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It is a pleasure to write my first welcome to you as Director of the Institute. There is no denying the challenges ahead in order to maintain the reputation of the Institute as a world-leading research facility, but I also feel that I am taking the helm at an exciting time for aquaculture science, with the application of new technologies to classical fields. As the articles in this edition show, the Institute of Aquaculture remains at the forefront of scientific development and application.

Looking at scientific achievements during my professional lifetime, in the mid-1970's, there was the development of monoclonal antibodies, which have become firmly established as important tools for disease diagnosis. The 1980's ushered in the era of molecular biology, and the greater understanding of the genetics of life. The era of -omics is clearly with us now at the start of the twenty first century. The subjects of genomics and proteomics feature in any modern scientific discussion.

These technologies have been embraced by aquaculture and are at the forefront of research at the Institute of Aquaculture. With these modern approaches, scientists are gaining greater understanding of the genetics and, in particular, gene expression of aquatic species and their pathogens. In this edition of Aquaculture News, our scientists provide a range of articles describing their recent research activities. The subject of -omics is introduced by a team working with Michael Leaver. Other colleagues go on to describe the application of these technologies in familiar areas of disease, nutrition and genetics; covering such topics as resistance to IPN, genetic sterility, vegetarian salmon and hormonal control. Janet Brown and Liz Ashton introduce their work on native oyster conservation, while Lindsay Ross and Carlos Martinez Palacios describe their work towards the conservation of indigenous species in Latin America.

The diversity of articles in this edition is certainly a testament to the high powered research of our scientists.

PROFESSOR BRIAN AUSTIN, DIRECTOR

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FRONT COVER: Scanned microarray image  
Image courtesy of James Bron and John Taggart  
BACK COVER: Reflection in a new tank at Howietoun hatchery  
Image courtesy of John Bostock

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### Future e-Aquaculture News

Aquaculture News is now an electronic publication. We will be publishing short electronic editions on a quarterly basis. We would like to keep our e-mailing list up to date and, of course, welcome new subscribers. We continue to welcome short articles from readers and would be grateful if you could circulate this copy to anyone you feel would find it interesting. Aquaculture News will remain free of charge. All editions can be viewed at [www.aqua.stir.ac.uk/aquanews](http://www.aqua.stir.ac.uk/aquanews)

To join the mailing list or update your e-contacts, please e-mail Anton on [a.j.immink@stir.ac.uk](mailto:a.j.immink@stir.ac.uk) - with 'Aquaculture News' in the title.

# 'omics' in the Institute

Michael Leaver, John Taggart, James, Bron, Douglas Tocher and Stephen George

**'Omics' disciplines** and technologies, seek to simultaneously measure many hundreds to tens of thousands of biological molecules. For example, transcriptomics involves the measurement of messenger RNA (mRNA) levels in cells, tissues or whole organisms for defined biological states e.g. stress, disease, nutrition. mRNAs are regarded as a proxy for all the proteins being produced by an organism in a given state or states, potentially reflecting all of the functional genes possessed by an organism. Similarly, proteomics involves direct measurement of the levels of several thousand distinct proteins simultaneously in a single experiment. Such measurements enable the researcher to ascertain which individual molecules, molecule classes and biochemical/physiological pathways are most important to the biological process under investigation, often without any recourse to preconceived ideas.

Over the last few years, staff at Stirling have been leading the development of transcriptomic microarray platforms for Atlantic salmon and European flounder, to address key issues in aquaculture and ecotoxicology. The development of these platforms or tools is labour-intensive and expensive and has been carried out in collaboration with other groups in the UK and Europe. A microarray is a glass slide, the size of a microscope slide, onto which have been microscopically printed thousands of separate spots of DNA (< 0.2 mm diameter). Usually each spot represents a different gene and is capable of binding the matching gene product from the organism. Originally each spot (microarray feature) comprised a cloned cDNA fragment, but with improvements in technology and increasing availability of species-relevant transcriptome sequence data, current platforms usually employ short gene specific oligonucleotide sequences (25-70 bases in length), which are printed or synthesised *in situ* on the slide. A typical analysis involves taking the

gene products derived from two biological samples, labelling them with different coloured fluorescent tags, and competitively binding them to the DNA spots on the microarray. Relative gene expression can be determined by examining the fluorescence intensity of the two samples for each of the thousands of features on the array.

