estimated 2-5% loss in annual production, amounting to £2 million in lost revenue. The DEFRA-BTA funded research undertaken by Andy Shinn and Nick Taylor continues to develop effective farm management strategies in combination with a regime of chemotherapy to minimise the impact of this highly pathogenic parasite.

Miss Adriana Garcia-Vasquez has recently joined the Parasitology Group from Colombia to begin a PhD investigating the host-parasite interactions between the ectoparasitic monogenean *Gyrodactylus cichlidarum* and species of tilapia fish. It is anticipated that the information gathered from the project will lead to improved management and control strategies, which would in turn minimise the impact that this parasite has in tilapia hatcheries and juvenile rearing ponds.

Continuing the group's research interests in image analysis and the semi-automatic identification of parasite species, Dr Shinn and Dr Bron have received a grant from the University of Adelaide to develop an enumeration and segmentation technique for the determination of parasitic Monogenea on kingfish. It is proposed that the developed system will help farm managers monitor the levels of infection on farm stocks.

Four scientists from the Institute of Parasitology, Czech Academy of Sciences led by Professor Tomas Scholz made study visits to the Parasitology Laboratory in 2004 under the European Union's Access to Research Infrastructures Action of the Improving Human Potential Programme and further visits are planned for 2005. They investigated the taxonomy and biology of cestodes and myxozoans from freshwater and marine fish.

The Aquatic Vaccine Unit and Aquatic Research Facility (disease aquarium), active partners in the Scottish Fish Immunology Research Centre (SFIRC), continue to provide a platform for research in rapid diagnostics and vaccine development for aquaculture.

The AVU research laboratory focuses on two main areas of disease control -the development of rapid methods for the detection of fish pathogens, and the development of fish vaccines. Research grants held for studies on the immunology of proliferative kidney disease (PKD)(DEFRA), standardisation of methods for the detection of infectious salmon anaemia virus (ISAV) (EC), and the collection of data to develop an automated fish vaccination machine (SMART) have been completed.

Dr Morris and Professor Adams have continued their research into PKD, a parasitic disease that is a major constraint on the rainbow trout industry. A 3 year DEFRA project examining

culture techniques and humoral immunology identified antigens that may be important in conferring immunity to fish and led to a continuation in funding to express these antigens and examine them in a series of vaccination trials, with the ultimate aim of developing a vaccine against PKD. A new three-year grant (DEFRA/Schering-Plough Aquaculture) will therefore start shortly with Charles McGurk as the post-doctoral scientist. Dr Morris and Professor Adams have also been awarded a new three-year BBSRC funded project to complete the life cycle of *Tetracapsuloides bryosalmonae*, the myxozoan parasite that causes PKD. The life cycle of *T. bryosalmonae* has only been partially elucidated, with infected freshwater bryozoa known to release stages that infect salmonids. This project aims to fill the gap in our knowledge by identifying how bryozoa become infected and the role that salmonids have in the life-cycle. This will be achieved by a combination of transmission trials, molecular and immunological techniques. It is envisaged that this research will provide important information into the life cycle of *T. bryosalmonae* that will aid future studies into the parasite epidemiology and the control of the disease.

Much of the other research in the AVU involves bacterial pathogens (*Flavobacterium psychrophilum*, *Renibacterium salmoninarum*, *Photobacterium damsela* subsp. *piscicida*, *Mycobacterium* spp., *Piscirickettsia salmonis* and *Aeromonas hydrophila*), with one project on nodavirus. Dr Thompson and Professor Adams were recently awarded a new three-year grant (DEFRA/Schering-Plough Aquaculture) to develop a vaccine against rainbow trout fry syndrome caused by *Flavobacterium psychrophilum*. The project will start in December 2004 with Dr Alison Morgan as the post-doctoral scientist. RTFS continues to be one of the most significant diseases affecting the rainbow trout fry and fingerling industry in the UK. Antibiotic treatment of infected fish is currently the only method of control as no commercial vaccine is available to prevent the disease. This proposal aims to develop control strategies for RTFS to assist the trout industry.

Basic immunology research is also continuing with a new three-year BBSRC grant awarded to Professor Adams and Dr Thompson in collaboration with Professor Secombes from the University of Aberdeen (partners in the Scottish Fish Immunology Centre). This project will develop methods to measure specific-cell mediated immunity in fish and its role in disease resistance. From a fundamental perspective, this information will contribute to our understanding of the evolution of the immune system within vertebrates, and whether immunoregulation in a fish is significantly different from that in a mammal. It will also assist fish vaccine research. The ability to measure whether a pilot vaccine can effectively stimulate cell-mediated responses would be a major step forward and would potentially allow methods to be established that can avoid challenge of fish by virulent pathogens as a primary means of assessing vaccine performance. A new one year grant, awarded to Professor Adams and Dr Thompson, from the Home Office Animal Procedures Committee (APC) complements this research. The APC project aims to validate serological testing of fish vaccines so that this can replace disease challenges for vaccine efficacy tests where an antibody response correlates with a protective response.

Three PhD students successfully completed their theses this year: Rubina Sheriff on 'The identification, cloning and development of an ELISA for gamma interferon from fish', Alison Morgan on 'The effects of seasonality on the immune response of salmonids' and Joanne Good on

the "Assessment of immune response in Atlantic salmon receiving alternative oil diets". The Aquatic Vaccine Unit continues to support a large number of research students engaged in studies on the detection and control of a wide variety of fish pathogens.

Visiting scientists to the Aquatic Vaccine Unit this year included Drs Galina Jeney and Zsigmond Jeney, from the Research Institute of Fisheries, Aquaculture and Irrigation, Szarvas, Hungary, and their research assistant Timea Raez. They visited the AVU as part of the British Council link with Professor Adams and Dr Thompson. The aim of the project is to examine the effect of immunostimulants on immunisation of genetically different lines of carp against erythrodermatitis.

Dr Tom Wiklund from the Laboratory for Aquatic Pathobiology, Åbo Akademi University, Finland visited under the EU Improving Access to Infrastructures Programme to carry out preliminary studies examining the infection of rainbow trout eggs with *Flavobacterium psychrophilum* using confocal microscopy with the help of Dr James Bron.

Staff in the AVU are also involved in promoting science in local secondary schools. Professor Adams was invited to hold masterclasses in Biotechnology at Falkirk College (funded by BP) where students from 6 schools attended. Dr Morris also hosted Jodie Foster from Dollar Academy on a Nuffield bursary studentship examining the invertebrate parasites of Airthrey Loch.

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, a spin out company established 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.

This area of research has been very successful over the last year obtaining funding for 3 major research projects from DEFRA and the Niall Bromage studentship from the British Trout Association (BTA).

A paper is now in press from work conducted with the University of Glasgow and Scottish Quality Salmon (SQS), describing the development and application of a novel multivariate technique to analyse the effects of stocking density on the welfare of Atlantic salmon in marine cages. This significant development in the evaluation of fish welfare has already been applied in a DEFRA funded project on trout welfare that was successfully completed in 2003 and is now being used in two new DEFRA grants in collaboration with CEFAS Weymouth and the University of Bristol. These two projects on the impact of water quality and fin damage on the welfare of rainbow trout have the support of the BTA, which has also created the Niall Bromage studentship in recognition of Niall's contribution to the trout industry. The first award of this studentship has come to Stirling for a PhD on trout welfare and has been awarded to Mr Craig MacIntyre, a Stirling honours graduate who will be jointly supervised by Drs Turnbull and Bron. In addition to the staff already mentioned Dr North is the main post-doctoral researcher on the DEFRA grants.

A project has been funded by DEFRA to model the potential spread of exotic fish pathogens in the UK. This ambitious

project in collaboration with the Universities of Liverpool and Lancaster, and in association with CEFAS Weymouth, ultimately aims to use both statistical and mathematical models to test and refine disease control strategies. A conceptual model of potential disease spread has been constructed. The group have already improved existing mathematical models of disease spread and adapted them for aquatic systems. Dr Thrush (a PhD graduate from Stirling) has also developed a spatially explicit contact model for farms and watercourses in England and Wales.

A project on the histopathology and epidemiology of category II parasites in collaboration with the Environment Agency (EA) supervised by Dr Turnbull and Professor Ferguson has made excellent progress. It has quantified some of the risks associated with movement of coarse fish and improved our understanding of the impact of these pathogens.

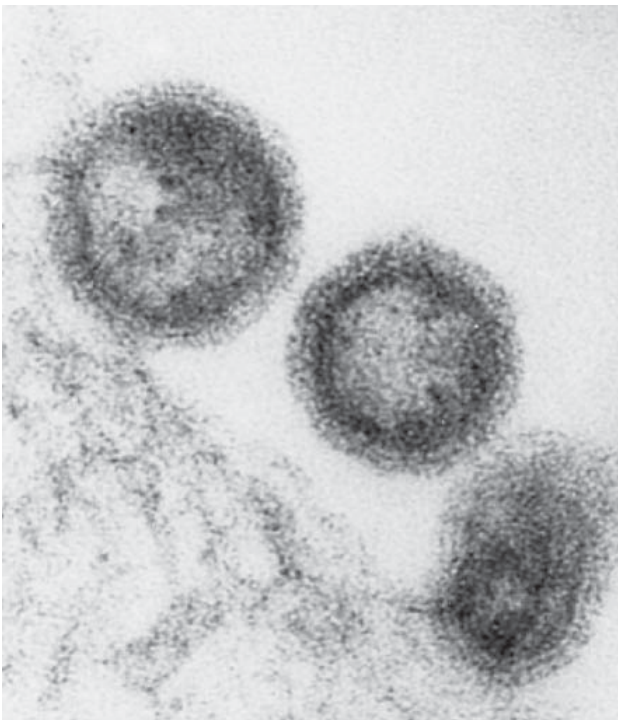
A DFID funded project on a disease of farmed *Pangasius* spp. catfish in the Mekong Delta of Vietnam has been completed recently under the direction of Dr Crumlish and in collaboration with Professor Young of the Marketing Department at Stirling, Scottish Agricultural Colleges, Edinburgh and Can Tho University, Vietnam. The associated bacterial pathogen has been identified and the pathology described. In addition the epidemiological component of the project demonstrated that the problem was a propagating epidemic within cage groups and proposed methods to improve detection and limit the spread of disease outbreaks. This information was combined with marketing and social science expertise to provide practical disease control strategies for farmers and fish health services throughout the Mekong Delta. Dr Crumlish visited Vietnam during August 2004 and in a series of workshops and seminars discussed the findings with stakeholders and developed proposals for the dissemination of the findings and monitoring of the impact of the project on the livelihoods of families in the area.

