

Fish Metabolism

Dr Michael Leaver and Dr Douglas Tocher of the Institute of Aquaculture recently completed a project funded by the BBSRC

The background to this study revolved around the apparent requirement to feed farmed fish diets containing oils derived from wild fisheries. This practice is not sustainable in the longer term, and will constrain further growth of aquaculture activities, particularly with marine fish. The researchers reasoned that a greater understanding of how fish metabolise and biosynthesise lipids (fat and oil) may enable us to produce more sustainable and environmentally friendly aquaculture diets based on plant products. Therefore, they undertook a molecular biological study to isolate and characterise genes involved in regulating lipid metabolism and homeostasis.

This study has now been successfully completed, and has resulted in the isolation, from a marine fish species, of three genes which are involved in regulating many lipid homeostatic processes. The genes encode receptor proteins, termed PPARs, which have an important and central role in transferring extra- and intra-cellular signals into the nucleus. There, the PPARs bind to DNA and, in doing so, modulate cellular functions by "switching on" or "switching off" suites of genes that are involved in metabolising fat. Alteration of gene expression in this way determines how the cells use dietary fat, including either burning it for energy or storing it as fat deposits. The characterisation of the genes and their protein products has shown that the presence of certain specific fatty acids is crucial in determining the receptors' activities and also gives a number of options for the development of novel aquaculture diets.

Careful formulation will produce feeds containing specific components targeted at altering PPAR functions enabling fish to more efficiently utilise dietary fat. Further funding from the European Union, and collaboration with the aquaculture industry will now allow Dr Leaver and Dr Tocher to test these new ideas in dietary trials.



Environment Agency/SNIFFER Project: Development of a predictive system to assess the ecological status of rivers and lakes using macrophytes, £91,353

The name may not suggest it but in terms of protecting and enhancing aquatic environments the European Water Framework Directive is one of the most exciting pieces of environmental legislation of the last 30 years. It will also be one of the most challenging to implement. The long standing preoccupation with water chemistry as the universal determinant of water quality is replaced by a more holistic view of quality based on the condition of different ecological elements – phytoplankton, macrophytes, macroinvertebrates and fish – relative to an undisturbed reference condition.

Sites must be monitored and their condition assessed and causes of degradation diagnosed, and, in theory, put right by 2015, unless exceptional extenuating circumstances can be presented. This project does exactly what it says in the title! Over the next 3 years a robust and user friendly tool must be developed that allows agency biologists to predict the expected macrophyte community at any river or lake site, classify the condition of the site based on the deviation between the expected reference community and what they actually find, and diagnose what the anthropogenic pressures are on the site.

This project is being co-ordinated by Nigel Willby and is a partnership between other Stirling University staff in Environmental Science and Computing Science, plus the University of Ulster and the Centre for Ecology and Hydrology.

NIGEL WILLBY

PROFESSOR NIAL BROMAGE has been awarded a £150,000 grant as part of a three year collaborative European Project (Pubertiming) on photoperiod control of puberty in farmed fish. Dr. Herve Migaud was appointed to work on this project. The overall objective is to develop improved photoperiod protocols for delaying first sexual maturation (puberty) in commercially farmed European fish species. At Stirling, the Reproduction Group will carry out *in vitro* and *in vivo* studies on the pineal sensitivity to light of different intensities and spectral compositions through melatonin measurements. This will allow us to determine threshold values for artificial light to control puberty in salmon and sea bass in outdoor fish farms/ sea cages.

Towards a molecular genetic tool for health and performance monitoring of Atlantic salmon **Alan Teale, Douglas Tocher, John Taggart £453,042 BBSRC**

This new project is funded for four years from 1st March 2003 by the Biotechnology and Biological Sciences Research Council (BBSRC), UK, as part of the *Exploiting Genomics* initiative. The proposal to BBSRC, which was co-ordinated by Prof. Teale, was made jointly by the University of Aberdeen (Prof. D. Houlihan, Prof. C. Secombes and Dr. S. Martin), University of Cardiff (Dr. G. Sweeney) and the Institute of Aquaculture, University of Stirling. The total grant to the three universities is for almost £1.1 million. The application to BBSRC received strong support from Scottish Quality Salmon, Marine Harvest and Qiagen Ltd.