Initial, large-scale transcriptomic studies carried out at the Institute of Aquaculture (IoA) used directly-spotted cDNA microarrays. These had a number of recognised technical shortcomings, including lack of target specificity and poor printing quality and have been largely superseded. We are now using cutting-edge Agilent 44,000 (44K) feature oligo arrays, these having a near-perfect spot morphology and optimal target specificity. The latest 44K microarray developed by IoA, in collaboration with Aleksei Krasnov of NOFIMA Norway, Bjørn Høyheim of Oslo Veterinary School, and Christophe Klopp of University of Toulouse, targets the Atlantic salmon transcriptome and has proven highly successful in its first experiments (see next article).

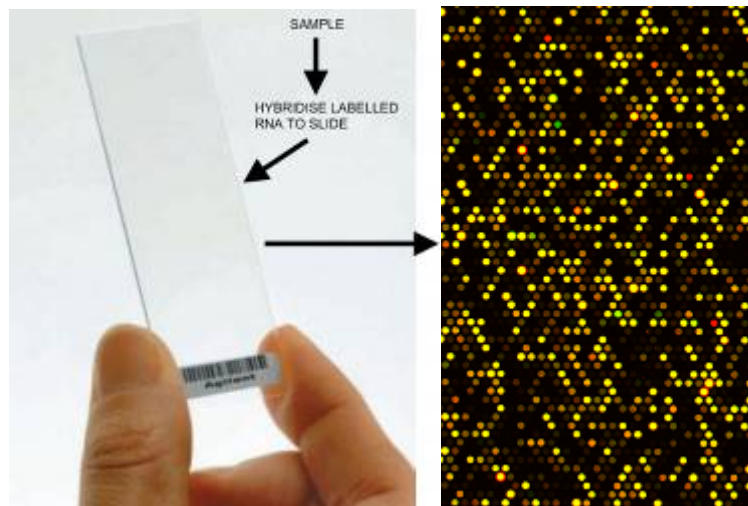
Each microarray may contain tens of thousands of different probes, hence one of the principal challenges of such

hybridisation devices, fluorescent scanners and image analysis and bioinformatics software.

Research with these microarrays has been targeted towards understanding disease and nutrition in Atlantic salmon aquaculture and towards exploration of fish responses to chemical contamination e.g. flounder inhabiting polluted estuaries. A current issue in aquaculture concerns the need to find alternatives to fish oil and fish meal included in aquafeeds. Currently the best candidates for replacement are meals and oils derived from plants e.g. rapeseed oil and soya meal. From empirical feeding trials it has been established that under aquaculture conditions Atlantic salmon can tolerate up to 70% replacement of fish oil with plant oils in their diets. However, although growth is not affected, some such replacements have been associated with impaired disease and stress resistance, and with abnormal gut function. By comparing gene expression in salmon fed different plant oil based diets with expression in those fed fish oil diets, we have discovered major effects on fatty acid and cholesterol biosynthesis, which can be linked to some of the pathological conditions observed under aquaculture conditions.

Consideration of the genes involved implicates certain mechanisms, which suggests that the primary cause of these effects may be due to deficiencies in cholesterol in plant oils. This in turn provides clear possibilities for improving aquafeed quality.