A DFID funded project "Impact of Aquatic Animal Health Strategies on the Livelihoods of Poor People in Asia", was jointly directed by Dr Turnbull and Dr Chinabut (Department of Fisheries, Thailand) and involved participants from Mangalore, India, Ho Chi Minh City and Can Tho, Vietnam and the Universities of Sussex and Liverpool. This project improved our understanding of provision of aquatic animal health expertise in India, Vietnam and Thailand. While it was demonstrated that the participating institutes were very effective at providing expertise directly benefiting poor families, some areas for potential improvement were identified. Retaining diagnostic expertise and the lack of rigorous scientific field-tests of disease control strategies were two of the main challenges. Potential approaches to address these challenges have been proposed, including an online journal to develop a repository of case study material in a self-study format.

Dr Starkey and Professor Richards continue to direct research on the development of diagnostic procedures and vaccine design for viral diseases. A three-year European Union funded project aimed at the development of real-time diagnostic procedures for infectious salmon anaemia virus (ISAV) is nearing completion. Infectious salmon anaemia virus has been isolated from salmonid fish in several countries including Scotland, Norway, Canada, the United States, Ireland, Chile, and the Faroe Islands. 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 real-time nucleic acid sequence based amplification (NASBA) procedures have now been developed for ISAV. Both of these molecular detection methods have been found to be approximately 1000 fold more sensitive than the conventional RT-PCR methods currently used for ISAV detection, with detection limits of between 100 and 10 target copies. The closed-tube format of the real-time assays is less susceptible to amplicon-contamination associated false positive reactions that are an inherent complication of conventional nucleic acid amplification assays. The real-time RT-PCR and NASBA assays have now been used to test extensive panels of clinical samples obtained from ISA outbreaks. Both real-time assays gave excellent results in these studies.

Janina Costa's PhD project aimed at the identification of



Infectious salmon anaemia virus (ISAV) particles viewed by electron microscopy. ISAV has been the subject of an EU FP6 programme on diagnosis and vaccine design.

B-cell epitopes on fish nodaviruses is nearing completion. The results of this project will advance understanding of the immune response to betanodaviruses, and contribute towards the development of vaccines to counter these increasingly important pathogens. In previous years, 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 has now been carried out. The peptide-based epitope mapping study has facilitated the identification of a number of regions of the nodavirus particle that may represent important B cell epitopes, and thus be of importance for vaccine design. The project represents one of the first flow cytometry based epitope-mapping studies of an aquatic viral pathogen.

Gavin Mckinley has continued his PhD project aimed at the characterisation of the role of innate immune responses in infectious pancreatic necrosis virus (IPNV) infection of Atlantic salmon. This project is run in collaboration with Dr Tony Ellis of the FRS Marine Laboratory, Aberdeen. IPNV is currently the most serious viral disease affecting the UK aquaculture industry. IPNV associated losses incurred by UK fish farms have been estimated to be greater than five million pounds annually and the incidence of the disease is rapidly increasing. The aims of the project are to investigate the role of the interferon system in acute and persistent IPNV infection. Gavin has now developed techniques for detection and gene quantification analysis of important components of the innate immune response to viruses, including the interferon inducible Mx gene. The gene expression analyses will be performed in salmonid macrophages, postulated to be an important site of IPNV persistence. The results of the project will facilitate the design of IPNV vaccines.

A project aimed at the development of vaccines against a range of aquatic viruses of economic importance to the aquaculture industry is also ongoing in the virology laboratory. This study is 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. A variety of novel vaccines based on either inactivated whole virus or recombinant proteins have been produced at Stirling and initial efficacy testing of these candidate vaccines has now been completed.

This past year has been one in which cardiomyopathy seems to have dominated our thinking. Mostly this is due to the first diagnosis in Scotland of a disease that is presently causing a lot of concern in the Norwegian salmon industry, namely heart and skeletal muscle inflammation or HSMI. So far we have only one diagnosis in Scotland, but if similar to the situation in Norway, it could easily spread. This disease has been shown to be infectious and is presumed to be viral. The lesions that accompany the disease differ from those seen in pancreas disease and also from cardiomyopathy syndrome, another condition of unknown aetiology found both in Norway and Scotland. It is somewhat ironic that we are being encouraged to eat fish for the benefit of our own cardiovascular system, while the fish themselves are dying from heart failure!

Another interesting diagnosis involved cultured Asian seabass fry (barramundi) from Singapore that were developing severe necrotizing bacterial enteritis leading to perforation of the gut wall, and subsequent peritonitis. Using immunohistochemistry the bacteria associated with the lesions were shown to be *Edwardsiella ictaluri*, a bacterium we have previously shown to be involved with serious outbreaks of disease in *Pangasius* culture in Vietnam.

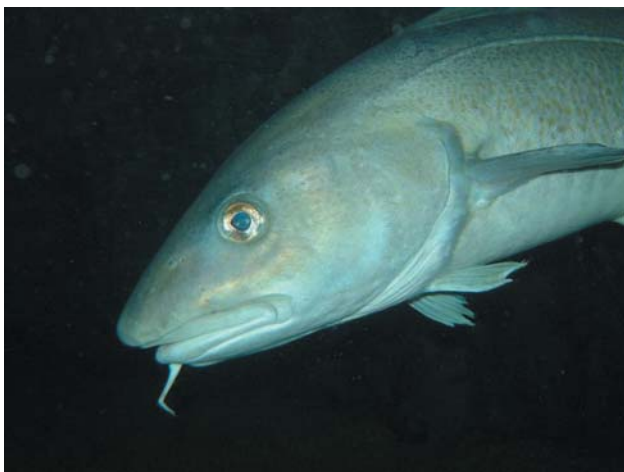


Staff and postgraduate students in Reproduction and Genetics have continued to focus their attention on the management and improvement of broodstock and the production of fish as a controllable and sustainable resource. This involves studies of the photoperiodic control of maturation, puberty and growth, 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.

Andrew Davie is now nearing completion of his 4 year study into photoperiod regulation of growth and maturation in Atlantic cod. In its final year this work has developed close ties with the UK industry to investigate the commercial realities of applying the recommendations the work has developed. The results in winter 03/04 have been promising with management strategies now being adopted at a number of commercial sites both in the UK and North America. This work has resulted in commercially sponsored investigations into the development of lighting technology for use in the commercial management of marine finfish.

Herve Migaud and Andrew Davie were funded to continue



Cod aquaculture is expanding rapidly and this species is the subject of a number of new Institute research projects.

their long running collaborations between Stirling and SAMS Ardtoe marine farm unit in conjunction with Dr Jim Treasurer and Dr Carlos Mazorra through a project sponsored by HIE, SAMS and SEAFISH, on photoperiod control of growth and maturation in haddock. The experimental work is completed with laboratory analysis ongoing. This, in conjunction with the photoperiodic work in Atlantic cod, will inform a commercially orientated study of the management of farmed haddock starting in winter 2004 in collaboration with Canadian research partners.

In October 2002, a 3-year project (Pubertiming) funded by the EC Framework 5 Programme was awarded to Professor Niall Bromage with colleagues from IMR in Bergen (Norway), INRA in Rennes (France), IATS in Torre de la Sal (Spain) and Utrecht University (Netherlands). Dr. Herve Migaud is now the principal investigator for Stirling. This project aims to investigate the photoperiod control of puberty in farmed fish and to develop new techniques and research into underlying physiological mechanisms. The project focuses on three species: salmon, sea bass and trout. It also involves the support of the British Trout Association (BTA) and collaboration with a number

of Marine Harvest 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. John Taylor has been appointed to the project as a Research Assistant. At Stirling the third year will focus on the application of the *in vitro* results on a commercial scale in collaboration with IDEMA Aqua, a lighting manufacturer, and Marine Harvest and project partners to analyse samples.

A project funded by Norwegian enterprises (Intravision and EWOS Innovation) on control of growth and maturation in salmon using new lighting technology was awarded to Dr Herve Migaud and was completed in September with Andrew Davie as a research assistant. This work has led to the development of more formal associations with Intravision and Bodo University (Norway) investigating the development of this novel lighting technology for application in photoperiodic management of marine species like cod. This work will complement the group's existing associations in this field with the IMR Bergen which at present is focusing on investigating the effects of spectral composition and light intensity on melatonin production in cod.

Funded initially by grants from NERC (ROPA Award), the BTA and a Broodstock Research Club to Professor Bromage, John Taylor finished 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. A new European CRAFT project "Effect of photoperiod on feed efficiency, growth and quality traits in diploid and triploid rainbow trout (Luxtrout)" has been submitted in 2004 with Dr Herve Migaud as coordinator and colleagues from Norway (IMR, Bergen), France (INRA and SYSSAF) and 6 SMEs throughout Europe. The main objective of the projects is to evaluate the potential effect of constant light during on-growing as a means to increase productivity, quality and competitiveness of large trout production in Europe.