The objective of the project is to identify key genes of Atlantic salmon that control four important traits – lipid synthesis, protein catabolism, immune function and smolting. The team at the Institute of Aquaculture will focus on genes controlling synthesis of highly unsaturated fatty acids (HUFA). The ability of Atlantic salmon to synthesise HUFA is of increasing importance as the supplies of fish oils for salmon diets come under increasing pressure. This pressure is due to the decline in the fisheries that have been the source of HUFA for the salmon-farming industry. Fish such as Atlantic salmon provide the human consumer with omega-3 fatty acids, a particular type of HUFA, that are required for normal health and development. Therefore, the threatened supply of fish oils for salmon diets is an indirect threat to the human consumer. The rationale underpinning the Institute effort in this project is that if the molecular genetic control of HUFA synthesis in salmon could be elucidated, it may be possible to improve the ability of salmon to synthesise HUFA, and thus reduce the dependence of the salmon farming industry on fish oils.

The approach that is being taken makes use of the latest technologies to identify genes that are up- and down-regulated in response to informative interventions. In this case the group will search for genes that change their expression in salmon livers in response to changes in the dietary content of HUFAs and their biochemical precursors. This will initially involve the interrogation of the salmon liver transcriptome on cDNA microarrays comprising many thousands of salmon genes. The partners in Aberdeen and Cardiff will similarly search for differentially expressed genes of relevance to their traits of interest, and it is here that the important synergy of the partnership is created. The three groups will use common microarrays that will be constructed and used to probe gene expression in appropriate salmon samples at the Roslin Institute's *ARK-Genomics* facility. Dr. Taggart of the Stirling group will work closely with colleagues at Roslin on this aspect of the project on behalf of all three university partners.

In a second phase, differentially regulated genes will be identified and sequenced, and a second-generation gene array comprising synthesised oligonucleotides will be constructed and tested. Ultimately, a development of this "oligo" array will be trialled as a tool for monitoring expression of key genes in farmed and wild salmon populations. The project thus anticipates that this type of gene technology is set to supplement, or in some cases replace, current tools for monitoring health and productivity such as biochemical tests for metabolites, antibody assays, hormone assays etc.. It thus looks forward to providing the salmon farmer and the wildlife biologist with a simple way to measure gene expression, and thus monitor the health, development and productivity of farmed and wild Atlantic salmon populations with a depth and precision that has been unobtainable hitherto.

The Stirling group gratefully acknowledges the valuable input of Institute colleagues Drs. Morris Agaba and Margaret Cairney in the development of the project proposal.

Utilisation of byproducts from shrimp peeling for feed addition to commercially important fish and shellfish species (BYPROFEED) £67,667

Dr Gordon Bell & Fiona McGhee

The Nutrition Group was awarded this Cooperative Research (CRAFT) award under the EU FP5 in July 2002 for 2 years. The processing of prawns and shrimps generates a considerable amount of waste material which would normally be disposed of in wastewater. The world wide shrimp processing industry is valued at several hundred million dollars per annum and the waste produced from processing represents approximately 70% by weight of the total catch. The wastewater produced from shrimp processing represents a considerable organic load if discharged directly to sea and current regulations demand that an effective treatment be introduced to reduce the environmental impact of nitrification. In recent years, rather than dump waste directly to sea or in land-fill sites, methodologies have been developed to recover solid material from crustacean processing plants and prepare a product rich in protein, lipid and carotenoids which has potential as a feed ingredient.

The Byprofeed partners include two shrimp processors, in Norway and Germany, who, in partnership with two Food Technology Institutes in the same countries, have developed equipment to remove a large proportion of the waste materials and produce a dry product called

Protein Omega Concentrate (POC). The POC contains 60-70% protein, 18-27% lipid and up to 350 ppm of carotenoid (astaxanthin; Ax) and, depending on the method of shrimp peeling, relatively low levels of ash. In addition to providing a source of high quality protein, lipid and carotenoid, crustacean meals contain a variety of taste attractant molecules which are known to enhance feed palatability and consumption.

The Nutrition Group have been involved in testing the composition of various POC products prepared and dried using different methodologies to find an optimal POC product for inclusion in fish feed formulations. Formulations developed and produced in partnership with Bentham Trout will be tested in feeding trials with Atlantic salmon and Arctic char to assess the influence of POC on growth and feed utilisation, as well as proximate, fatty acid and carotenoid composition. Diets containing POC will also be tested in weaning diets for halibut larvae and in juvenile lobsters where the feed attractant properties of POC will be important for weaning success in the former and in reducing moult cycle times in the latter.