Similarly, by comparing gene expression in flounder living in uncontaminated environments, polluted estuaries and under controlled contaminant exposure conditions, we have shown that it may be possible to predict which pollutants are affecting wild fish. Such knowledge can be used to target remediation activities, and to derive 'biomarkers' for monitoring environmental quality. Microarray technology has also proven to be a powerful tool for undertaking disease studies and has allowed IoA researchers to investigate the mechanisms employed by



work is the analysis and interpretation of the vast quantity of data generated, based on the difference in expression levels between experimental conditions and upon the known identities of the genes. At IoA we are now at the stage of performing such experiments routinely, having invested in the development of microarrays, and the necessary equipment such as

pathogens, e.g. infectious pancreatic necrosis virus in Atlantic salmon, to subvert the host immune system and the mechanisms employed by the host to defend itself against disease (see article below).

Such studies are providing a better understanding of the disease process itself and of host resistance mechanisms and promise to improve management of disease by a range of

methods including improved disease resistance breeding programmes and vaccine development.

For list of recent references see p12

## IPN resistance isn't futile!

James Bron, John Taggart, Jacquie Ireland, William Starkey and Brendan McAndrew

**IPN is** caused by infectious pancreatic necrosis virus (IPNV), which damages the pancreas, intestine and liver of infected Atlantic salmon (*Salmo salar*). This highly contagious disease has the unusual characteristic of affecting farmed salmon during two specific windows of the life cycle. In the freshwater phase of the salmon life cycle, IPN outbreaks in fry have been observed for several decades, with up to 70% mortality. In the marine environment, the emergence of problematic IPN outbreaks (up to 40% mortality) is more recent, coinciding with the dramatic expansion of salmon aquaculture. The annual economic loss to the UK aquaculture industry from IPN is estimated to be £5-10 million. Stirling staff, together with researchers from the Roslin Institute (S. Bishop, C. Haley and Ross Houston) and an industrial collaborator (Landcatch Natural Selection Ltd) have been awarded a BBSRC grant (> £793,000 combined; duration 2008-2010) to investigate genetic aspects of IPN resistance in Atlantic salmon fry. The project has three major objectives: 1) to describe genetic resistance to IPN in salmon fry and identify specific regions of the genome affecting resistance; 2) to determine which salmon genes work differently between genetically resistant and susceptible fish following infection; 3) to identify specific genes and gene pathways that may be responsible for the genetic resistance.

A number of large freshwater IPN challenge experiments, involving fish of known pedigree, have been undertaken (at CEFAS, Weymouth) to provide samples for a thorough investigation of the genetic basis of resistance to IPN in salmon fry. Differential IPN-mediated mortality among the family groups was observed (Fig 1), with almost all the genetic variation in IPN mortality being explained by a single resistance marker (quantitative trait locus or QTL) on a chromosome (linkage group 21). This appears to be the same major QTL implicated in IPN resistance in marine Atlantic salmon post-smolts (Houston *et al.* 2008).

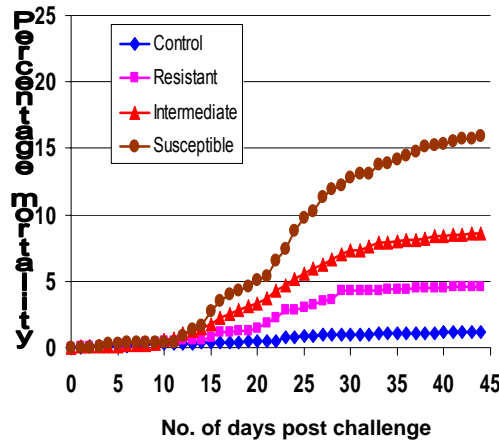


Fig 1. Cumulative mortality curves for 20 Atlantic salmon families experimentally infected with IPNV. Families were initially selected from known pedigrees based on their predicted IPN mortality at the post-smolt seawater lifecycle stage and are grouped accordingly. The graph shows that fry mortality was correctly predicted by smolt mortality.