Funded jointly by Nutreco Aquaculture Research Centre, Marine Harvest and the Faculty of Natural Sciences, Matthew Sprague, under the supervision of Herve Migaud and Gordon Bell, is continuing his investigations on the influence of environmental manipulations (principally light) on the underlying mechanisms governing growth and feeding responses in salmonids. His most recent work has charted a feeding/growth dip following the application of artificial light regimes, a phenomenon commonly reported within the salmon farming industry. Furthermore, the onset of continuous light regimes have led to short-term elevations in the "stress hormone" cortisol, although this is less pronounced in fish where light has been superimposed onto the natural photoperiod. Currently, with the aid of a small research grant from the Fisheries Society of the British Isles, Matthew is studying various growth-promoting hormones (growth hormone, insulin-like growth factor and thyroid hormones) to determine the underlying physiological causes behind this dip. This has led to a collaboration with Dr Jean-Charles

Gabillard (INRA-SCRIBE, France) in order to process samples using techniques which are currently not performed within the Institute. Matthew will begin his final study shortly by applying his tank-based trials into a commercial cage study using new lighting technologies in collaboration with Marine Harvest and IDEMA Aqua.

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 been extended until March 2005 to allow further evaluation of the performance of carp stocks in small farm ponds in India and Vietnam, and the impact on the livelihoods of farmers. The first generation of mass selection for faster growth rate in a synthetic base population of common carp was undertaken, and the selected fish are being grown on to be used as broodstock.

Almas Ara Gheyas completed the second year of a PhD, working on applications of molecular genetic markers to management of aquaculture stocks of carps in Bangladesh. Much of her research is linked to the DFID-AFGRP carp genetics project described above, applying microsatellite DNA markers to estimate heritability for harvest weight in a silver carp stock in Bangladesh and to analyse the first generation of selection for harvest weight in common carp. She is also isolating microsatellite markers from a rohu DNA library.

The EU BASSMAP project is now in its third year and the development of expressed sequence tags (ESTs) has been completed by Dr Helen Whitaker. The next stage of the project is to identify variation associated with the ESTs so that they can be added to the growing gene map. Mapped ESTs are essential to developing comparative genomic approaches as they act as landmarks between maps in different species. We will compare the levels of conservation groups of genes in other mapped species to identify synteny that may have important commercial implications.

The LINK Aquaculture trout selective improvement project has undertaken a major processing trial of commercially reared trout originating from a genetically structured group of families. This was a collaborative project between Glenwyllin Trout Farm in the Isle of Man, Houghton Spring Farm in Dorset, Trafalgar Fisheries and Test Valley Trout in Hampshire. Nearly 3000 fish were individually measured for a variety of traits that included overall growth, fillet weight and an assessment of flesh colour. These fish have been assigned to one of 560 families and heritabilities and genetic correlation between the various traits have been calculated. The results show that the approach adopted in these experiments of communal rearing of all families and subsequent family assignment using genetic markers resulted in significant heritabilities. This information is now being used by the partners to establish their own breeding programmes.

A new BBSRC project on QTLs for IPN resistance will look for markers associated with resistance to IPN infection in young salmon transferred into the sea. This is a collaborative project with Landcatch Natural Selection, the Roslin Institute, and the Marine Laboratory, Aberdeen. The project will look for genetic markers that are associated with fish that die or survive a natural IPN challenge. Landcatch have good evidence of

IPN resistance from their selective improvement programme over 4 generations. The project will undertake a total genome scan using molecular markers from all known linkage groups. Linkage groups which show a close association with IPN will be studied in more detail to identify markers close to the gene that may be causing the effect or even the gene itself. The identification of a QTL would improve the rate of further improvement of IPN resistance in Atlantic salmon.

Mr Anu Frank-Lawale is in the final stages of writing up his thesis on the development of a broodstock replacement and improvement strategy for Atlantic halibut. His work showed that microsatellite genotyping approaches have proved useful in the pedigree analysis of replacement broodstock and in the assessment of growth performance and age of maturation in this long lived species.

A number of PhD students have continued research on sex determination and control. Chuta Boonphakdee is researching the identification and physical mapping of sex-linked and potentially sex-determining genes and DNA sequences in the tilapia genome, whilst Jose Cuitlahac Gallardo is working on physical and linkage mapping of genetic markers in tilapia. He is studying the relationship between physical and linkage distances, and the influence of genotype and phenotype on sex-specific differences in recombination. Marine Herlin is investigating sex determination in cod, and developing molecular genetic tools to analyse contributions to egg and fry production from mass spawning tanks, and the effects of size grading on genetic variation, with a view to developing a selective breeding programme suitable for commercial hatcheries.

During the past year, the molecular genetics research group led by Professor Alan Teale has continued research on the molecular basis of aspects of fatty acid synthesis in teleosts and on antibiotic resistance in aquaculture systems. A major effort has also been put into development of Atlantic salmon cDNA microarrays for studies of transcriptome functions in the context of commercially important traits, including those relating to lipid metabolism.

Research on the desaturase and elongase genes involved in fatty acid synthesis in fish, and on their expression, is conducted in close collaboration with Dr Douglas Tocher and colleagues, and is outlined in the report of the Nutrition Group.

During the past year the major focus of the TRAITS project (Transcriptome Analysis of Important Traits in Salmon, www.abdn.ac.uk/sfirc/salmon), has been production, collection, quality assessment, bioinformatic analysis and archiving of Atlantic salmon ESTs preparatory to development of an Atlantic salmon cDNA microarray. TRAITS is funded under the Exploiting Genomics programme of the Biotechnology and Biological Sciences Research Council, UK through a grant to Professor Teale, Dr Douglas Tocher and Dr John Taggart. The project is a joint one with the Universities of Aberdeen and Cardiff, in partnership with the Norwegian Salmon Genome Project co-ordinated by Prof. Bjørn Hoyheim of the Norwegian Veterinary School, Oslo and ARK-Genomics at the Roslin Institute. The objectives are to characterise the transcriptomic aspects of fatty acid synthesis, protein metabolism, immune response and smoltification in Atlantic salmon. To date, probes for approximately 17,000 salmon genes have been archived, among which are a number

that have been specifically developed by the project to be informative in studies of the traits of interest. Dr Taggart manages the research in Stirling, and during the past year he has co-ordinated the combined effort to make and characterise the clone collection. The major contribution of the Norwegian project to the collection has been particularly valuable. Also during the year, all of the RNA samples that will be needed by the project have been generated in appropriate trait "challenges" and stored. In the coming year the 16k-17k trait-targeted cDNA microarrays will be constructed and used to interrogate these samples.

During the third year of the EU-funded project "Asiarest" Dr Kerry Bartie and Mrs Cathryn Dickson have continued and extended molecular studies of antibiotic resistance occurring in bacteria in aquaculture environments in South East Asia. The primary focus in this project is resistance to chloramphenicol, although resistance to a number of other antibiotics is also routinely examined. The project, which is a collaborative effort involving partners in the University of Cantho in Vietnam, University Putra Malaysia, AAHRI in Thailand, the University of Ghent in Belgium and RILAB in Genoa, has completed a collection of approximately 800 antibiotic resistant bacterial isolates across the three SE Asian countries. The Stirling group leads the research aspects of bacterial strain definition and research on the molecular basis of resistance among the collection. Strain typing has been completed by repetitive PCR, and macrorestriction analysis by pulsed-field gel electrophoresis has been employed to confirm clonal lineages. With respect to antibiotic resistance factors, the majority of chloramphenicol resistance has been found to be mediated by CAT enzymes. Work is now progressing to elucidate mechanisms of chloramphenicol resistance in the minority of isolates not found to possess CAT activity. Preparatory to the final phase of the bench studies, preliminary conjugation studies have revealed the presence of large conjugative plasmids in a proportion of Enterobacteriaceae isolates capable of transferring multiple resistance to an *E. coli* laboratory strain. Dr Bartie will now lead studies on the characterisation of the mobile genetic elements associated with the antibiotic resistance genes with the aim of assessing the potential risk of gene transfer occurring within the aquaculture environment.

Continuing an area of research that was initiated in 2003 with a Natural Environment Research Council Studentship, Ms Karen Frake is applying molecular genetic approaches to study population dynamics among Atlantic salmon in the Dee system of NE Scotland. This work is being undertaken in collaboration with the Fisheries Research Services Freshwater Laboratory, Pitlochry, and the Atlantic Salmon Trust. To date Ms Frake has screened three major sample sets of parents and offspring from the main study site, and progeny assignment levels have been satisfyingly high.



Lipids are the major focus of the Nutrition Group with research continuing into the requirements, metabolism and functions of polyunsaturated fatty acids (PUFA) in aquatic organisms. Fish are the major dietary source of n-3 highly unsaturated fatty acids (HUFA), eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA) acids for humans. With declining food grade fisheries, aquaculture currently supplies over a third of food fish to human diets (24m tonnes of a 71m tonne

total) and this is likely to increase in the future. However, the current high use of fishmeals and fish oils, derived from feed grade marine fisheries, in aquaculture feeds is not sustainable in the longer term, and will constrain continued growth of aquaculture. This 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, very importantly, do not compromise the final product as a high quality food providing high levels of the important and beneficial n-3 HUFA for the human consumer. 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.

The EU FP5 project, "Researching Alternatives to Fish Oil in Aquaculture (RAFOA)" is in its final year and progressing well. In the second phase of the RAFOA project, a diet containing a blend of vegetable oils and fish oil (75/25 w/w) was fed to groups of salmon from first feeding through to harvest, followed by a finishing diet phase. In a parallel trial conducted in Norway, salmon were fed a similar blend but with the vegetable oil (VO) replacing all of the FO. The blend of vegetable oils was chosen to match the ratio of saturated, monounsaturated and polyunsaturated fatty acids found in fish oils, but without the long-chain HUFA. The trial started in March 2002 with first feeding fry and no differences in growth were seen up to seawater transfer in April 2003. The fish continued to grow well in the sea and were harvested at 2.4kg in March 2004. Good growth was achieved during this phase and fish fed the 100% VO treatment had significantly higher final weights than fish fed 75% VO or 100% FO. Specific growth rate and feed conversion ratios were good for all treatments and there were no significant differences between treatments. The fish fed the 75% VO diet were then switched to a 100% FO finishing diet for a further 24 weeks to follow restoration of n-3 HUFA levels and wash out/dilution of the C18 PUFA. After the finishing diet phase the fish had attained a final weight of 5.5kg.

There were no effects of diet on flesh lipid or carotenoid content but the n-3 HUFA, EPA and DHA, were reduced by around 50% and the C18 PUFA, 18:2n-6 and 18:3n-3, were increased by 3- and 6-fold, respectively. However, the decrease in n-3 HUFA and the increases in C18 PUFA were considerably less than when either linseed oil or rapeseed oil were used as single replacements at the same percentage in the 1st RAFOA trials. This result suggests that the balanced dietary fatty acid blend used in the 2nd RAFOA trials was less physiologically challenging and allowed better retention of the n-3 HUFA than previous single oil blends. This is supported by the analysis of non-specific immune parameters which suggested that immune function was less disrupted by using a blend of VO compared to single oil replacement. There were no significant differences in antibody response due to dietary treatment after vaccination against furunculosis. Post-smolts from each treatment were subjected to a challenge trial with *Vibrio anguillarum* but there were no significant differences in mortalities between dietary treatments. Organoleptic analysis of samples of fresh and smoked Norwegian and Scottish salmon was conducted by the Food Industry Forum at Queen Margaret University in Edinburgh. In the fresh salmon analysis, the 100% VO fish were most liked for colour, appearance,

texture and flavour. The result for smoked salmon was even more conclusive with fish fed 75% VO being favoured over fish fed 100% FO for all six taste, texture and appearance categories. Concentrations of dioxin and dioxin-like PCBs were significantly lower in fish fed 75% VO compared to those fed FO.

The Nutrition Group forms an integral part of a European thematic network, "Fish Oil and Meal Replacement (FORM)" funded by the EU 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. 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 2nd consortium meeting was held in Gothenburg, Sweden in June 2004. In addition to updated presentations on the five consortium projects invited speakers gave presentations on traceability and physiological effects of GM feed ingredients, alternative protein sources in relation to fish meal quality, an overview of GMO in aquaculture and genetic modification of oilseed plants to produce n-3 HUFA.

It is important that the more applied dietary trial led projects are supported by studies to investigate the basic biochemical and molecular mechanisms that underpin the applied research through studies aimed at identifying and characterising the factors controlling and regulating lipid and PUFA metabolism in fish. The aim of these studies is to apply modern and emerging biotechnological techniques to the current major problems in lipid and fatty acid nutrition and to provide a new level of information at the molecular and genetic level, along with methodologies and tools necessary to develop novel approaches to the design of new and alternative aquaculture diets. The two areas of current interest are (i) the quantity of oil in the diet driven by the increasing use of high energy (oil) diets, and (ii) the quality of oil in the diet driven by the need to replace the fish oil traditionally used in marine and salmonid fish diets with sustainable, vegetable oil alternatives.

High energy diets can have benefits in terms of cost efficiency through increased growth and decreased production times due to increased energy provision from fat/oil enhancing protein sparing. However, they also increase problems of "fatty fish" due to increased, and undesirable, deposition of fat in tissues. Our aims here are to determine what regulates lipid oxidation and deposition in fish, and how we can affect this and alter the balance between oxidation and deposition of dietary lipids. Wild capture marine fisheries have, at best, reached their sustainable limits, and so the current high use of fish oils in aquaculture feeds is not sustainable in the longer term, and will constrain continued growth of aquaculture activities. The only sustainable alternative to fish oils are vegetable oils, which can be rich in C18 polyunsaturated fatty acids (PUFA) but devoid of the n-3HUFA, EPA and DHA that are abundant in fish oils. The extent to which fish can convert C18 PUFA to C20/22 HUFA varies with species, and is associated with their capacity for fatty acyl desaturation and elongation. Our aims here are to determine what regulates HUFA biosynthesis and how it can be optimised to enable fish to make effective use of dietary vegetable oil.

Over the past several years, the Nutrition Group have employed a candidate gene approach to the study of these two problems specifically focusing on peroxisome proliferator-activated receptor genes, and fatty acid desaturase and elongase genes, respectively. The latter being greatly facilitated by collaboration between Dr Douglas Tocher of the Nutrition Group and Professor Alan Teale, head of Molecular Genetics, to form the Molecular Nutrition Group. As described below, this has been a very successful approach resulting in the cloning of over 20 desaturase and elongase genes from a variety of freshwater and marine species including Atlantic salmon. This represents 2/3 of the total number of these genes so far cloned from fish anywhere in the world and this has placed the Molecular Nutrition Group in an ideal position to exploit the rapid advances and investment being made in the Institute in the application of both transcriptomic and proteomic analyses. The emphasis now is on the identification and quantification of traits at a genotypic level that are critical in determining important phenotypic responses to nutritional change.

Understanding the mechanisms whereby fish regulate lipid homeostasis is particularly relevant to the formulation of aquaculture diets and the optimization of dietary oil contents. Over the last decade it has become apparent from studies on lipid dysregulatory states in human conditions that a family of protein transcription factors, the peroxisomal proliferator-activated receptors, or PPARs, are critical to the regulation of mammalian lipid homeostasis. PPARs are ligand-inducible proteins that affect the transcription of genes. There are three isotypes of PPARs, alpha, beta and gamma, that are coded by separate genes, and have specific tissue distributions and target genes. The target genes for PPARs are predominantly involved in energy metabolism, specifically glucose and lipid metabolism, including genes involved in fatty acid biosynthesis, transport, deposition and storage, oxidation and metabolism. Following research grants from the BBSRC and the EU 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. PPARs can only function as gene regulatory factors after binding fatty acids and other structurally related compounds, and so a major aim of our work was to determine the lipid activation profiles and DNA binding characteristics of fish PPARs. PPAR alpha from all of the fish species displayed a broader unsaturated fatty acid activation profile, and also binds to a broader range of potential DNA sequences (cis-elements) present in the regulatory regions of various genes, than either PPAR beta or PPAR gamma. Specific fatty acids particularly potent in activating fish PPAR alpha such as conjugated linoleic acid (CLA) were identified, and these observations formed the basis of *in vitro* experiments investigating fatty acid- and PPAR-dependent gene expression profiles in primary fish cell cultures. Subsequently, the role of PPARs in regulating lipid and fatty acid metabolism in fish *in vivo* were investigated using diets formulated on the basis of the molecular genetic data summarised above. The dietary trials were carried out in Norway in partnership with Nutreco ARC, and Spain in partnership with Cripesa SA, commercial fish producers, on Atlantic salmon and sea bream, respectively. These studies were designed to correlate dietary dependent-PPAR activation potential and tissue-specific gene expression data with important farm characteristics such as growth performance and feed efficiency, tissue lipid levels and fatty acid compositions. The data are currently still being processed but it is already clear that CLA in particular has significant effects on lipid homeostasis in Atlantic salmon, consistent with it being an

activator of PPAR alpha. This result confirms that PPARs have important roles in the regulation of lipid metabolism in fish and that this can be modulated by dietary/nutritional intervention. Once completed, these studies will provide a mechanistic framework to underpin the formulation of lipid-optimised cost-effective aquaculture diets from readily available and sustainable ingredients.

Conjugated linoleic acid (CLA) is a term used to describe positional and geometric isomers of linoleic acid (18:2n-6) that are known to influence lipid homeostasis and have significant health benefits including decreasing body fat and increasing lean body mass, improving feed efficiency, protection against atherosclerosis, enhancing immune function, and anticarcinogenic properties in mammals. CLA has been shown to be a powerful PPAR agonist suggesting PPARs may be involved in the mechanism of CLA action. Therefore, linked to the work on PPARs, a project is underway to study the effects of dietary CLA in Atlantic salmon. Specifically, the roles of CLA as a potential dietary supplement in fish diets affecting lipid and fatty acid metabolism, and immune system function are being investigated in a BBSRC-funded studentship in collaboration with Prof Sandra Adams of the Aquatic Vaccine Unit of the Institute, with additional support from BioMar Ltd. Mr Sean Kennedy was appointed to the studentship and has carried out a large dietary trial at the Marine Environmental Research Laboratory, Machrihanish. The trial investigated the effects of dietary oil content and graded levels of CLA on growth performance, tissue lipid and fatty acid content, composition and metabolism, and immune function in Atlantic salmon post-smolts in seawater. The trial has produced a very large data set which is still being processed but which should substantially increase our knowledge of the nutritional effects of this interesting fatty acid in Atlantic salmon.