Work is currently underway to investigate the transcriptomic response of whole fry from these families to IPN infection. For this, an oligo-array platform (Agilent Technology) is being employed. The 44K feature microarray was custom-designed in collaboration with Aleksei Krasnov, NOFIMA, Norway and others. The first of these experiments, comparing families of known

phenotype (IPN resistant versus IPN susceptible) has been successfully completed and a range of differentially responding genes identified (Fig. 2). A second major transcriptomic experiment, investigating differential gene expression among siblings harbouring different IPN genotypes (RR, Rr and rr – from Rr × Rr parental crosses) is currently in progress.

The results of the study will strengthen salmon breeding programmes by providing genetic marker tests to identify IPN resistant fish early in the salmon life-cycle, thus reducing costs and reducing the number of diseased fish. The improved knowledge of the crucial genes determining IPN resistance may also contribute to the rational development of control measures against IPNV infections, including vaccination, and provide sensitive diagnostic tests.

### Reference

Houston, R.D., Haley, C.S., Hamilton, A., Guy, D.R., Tinch, A.E., Taggart, J.B., McAndrew, B.J. and Bishop, S.C. 2008. Major QTL affect resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*). *Genetics* 178: 1109-1115.

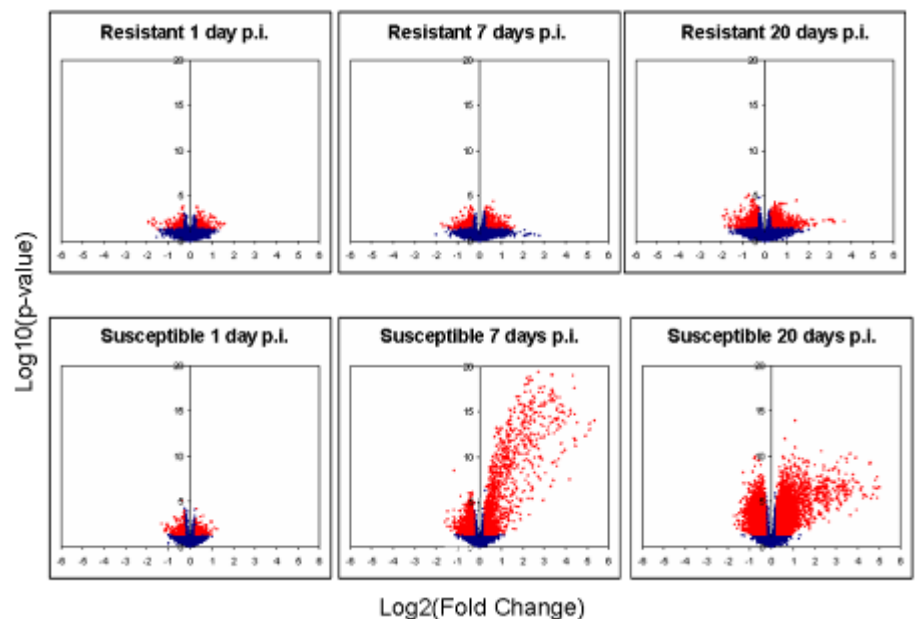


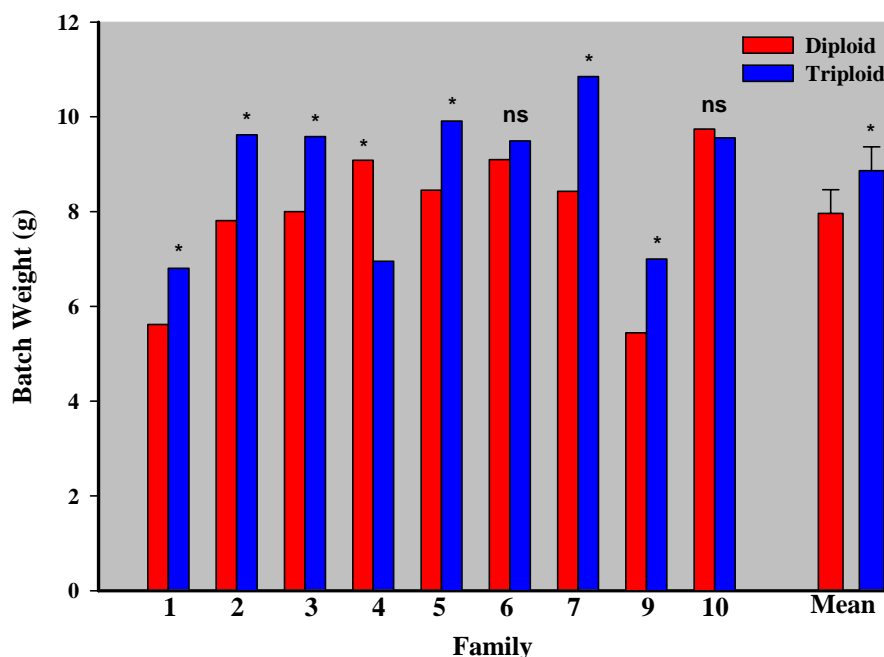
Figure 2. Volcano plots depicting the extreme transcriptome response observed in susceptible fish by seven days post infection (p.i.) with IPNV. Red dots represent genes significantly differentially expressed in IPNV-exposed fish relative to uninfected controls. X axis denotes level of up / down regulation relative to controls; y axis denotes increasing statistical significance.

**The Atlantic salmon** (*Salmo salar*) farming industry is under increasing public and regulatory pressure to negate the impact that escapees have, particularly potential breeding between wild and farmed stocks. One option is the production of sterile stocks by triploid induction. Triploidy is not a new concept, originally tested in the early 1990's as a means to prevent maturation. Unfortunately, poor performance, higher mortalities and deformities led to the industry abandoning triploidy in favour of photoperiod control of maturation prior to harvest. However, although photoperiod does reduce maturation in culture, farmed stocks remain reproductively competent and the threat of genetic pollution following escapees persists. Triploidy is therefore at present the only non-GMO method that can produce sterile fish and the industry is now keen to re-explore this avenue.

It is for this reason that a transnational collaborative project between key R&D (Stirling University, Institute of Marine Research: Bergen and Wageningen University) and industry partners (Landcatch Natural Selection, Aquagen, Marine Harvest, Centre of Aquaculture Competence and Salmo) in Scotland, Norway, Netherlands and France was established in June 2008 as part of the EC funded Capacities Program "Research for the benefit of SMEs" to explore the feasibility of commercial triploid Atlantic salmon production. The project entitled SALMOTRIP is being lead and co-ordinated by Dr Hervé Migaud and Dr John Taylor at the Institute of Aquaculture. The project focuses on six key areas regarding triploid salmon production:



**Figure 1.** The first phase of production of triploid Atlantic salmon parr at Howietoun Fish Farm



**Figure 2.** Evidence of family and ploidy differences in hatchery growth performance

optimise induction protocols; identify physiological requirements and sensitivities; develop out-of-season smolt regimes; explore selective breeding programmes specific to triploid; commercial field trials; and examine public perception, marketing and consumer acceptance. Data generated will also aid legislative decision making regarding future aquaculture policies.

Partners have established a series of experimental and full-scale field trials at all levels of production. The first batches of triploid salmon were produced in early 2008, with second year classes produced for 2009 (Figure 1). During hatchery rearing survival to hatch did not differ between ploidy but was significantly affected by family. Survival was found to strongly correlate with gamete quality, particularly in triploids. As with survival, growth appears to be strongly affected by family, and through correct selection, triploids were found to outperform their diploid siblings with minimal deformity rates (Figure 2).