The Molecular Nutrition Grouping of Dr Douglas Tocher and an EU-funded post-doctoral researcher, Dr Xiaozhong Zheng, in collaboration with Professor Alan Teale and Mrs Cathryn Dickson of the Molecular Genetics Group have been applying a variety of biochemical and molecular techniques to the study of HUFA synthesis in fish. Initially, the HUFA synthesis programme aimed to clone and characterize enzymes of paramount importance in the conversion of 18:3n-3 to EPA and DHA. Thus, a range of fatty acid desaturases and elongases, critical enzymes in the pathways for the biosynthesis of the long-chain C20/22 HUFA from shorter chain C18 PUFA, were cloned from a range of freshwater and marine teleosts. Functional characterisation by heterologous expression in the yeast *Saccharomyces cerevisiae* showed that fish fatty acid elongases generally have broad substrate specificity for PUFA with a range of chain lengths from C18 to C22 with activity decreasing as chain length increases. In contrast, fatty acid desaturases in fish, like desaturases from other organisms, are usually monofunctional displaying either $\Delta 6$ or $\Delta 5$ activities, although the desaturase cloned from zebrafish was unique in being bifunctional. Desaturases cloned from carp, trout, turbot and sea bream were all shown to be $\Delta 6$ desaturases, whereas the first desaturase cloned from Atlantic salmon was found to be a $\Delta 5$ desaturase, to date the only such single function $\Delta 5$ desaturase cloned from fish. The search for further fish desaturase and elongase genes continues. Within the last year, a $\Delta 6$ desaturase has been cloned and characterized from Atlantic salmon. Quantitative real time PCR assay of gene expression showed that both $\Delta 6$ and $\Delta 5$ fatty acyl desaturase genes, and a fatty acyl elongase gene in salmon were highly expressed in intestine, liver and brain and less expressed in kidney, heart, gill, adipose, muscle and spleen.

The expression of fatty acyl desaturase and elongase genes in salmon fed vegetable oil diets has been studied through real time quantitative PCR (Q-PCR) and both desaturase and elongase genes in liver have been demonstrated to be under nutritional regulation, being up regulated in fish fed diets containing vegetable oils rich in C18 PUFAs and devoid of C20/22 HUFA. In addition, expression of both $\Delta 6$ and $\Delta 5$ fatty acyl desaturase genes in intestine, red muscle and adipose tissue was significantly increased in salmon fed vegetable oil compared to fish oil. Further studies have investigated the relationships between $\Delta 6$ and $\Delta 5$ desaturase and elongase gene expression and HUFA synthesis through an entire production cycle identifying regulation of gene expression by environmental factors, particularly in relation to smoltification, and nutrition. These studies are elucidating the mechanisms that underpin the nutritional regulation of fatty acyl desaturation and elongation in response to feeding vegetable oil and will facilitate identification of the molecular differences in the pathway between freshwater/salmonid fish, which can grow and survive on diets containing vegetable oil, and marine species which must have significant levels of dietary fish oil for optimum growth and survival.

Marine fish nutrition is an important area that has been less studied, compared to salmonid nutrition, in the UK. However, with the renewed interest in both cod and halibut nutrition this situation will surely change in the near future. Two projects currently address the largely neglected role of arachidonic acid (ARA) in marine fish nutrition. Emma Alorend will shortly submit her PhD on the importance of ARA in egg and larval quality in halibut broodstock. Data collected so far indicates that supplementation of broodstock diets with ARA leads to improvements in egg and larval quality.

In further studies on ARA nutrition, Gordon Bell and Jim Henderson have been involved in trials investigating the role of ARA in larval growth and development in three species of marine fish that show promise as new aquaculture species. This work was supported by DSM Nutritionals and the Australian government and involved researchers from Tasmania, Spain and Scotland working on striped trumpeter, Senegalese sole and Atlantic cod. These studies have recently been completed and initial data analysis suggests that dietary ARA is involved in modulating stress reactions and skin pigmentation, probably in relation to cellular eicosanoid production.

The EU-funded CRAFT project, BYPROFEED, has tested the suitability of Protein Omega Concentrate (POC), which is derived from waste material produced in prawn and shrimp processing, as a replacement for fish meals, FO and carotenoids in commercial aquaculture feeds. This two year programme involving Gordon Bell and Fiona McGhee has studied the inclusion of POC in feeds for Atlantic salmon parr, Arctic char, rainbow trout, cod post-larvae, and lobster juveniles. The dried POC product derived from *Pandalus borealis* contains high levels of astaxanthin and should be a useful raw material for pigmenting salmonid flesh, and should also be highly suitable for inclusion in organic feed formulations. Another POC, derived from *Crangon crangon*, is much lower in carotenoids but both products should still provide important taste attractant properties. Inclusion of POC, at between 5 and 15% in the diet of juvenile char resulted in significantly increased final weight and SGR, and reduced FCR, compared to char fed no POC. Salmon parr

and rainbow trout fed POC from *Pandulus borealis* showed increased flesh pigment deposition and in post-larval cod POC inclusion significantly increased SGR values. The feeding trials conducted during the BYPROFEED project provide evidence that both attractant and pigmentation characteristics could be attributed to POC products.

The Nutrition Group has continued their involvement in projects in human nutrition involved with the role of n-3 and n-6 HUFA in neurodevelopmental disorders. In the past year Gordon Bell has been collaborating with researchers at the Mansfield College and University Laboratory of Physiology, University of Oxford on the role of HUFA in dyslexia. In the dyslexic group only, high concentrations of ARA were significantly associated with visual symptoms when reading, and in both groups a very strong negative correlation was found between n-3 and n-6 concentrations. Better word reading was associated with higher total n-3 concentrations in both dyslexic and control groups. In dyslexic subjects only, reading performance also correlated negatively with ratios of LA/ALA and AA/EPA and with total n-6 trend level. Better spelling was related to higher DHA status only in controls, and to lower LA/ALA ratios only in the dyslexic group. The niacin skin test reflects a prostaglandin mediated flush and oedema reaction owing to the production of prostaglandin D2 from arachidonic acid. A diminished response to niacin may indicate potential abnormalities in membrane phospholipid pathways. Flushing activity was significantly reduced in dyslexia patients compared to controls. These results are consistent with the idea that dietary supplementation with n-3 fatty acids may help some dyslexic adults and controlled treatment trials to investigate this possibility are currently in progress.

Nutritional Analytical Services (NAS) has continued to be very busy. The NAS analysis largely concerns constituents of fish feed and tissues, mostly fatty acids, vitamins and carotenoid pigments. NAS continues to take part in the Roche ring test for carotenoid analysis and the Nutreco Ring test. In addition, NAS will soon qualify as an accredited laboratory as part of the Bureau Inter-Professionnel d'Etudes Analytique (Bipea) Proficiency Testing Scheme for Aquaculture Feeds and Raw Materials. The Nutrition Group has recently taken steps to develop analytical methods for the analysis of persistent organic pollutants (POPs) including dioxins, PCBs and polybrominated diethyl ether (PBDE) flame retardants and it is hoped that analysis of dioxins and PCBs will be available as part of the Nutrition Group research and commercial activities early in 2005.



Fisherfolk from Lake Patzcuaro in the Mexican altiplano examining cultured juvenile whitefish, *Chirostoma estor estor*, the subject of a DARWIN initiative project aimed at protecting this endangered species.

Jutaporn Bundit, working under the supervision of Dr Jauncey, has made a good start on a series of field-based surveys to establish current on-farm feeding practices in Thailand for the locally very valuable marble goby (*Oxyeleotris marmoratus*). Jutaporn has also conducted some initial feeding experiments on marble goby that serve to illustrate how unusual this species is in its' response to formulated feeds. The Nutrition Group continues to work closely with other research activities in the Institute. Examples include the work being done by Carlos Yanes-Roca on the nutrition and larval development of common snook (*Centropomus undecimalis*) and Ali Hajizadeh Kapateh on the effects of lipid quality on reproductive development in tilapia.

Dr Jauncey is part of the supervisory team of Gisela Rios Duran, one of the research students working on the British Council funded project based at INIRENA, University of Michoacan, Morelia, Mexico that is looking at the development of sustainable aquaculture of the endangered fish *Chirostoma estor estor* in marginalised indigenous communities around Lake Patzcuaro in Central México, as well as providing more generalised nutritional support to this project. Gisela is investigating a number of important dietary factors in feeds for the early life history stages of this very fragile species especially those, such as ascorbic acid, that might interact with stress-related mortalities.



Juveniles of *Chirostoma estor estor* being released into a lake in the Mexican altiplano as part of the programme to conserve this endangered species.

Dr Amer Diab and Professor Steve George, together with Dr Tim Williams from the University of Birmingham have utilised their 13000 clone flounder liver DNA microarray in the EU-funded GENIPOL project (www.genipol.stir.ac.uk) to identify diagnostic transcriptional responses to a variety of prototypical compounds elicited by carcinogenic polyaromatic hydrocarbons, polychlorinated biphenyls, chlorinated pesticides, heavy metals, peroxisomal proliferators, endocrine disrupting agents, oxidative stressors and inflammatory agents. From these results a diagnostic miniarray for analysis of chemical impacts in wild populations will be produced. This study, which is the first of its kind in the world, forms part of an integrated genomics and proteomics study and databases of protein expression profiles from 2D gel electrophoresis of these flounder samples have been compiled with Dr Adelina Roskowska of the Centre for Proteome Analysis in Denmark. With the forthcoming SRIF-funded refurbishment of our Institute molecular biology and proteomics facilities most of these studies will be now

carried out in house and the GENIPOL work will be extended with major additional funding from NERC. The genomic, proteomic and metabolomic responses in wild populations of flounders from severely polluted estuaries in the UK and Germany will be analysed to identify key genes and proteins which confer resistance or susceptibility to pollutants. The genetic basis of these responses will be investigated by analysis of neutral (population) markers, and genetic and phenotypic polymorphisms in these key genes.

Professor George as a member of the ecotoxicology committee of the UK National Marine Pollution Monitoring Group and the ICES Working Group on Biological Effects of Contaminants, presented field results obtained by Vicky Sabine using new diagnostic Q-PCR (Real time PCR) methods she has developed for long term status and trends analysis of effects of polyaromatic hydrocarbons, pcbs, dioxins, heavy metals and endocrine disruptive chemicals.

Dr Telfer and his colleagues have continued to pursue research on the impacts and environmental sustainability of aquaculture. Ongoing projects range from the use of modern feeds and feeding technology to an investigation of sustainability indicators in SE Asian aquaculture.

Patrick Reynolds has been based in Norway for much of his PhD investigating the relative environmental effects and nutritional quality of new formulation Atlantic salmon feeds. Data suggests that these will be of benefit to environmental sustainability, by producing less waste and lowering fish-



Richard Corner visiting fin fish cage sites in China as part of the EU funded SPEAR project which will develop a framework for interpreting coastal zone structure and dynamics.

based protein requirements. In addition, he is developing new facilities to collect waste from fish cages during feeding trials. This will be of considerable help in estimating waste and for environmental impact modelling.

Research on the environmental benefits of non-toxic antifoulants continues with further funding from Hyperlast UK Ltd and PRI Ltd, to investigate the efficacy of novel coatings for fish cage nets. This work has received some interest from the Scottish Environment Protection Agency, in that these coatings have the potential to limit distribution of dissolved and particulate copper in relation to aquaculture and therefore may be beneficial for the environment.

Richard Corner has completed his PhD research on the implications of new feeding technologies in environmental

mitigation of cage aquaculture in Scotland. The project has involved extensive recording of waste inputs within the environment using collection devices and has considerably improved dispersion modelling through the GIS medium by accounting for the physical environment in controlling the behaviour of fish cage waste. Steve Cross has studied the water quality interactions between fin- and shellfish and their potential for integrated aquaculture in British Columbia. This is vital work in bringing together the two main, and often competing, forms of coastal aquaculture on the western coast of Canada.

Robert Oliver has started a PhD project investigating waste and nutritional aspects of Atlantic cod culture. The project is co-funded by Seafish Ltd and Highland and Island Enterprise with participation by EWOS Innovation, studying nutrition, growth and environmental implications of cod farming both at the early stages of the life cycle (in tanks) and later on growing to harvestable sizes (in sea cages). Using the information derived, computer modelling of waste dispersion and environmental impacts will be achieved for cod farming to allow environmental sustainability to be taken into account at an early stage in this important new industry.

Dr Telfer's interests in fish cage waste dispersion modelling and coastal zone management models through GIS have expanded. In association with Professor Lindsay Ross, two new PhD projects have commenced looking at different aspects of coastal modelling and integration of all anthropogenic impacts for decision support systems development. This topic is becoming increasingly important in the wider field of coastal management and will be a major development for the future of effective environmental management of coastal aquaculture.

Two new EU 6th Framework funded projects have been funded. The "SPEAR" project is in association with 9 partners throughout Europe and China and investigates sustainable options for people, catchment and aquatic resources in China. This three-year project will develop and test an integrated framework for interpreting coastal zone structure and dynamics, in areas where communities primarily depend on marine resources. The framework accounts for watershed interactions, ecological structure and human activities, using an interdisciplinary approach combining natural and social sciences, and addresses the complex scaling issues inherent in integrated management. Two contrasting systems in China will be studied: Sanggou Bay, part of a rural watershed, and Huangdun Bay, located in an industrialized area south of Shanghai. In both systems, large-scale cultivation of seaweeds, shellfish and finfish are of paramount importance for community income and livelihood. The "CONSENSUS" project is an EU Concerted-Action project bringing together researchers, regulators, managers, consumer organisations and stakeholder organisations, with a view to developing and implementing aquaculture sustainable protocols based on low environmental impact, competitiveness and ethical responsibility with regard to biodiversity and animal welfare.

The TROPICA project, which will give practical guidance for the estimation and allocation of environmental capacity for aquaculture in tropical developing countries, concludes in the next few months. This has been successful in collecting data that allows estimates of carrying capacity for aquaculture and simple indicators of sustainability. At present these data are being analyzed and resulting publications will show the benefits of sustained and managed aquaculture production in Bangladesh and Vietnam.

Dr Willby has been much involved in policy-led research largely in relation to the needs of the EU Water Framework Directive. The focus of this research has been a project to devise a tool for predicting the abundance and composition of aquatic vegetation in reference condition lakes and rivers in the UK and to diagnose the causes of deviation from these conditions. The project involves extensive collaboration with CEH and the University of Ulster, and with scientists in the Environment Agency and SEPA and those undertaking other WFD related research elsewhere in the UK and Europe.

Other areas of research activity over the past year include advising on design and management of sustainable urban drainage systems in central Scotland (funded by SEPA); guidance on strategies for restoring navigation on ecologically sensitive waterways (Interreg); research on hydrological controls on riverine backwater biodiversity on the Upper Tay (jointly with Dr Gilvear of SBES, funded by Scottish Wildlife Trust and the Faculty); investigations of the causes of aquatic plant dieback on trout streams in southern England (English Nature); experimental studies on aquatic plant grazing by snails (National Institute of Ecology, Netherlands); studies on historical changes in aquatic plant communities in the Norfolk Broads (in collaboration with University College London). New funding has recently been acquired from Scottish Coal for a PhD and 15 year monitoring programme on the biological recovery of a newly engineered river channel in Ayrshire.

The past year has been a very busy time for the Environmental Services, the consultancy section of the Environment Group. Considerable success was achieved in maintaining contracts to monitor and provide environmental impact assessments to the Scottish and Irish aquaculture sector in both marine and freshwaters. The work of the analytical laboratories of the Environmental Services has been maintained at a high level with a record number of sediment samples being sent for analysis of fauna and chemical parameters. In addition, the advice given to the industry on environmental matters has increased considerably.

The future for environmental management of the aquaculture industry in Europe may change in the light of the forthcoming Water Framework Directive. To this end the Environmental Services have been keeping close contact with the Scottish Environment Protection Agency on the development and implementation of protocols for future environmental monitoring. In addition, Environmental Services have also been establishing informal links with other environmental consultancies to enable us to expand into new areas.



The Systems Group has maintained a diverse and active range of activities throughout the year, both in the UK and internationally. In close association with the DFID Aquaculture and Fish Genetics Research Programme, with EU funding, and through its varied contract and advisory work, it continues to develop and innovate in a range of themes at an international level. Within the Institute the group has continued its very significant inputs into teaching and PhD supervision, also leading and developing a number of new MSc initiatives. There is continued UK and international 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 staff and resource constraints, through the efforts of key staff, contributions from a range of partners, and the enthusiasm of our postgraduate students, we have been able to maintain significant outputs in diverse fields.

The DFID Aquaculture and Fish Genetics Research Programme (AFGRP), managed by Professor Muir, has provided further opportunities for international partnerships in aquaculture development, within and outside the University, and between the UK and developing country partners and institutions. Here, 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. The support of Professor McAndrew and Dr Penman in the Genetics and Reproduction Group and Dr Turnbull and Dr Crumlish in the Disease Group, have also been very significant. Dr Krishen Rana in particular has also increased our involvement in sustainable aquaculture in Sub-Saharan Africa.

The work and impact of the Group continues to be enhanced by the very active and productive linkage with Professor James Young of the Marketing Department. Important areas



Farmers and researchers in Bangladesh discussing improvements to natural resource management.

of collaboration continue with Dr Trevor Telfer and Dr Nigel Willby of the Environment Group, with the Applied Social Science Department (Dr Samantha Punch) and with the Dept of Nursing in areas of social research, and community health and nutrition, respectively. The Group was also pleased to retain its links with former colleagues Dr Malcolm Beveridge, Director, Freshwater Fisheries Laboratory, Pitlochry, and with Professor Donald Baird, Fredericton, New Brunswick.

The Group maintains a wide range of international contacts and collaborating researchers, and partly in association with the AFGRP, has developed these further in a range of initiatives

and networks. New links include visits from the French CIRAD Aquaculture Research Team to Stirling and proposals for joint projects are being developed, as are further connections with other European and Asian networks.

Through the Group and via the AFGRP, the Institute is a lead partner in the Aquaculture Compendium - an electronic global databased, encyclopaedic reference tool spearheaded by CABI. Several staff at the Institute have contributed lead articles for inclusion. With DFID funding Anton Immink is

working closely with CABI and AIT to develop an effective mechanism for the inclusion of country specific information in the compendium and testing its effectiveness in bringing benefits to poor people in developing countries. The two foci for the first edition are Bangladesh and Vietnam. Case study materials have been collated and are being entered into the large compendium framework for testing in early 2005.

Following a six and a half year period as Dean of the Faculty, Professor Ross was granted research leave for the 2003-2004 session. Much of the year was spent in Mexico and Latin America. The principal focus of the work was to continue projects running with Dr Carlos Martinez at UMSNH, Morelia, Mexico, on the endangered whitefish, *Chirostoma estor estor*, one of a flock of atherinid species unique to the Mexican altiplano lakes. We have now completed construction of pilot scale ponds on Lake Patzcuaro and we expect to stock with juveniles in November 2004. During the year we have been successful in obtaining further substantial funding from CONACyT (the Mexican Research Council) and government departments including SAGARPA (Department of Agriculture and Fisheries).