These observations are a major advancement over earlier studies and suggest that with correct broodstock selection, triploid salmon can perform as well and even better than their diploid siblings. It will also be important to determine if families that perform well in freshwater stages perform as well during saltwater grow out. Furthermore, we are evaluating the best families on traits of interest (growth, flesh quality, disease resistance etc.) observed in field trials by genotyping. Together these findings open up exciting new avenues for research into triploid-specific selection programmes and family-ploidy interactions.

### Seasonal issues

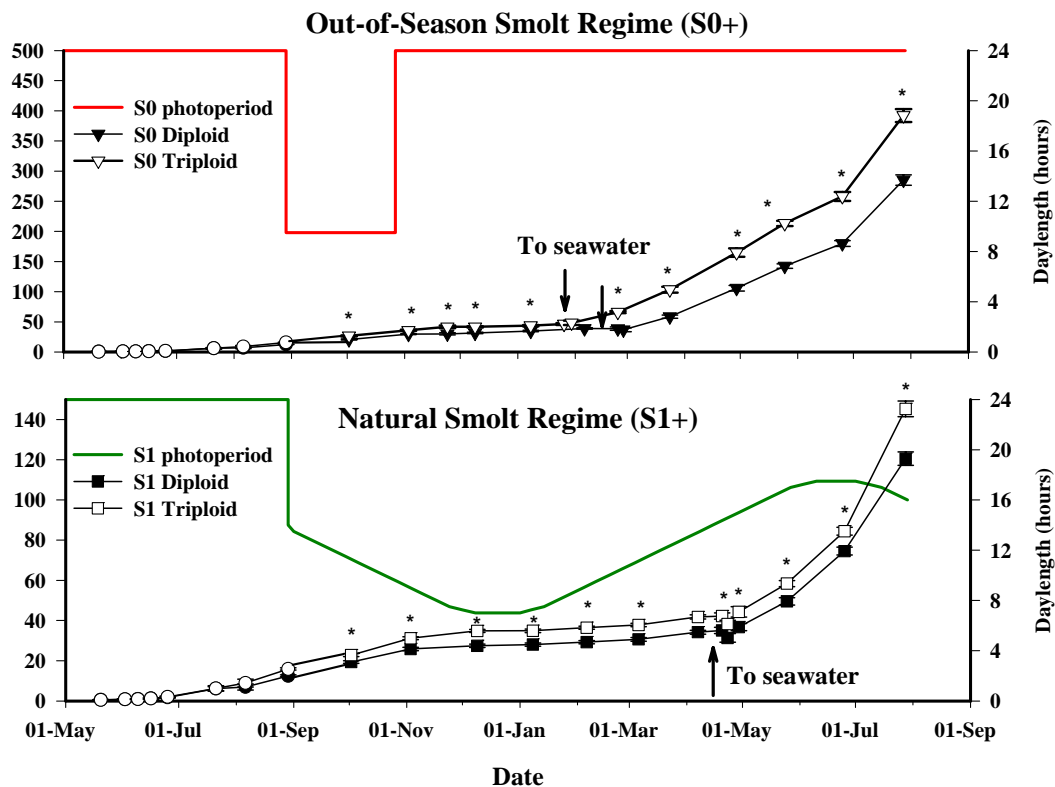
One of the fundamental successes of the salmon industry has been the ability to produce out-of-season (S0+) smolts to ensure year round availability. However, to date, studies in triploid Atlantic salmon have only focused on natural (S1) smolt production. It is thus essential that knowledge of how to produce S0+ triploid be available if farmers are to adopt this strategy. In this respect we have recently demonstrated for the first time that S0+ triploid smolts can be successfully produced, and that they show superior performance, attaining a larger size and allowing earlier seawater stocking (Figure 3). We are

continuing to investigate important criteria to consider when manipulating smoltification time in triploid salmon, particularly size-growth-lipid thresholds, and osmoregulatory physiology: in addition to refining the photo-thermal regimes themselves with regards to timing and duration.