Martin van Brakel completed his work on GIS-based models for focusing development interventions in areas of poverty. Martin has drawn together a substantial spatial database for the lower Mekong and its delta, focused on aquaculture development and poverty alleviation. His work is novel in that it combines environmental, biological and socio-economic data in models which enable decision making related to poverty targeting and use of natural and financial resources. He has also shown how the massive datasets required for such modelling can be sourced from the growing international provision on the internet. Manos Sagkarakis brought great energy and increasing expertise and understanding to a novel project examining the use of GIS models which examine spatial influences on sea lice infestation. The project was exploratory but with the assistance of Dr James Bron we were able to prepare some initial promising scenarios which are now under further development.

Based on the outputs from these concurrent projects, Professor Ross was awarded a DEFRA Darwin Initiative grant over the next three years for the project "Sustaining livelihoods and protecting biodiversity through development of pez blanco aquaculture". Our developing web site is along with other related work on indigenous species can be found at <http://www.aqua.stir.ac.uk/GISAP/Darwin/index.htm>. The project is designed to extend our technology into the field and to develop a sustainable production activity consistent with maintenance of biodiversity. Dr Antonio Campos Mendoza (PhD Stirling 2004) has been appointed as project manager in Mexico.

Following on from earlier work on livelihoods in peri-urban Kolkata in which the diversity of livelihoods and natural resources were understood within the DFID livelihoods framework, ongoing research by Dr Stuart Bunting, Dr Samantha Punch (Department of Applied Social Science) and Anton Immink, supported by DFID-NRSP, is allowing everyone involved in sustaining the wetlands a voice in its future management through a process known as action planning. Action planning will provide an opportunity for the Government of West Bengal, landowners, labourers and others to meet together and agree upon a set of actions that will benefit all

concerned. This is not just a list of hopes, but sets out the process by which these ideas can be implemented. Potential donors will be shown the action plan and asked to contribute to ensuring that the wishes of all those with an active interest in the management of the wetlands can be met. Active contributions will also be encouraged from those involved in developing the action plan. If the project is successful it is hoped that the action planning tools developed could be used to assist in the planned management of other wetlands and peri-urban areas in West Bengal, India and globally.

As the EU funded GENESIS project entered its final year, Dr Bunting worked with the SME partners involved in the project to help them analyse developments and trends in production and market data for species being cultured in prototype integrated land-based marine systems in Israel, France and Wales. Dr Bunting, in collaboration with colleagues from the University of Caen has also developed bioeconomic models for the prototypes which are being validated in consultation with the SME partners and used to assess the sensitivity of financial returns to changing scenarios.

The DFID KAR- funded project "Integration of Aquaculture within Irrigation Systems" has concluded. Gender issues raised through the KAR project have been disseminated at the AFGRP Eden Project event in August 2004 with further dissemination of project outputs planned. The outputs of this project are currently being written into Lindsay Pollock's forthcoming Ph.D. thesis which details the introduction of aquaculture within large-scale irrigation systems in water-stressed areas of Asia. This cross-disciplinary study addresses how aquaculture can be developed to meet the needs of the poor in areas where aquaculture is not normal practice. A socio-economic study in Sri Lanka follows the process of post-intervention aquaculture adoption and household-level impact on poverty.

In September 2003 Anton Immink attended an EU-funded workshop on the role of ecosites in sustainable development at Ecosite near Montpellier. Examples of 'good practice' exist around Europe and this network aims to consolidate and highlight the roles the sites currently have in demonstrating effective opportunities for sustainable development in Europe. The Ecosite at Montpellier is significant in its dominant inclusion of aquaculture.

Dr Rana was awarded funding by DFID for research on resource and product flows for sustainable aquaculture development in urban and periurban zones for livelihood appraisal in Sub-Saharan Africa. This project will be undertaken in collaboration with WorldFish based in Abbassa, Egypt. Plans are currently being developed to explore how DFID and the WF regional centre can integrate their resources for common goals.

A research project is also underway to investigate the role of traditional aquaculture and fish in livelihood strategies in two states in Nigeria. Household survey protein (including fish) consumption and types of dependency on fish have been conducted.

Dr Little has coordinated a successful bid to a new UK Research Councils initiative towards strengthening interdisciplinary research to benefit rural development in the UK. The project is funded under the sustainable food chain theme of the new Rural Economy and Land Use Programme

(RELU). A tri-faculty collaboration within the University 'Warmwater fish production as a niche production and market diversification strategy for organic arable farmers with implications for sustainability and public health' will combine the skills of the Institute with the Dept. of Marketing and Public Health Group.

Dr Little has also been active in developing a new initiative, the Scottish Centre for Seafood Quality and Sustainability, which the University has put forward for SHEFC support in collaboration with groups interested in flesh quality at St. Andrews and human health research at Dundee.

Funded for much of its time by various parts of DFID (and formerly ODA), the 25 year link between the Institute and Bangladesh Agricultural University was celebrated in 2004.



A traditional procession to open 25th Anniversary celebrations of the Stirling BAU partnership.

Professor Christina Sommerville was the first visitor from the Institute to BAU at the request of the British Council and joined celebrations with Professor James Muir. There are now more than 50 Stirling aquaculture alumni in Bangladesh, most of whom came together to discuss achievements over the last 25 years and investigate paths ahead. The event was widely covered in the Bangladesh media and attended by the Minister for Education.

Working with the Eden Project and with funding from AFGRP, the Systems Group put together an exhibition of their work

relating to aquaculture within rice-based agriculture systems in Asia at the Eden Project in Cornwall. The exhibition, called Fields of Fish, also marked collaboration between the Institute and the MacRobert Arts Centre in the University, who devised a play based on rice-fish farming. Life in Bangladesh was a focus of part of the exhibit; given this linkage we chose to take the drama and the exhibition to the Edinburgh Mela. More than 4000 people were interested in the exhibited stories of rice and fish and life in Bangladesh over the five days of exhibition and drama. A permanent display unit sits in the Humid Tropics Biome at Eden alongside their live paddy exhibit which has more than 100,000 visitors a month.

As a result of the 25 year celebrations and Fields of Fish, we established firm contact with the Bangladeshi community in the UK. The primary contact is Dr Wali Uddin, director of the Bangladeshi British Chamber of Commerce. The linkage has already lead to investigations into wider aquaculture potentials in Bangladesh and discussions on funding future graduates to come to Stirling.



Stirling Aquaculture (STAQ), the consultancy and project management arm of the Institute, were involved in a number of interesting projects throughout the year. A major study of the market for aquaculture produced sea bream and sea bass was carried out for the European Commission in partnership with Professor Jimmy Young of the Department of Marketing. This investigated the reasons for the price crisis in 2001-2002 and the likely future evolution of the market. To provide the required geographic coverage and local industry expertise, contributors were recruited from twelve other countries. We were also pleased to be rejoined by Mr David Scott who worked on the project as a Senior Associate Consultant to help develop a final report of around 450 pages. Work was also started with Professor Young on a study of the potential for a fresh fish hub at an English regional airport.

Two consultancy visits were made to Brunei Darussalam under a Commonwealth Fund for Technical Cooperation programme to support strategic developments under the Department of Fisheries. A team of consultants was involved including Mr Jonathan Grubb (Marine fish farming & STAQ team leader), Mr Tony Garthwaite (Processing & quality control), Mr U. Win Latt (Shrimp farming and environmental management), Professor Mohamed Shariff (Pathology) and Mr John Paton (Human Resources). A consultancy was also carried out at the request of the European Investment Bank to conduct a technical audit of a shrimp farming operation in Madagascar. This involved a visit to the site by Dr Matthew Briggs to inspect facilities, examine farm records and interview staff. Other overseas consultancies took Mr David Currie to Saudi Arabia for a shrimp farm pre-feasibility study, and Mr Jonathan Grubb to Australia to assess a cage farm site.

Closer to home, an aquaculture opportunity study was carried out for a Scottish estate owner and advice was also given to a company developing an innovative marine fish farming system.

At the end of 2003, the highly successful Aquaflow project came to an end. STAQ had acted as the UK National Network Leader for this research dissemination project for 6 years. However, some elements of this project are being continued in a new project that Stirling is coordinating – "The Aquaculture Innovation Network" (Project acronym CSN-INTRAN, 5th Framework Combined RTD and Demonstration Project). This project has 14 partners and is focusing on the processes of innovation and technology transfer in European aquaculture, using that analysis to help facilitate the strengthening and upgrading of aquaculture in the former Soviet New Member States of the EU. Mr John Nikolaidis investigated the status of carp processing and marketing in France and Hungary for his MSc thesis project and has subsequently joined the staff of STAQ to provide further support to this project. The group also continued to participate with the Department of Marketing in the project "GENESIS", which is investigating various approaches to integrated mariculture in the European context. Focus group work with consumers was conducted in France, and further economic modeling was carried out in Stirling.

The Aquaculture Technology Centre (ATC) is a broader grouping of commercial services within the Institute of Aquaculture, providing a single brand for such services and a clearer access point for new enquiries. STAQ continue to provide the front office for this service and joined with colleagues from other sections in promoting the ATC at Aquaculture International 2004 in Glasgow, and at an IRC Blue Biotech event in Oban.



The Institute External Facilities at Howietoun, Buckieburn, Machrihanish Marine Environmental Research Laboratory and Machrihanish Marine Farm Ltd have had another very successful year.

Smolt production of both S0's and S1's was similar to last year (630,000) and the stock has performed very well in the sea. However, a delayed delivery in the spring dramatically demonstrated the potential problems due to the lack of a cost effective efficacious fungicide. A fungal infestation occurred that was so rapid in its onset that, had the fish not been delivered to sea three days after the infestation started to increase, there would have been a significant loss. Over summer 2004 occasional treatments have been carried out with the only licensed fungicide available and whilst techniques for controlling fungus have been developed these are very costly. Fungal infections are the biggest problem that Scottish freshwater aquaculture currently faces and a cost effective solution is urgently required.

The market for farmed salmon over the last year has not been as good as anticipated and there have been several bankruptcies in the Scottish industry. The most significant was that of Kinlochdamph, one of the biggest smolt producers for the Scottish industry. Whilst this undoubtedly provides opportunities for the remaining smolt producers the bankruptcy highlighted some of the poor selling practices that have been prevalent in the Scottish smolt industry. There are hopes that prices will stabilise in the near future due to the efforts of the European Union Salmon Producers Group, a small but very focused group of independent Scottish and Irish producers, who have raised an anti-dumping action against Norway. The group has had extensive support from the Scottish Executive and the UK Government but the action has not been supported by interest groups within the EU whose goal has been to procure cheap salmon. Whatever the outcome of this action the salmon industry appears to be dividing between high volume/lower cost commodity producers, who operate globally, and smaller independent producers who supply into niche markets, generally at a higher value. There is a role for both, as long as there is mutual recognition of each others right to exist.

As a result of the "Strategic Framework for Scottish Aquaculture" a Code of Practice for Scottish Aquaculture is expected to be rolled out in 2006. Whilst not ideal the code will change the way finfish aquaculture is carried out in Scotland for the foreseeable future. The industry will have to learn how to work with the code and it will present opportunities for some companies and problems for others. It is hoped it will lead to a regulatory process that is fair and competitive with other countries such as Norway, who still benefit from a regime that allows the use of unlicensed vaccines and pharmaceuticals.

Brown trout production has been lower than previously at the fishery. There was extensive predation from a number of protected species, including osprey, kingfishers and otters, as well as herons and cormorants despite an extensive anti predator netting system. Discussions are underway with the Game Conservancy and others to try to obtain funding for a study to accurately quantify predation at Howietoun as, whilst the conservation of protected species is to be supported, it cannot be at the cost of production without some form of compensation. However, despite these problems fish quality has been excellent and the customer base remains solid.

The wet summer this year has been good for brown trout growth but not so good for the tourist industry. In particular it is worrying that fewer young people are taking up angling because of competing pressures from other, primarily indoor, activities.

The opening of the new office, laboratory and changing facilities, coupled with the excellent tank facilities and water supply has led to an uptake in use of facilities at Buckieburn. The GLP capability of the facility is a major draw for commercial clients.

Dr Herve Migaud is now responsible for the academic projects at Buckieburn and is actively applying for funding of new projects. Ben North's project on stocking density effects on fish welfare continues to provide very useful results for the trout industry and a new project on how deteriorating water quality affects rainbow trout has just commenced. John Taylor's PhD on the influence and interactions of photoperiod, growth factors and energetic status on growth and reproduction of trout is now in its final stages and John has commenced a related follow on project at Buckieburn.

A number of new contract research projects have either started or are about to commence. A major project investigating a novel injectable sea lice therapeutant, which has already shown great promise at Machrihanish, is being combined with commercial vaccines to ascertain if one injection prior to smolting will protect fish for the whole of their sea life against both furunculosis and sea lice. If successful this would provide a huge boost for health control for the salmon industry and significantly reduce the need for repeat treatments in sea water. A number of promising antifungals are being investigated at Buckieburn and trials on new first feeding diets and behavioural modification of smolts are about to commence.

The difficult economic conditions of the industry have certainly affected MERL this year. Despite the growth in cod farming in Scotland and Norway it has proved difficult to fund commercial cod trials at Machrihanish and much of the work in progress is internally funded in an effort to provide an impetus to this new industry. In addition the problems of the salmon industry has meant that suppliers of products such as feed, vaccines and pharmaceuticals have been reluctant to invest in new products. However, despite these difficulties MERL has continued to make good progress.

The majority of new projects concern cod, including studies on the effect of photoperiod on cod maturation. A project on cod genetics using facilities at both MERL and MMF has

shown the benefit of DNA technology for marking fish which are impossible to keep in family groups because of the very frequent grading and mixing necessary in juvenile cod. A new PhD project on nitrogen output of farmed cod, funded by Seafish and HIE, will contribute towards the parameters for cod consents which currently are only 2/3rds of those for salmon. MERL is also providing facilities for a PhD student from Glasgow, co supervised by Jimmy Turnbull. This project is investigating the feeding rhythms of farmed cod and is already providing information on the best time of day to feed for maximum food consumption. The information provided by these projects will be extensively used by the new industry.

Work continues on vibrio vaccines for cod and an ATC trial for Pharmak on a vibrio vaccine is underway. Unfortunately the development of the cod industry is hindered by lack of commercially available vaccines, and autologous vaccines manufactured from strains originating from cod sites are now being used. One advantage of this approach is that any new strains of bacteria can be incorporated into these emergency vaccines without the delay and cost of licensing. It is also anticipated *Pseudomonas anguillisepticum* will be incorporated in vaccines as this bacterium is expected to become an increasing problem in cod culture.

A grant has recently been awarded by AIE to develop Atlantic hake, *Merluccius merluccius*, as a new species for aquaculture in Scotland. Hake catch quotas are drastically reduced and prices are regularly higher than for cod so it seems opportune to carry out preliminary investigations on hake culture. The work is being carried out in the newly developed research hatchery in the old lifeboat station. Two broodstock capture trips have been mounted using a chartered purse seiner and around 200 broodstock were caught. As expected, it was virtually impossible to bring hake to the surface alive, nevertheless, mature hake were successfully stripped and the eggs were fertilised at sea and brought back to the new hatchery. A small number of viable eggs were incubated and some hatched and successfully fed on rotifers. A number of small scale trials have been carried out in the research hatchery and it has proved particularly suitable for testing near-market products such as rotifer feeds, enrichments and weaning diets, as well as protocols for new species.

Cod broodstock nutrition is emerging as a key research area. None of the commercially available broodstock diets contain the correct level or ratio of fatty acids, primarily EPA and ARA. A major trial is being put together to test ARA enhanced diets for farmed cod broodstock. Wild cod give excellent quality eggs but if a meaningful programme on genetic improvement of cod is to be developed eggs of farmed origin must be used. Up to now these have given much poorer results in terms of fertilisation, hatchability and vigour of offspring,

MERL is still heavily involved with salmon research, much of which is concerned with sea lice therapeutants. Current work with a very large pharmaceutical company aims to produce the data to licence an injectable anti-lice compound. The results look very promising so far and a small number of smolts injected with the compound, under an Animal Test Certificate, will go to sea cages in Spring 2005. If this is successful further large numbers of smolts will be put to sea, again under an ATC, within the next twelve months. Research continues with Slice, which is generally a very effective product, and close monitoring of the industry is in progress to see if there is any indication of genuine resistance to the

product. Consideration is also being given to its potential use in new aquaculture species.

Janet Stone has completed a project for the Australian Government to ascertain whether sea lice, *Lepeophtheirus salmonis*, can survive on iced salmon for the period taken to deliver salmon from Scotland or Norway to Australian food outlets. Whilst the lice could survive the trip the eggs that were produced were not viable. As a result Scottish salmon will stay on the menu of discerning Australian restaurants!

An interesting project has been the training of a hatchery manager for the Namibian government funded by the British Council. The manager studied with Bill Roy and at MMF for two months and learned techniques of live feed and juvenile production. The Namibian government hope to initiate a project on kob, *Argyrosomus japonicus*, culture with funding from the Commonwealth Fund in the near future.

During the year the building of a new seminar room on the first floor of the lifeboat station has been completed with funds from AIE. The room will be used primarily as a client meeting room and will have modern IT facilities, but it will also provide facilities for student seminars and community use.

MMF continues to establish itself as the leading cod hatchery in the EU. Production in 2004 is expected to be around 750,000 to 1 million juveniles with 500,000 so far delivered to our customers in Shetland, Wester Ross and Argyll. The quality of the fish has been excellent and they adapt very well to salmon farming techniques such as counting, grading, vaccination and well boat transportation.

The demand for juvenile cod is very strong and there is a significant customer waiting list. Johnson Seafarm Ltd, a major customer in Shetland, have been very successful in raising finance in the City to on grow juvenile cod following their starring role in the BBC's Money Programme. The prices being paid for farmed cod, £4.95/kg head on gutted, is now more than double that for farmed salmon. These are early days but the move by producers to quality and organic status so early in the development of cod farming is a clear indication of where our current customers wish to place this excellent product.

Relationships with Marine Farm, our partner in the project, are excellent and they are hoping to develop on-growing sites in Argyll and Orkney. MMF has also reached an agreement with Marx Mariculture Ltd, to on-grow all their production. Total combined production planned for 2005 is 2.5 million juveniles.



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Senior Stockworker

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Stockworkers

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 Stuart Galloway
 Robert Redfern
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Secretary
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Cleaner
Rhona Smith

Site Manager
Alastair McPhee BSc
Stockworker
John Gardiner

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Kevin Smith

Manager
William Roy BSc, PhD

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Mr J Botero MSc Colombia
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Ms J Bundit BSc, MSc Thailand (Royal Thai Government)
Mr G Butterfield BSc, MSc UK
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Mr R Corner BSc UK (NERC CASE)
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Mr S Cross BSc, MSc Canada (Private)
Mr A Davie BSc, MSc UK (NERC)
Mr A Derayat BSc, MSc Iceland (Icelandic Government)
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 Mr Emmanouil Tsagkarakis BSc Greece (Private)
 Mr Guillaume Salze BSc France (Private)
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