Currently, out-of-season triploid Atlantic salmon have been transferred to sea in the Institute of Aquaculture marine facilities (Machrihanish Environmental Research Laboratories) where they will be monitored for long-term seawater performance. Results so far are very encouraging with triploid post-smolts continuing to out grow their diploid siblings under both accelerated and natural regimes (Figure 4). However, long-term sea monitoring will continue to determine whether previously reported deformities and poorer growth are a function of the change in environment or are carried genetically. Colleagues in Norway are currently exploring the effects of sub-optimal environmental rearing conditions and dietary requirements on deformity prevalence and seawater performance in relation to family-ploidy interact-



**Figure 3.** Significant freshwater growth advantage allowed stocking of larger triploid smolts (Bottom 3 fish) compared to their diploid siblings (Top 3 fish)



**Figure 4.** Comparison of growth performance of diploid and triploid Atlantic salmon siblings under accelerated (S0+) and natural (S1+) smoltification regimes.

ions. Findings to date would indicate significant ploidy differences, particularly with regards to dietary nutrient availability.

Overall the project is at a very exciting stage with knowledge growing rapidly in terms of transfer of methodologies, knowledge on triploid biology and requirements and marketing strategies to SMEs.

However, this is only the tip of the iceberg and considerable research is required in the fields of nutrition, immune competence, vaccine development, and family selection criteria to name but a few. Only when this knowledge is available can the potential for triploidy be truly realised within the salmon farming industry.

For further information please contact Dr Herve Migaud ([hm7@stir.ac.uk](mailto:hm7@stir.ac.uk)) or visit <http://www.salmotrip.stir.ac.uk/>



Ann McKechin MP, Parliamentary Under Secretary of State in the Scotland Office, visited the Institute on 28<sup>th</sup> July as part of a wider visit to the University. She was welcomed by our new director, Professor Brian Austin (centre), and is shown here about to start a tour of the tropical aquarium with Professor Brendan McAndrew (right).

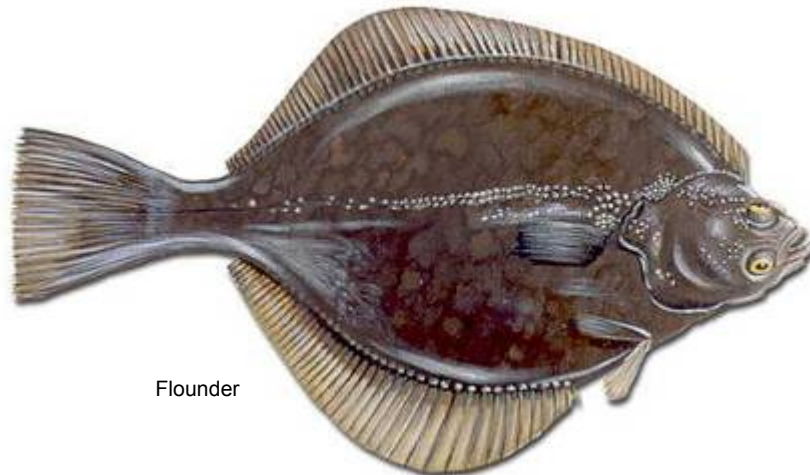
# Assessing pollution effects in aquatic environments

Michael Leaver and Stephen George

Many estuarine and coastal aquatic environments have been sinks for industrial and agricultural effluents for hundreds, perhaps thousands of years. Over the last 100 years the number and diversity of man-made chemicals which find their way into aquatic environments has increased enormously. There are now estimated to be over 100,000 distinct chemical compounds marketed in the European Union alone, and knowledge of the environmental fates and concentrations is restricted to only a small proportion known to be particularly harmful and persistent. Against this background there has been concern that long-term exposure to complex cocktails of chemical contaminants may be affecting stocks of fish and other animals which live in estuarine and coastal areas. In fact surveys of flounder have shown higher incidences of tumours and fin rot in some polluted estuaries in the UK, other parts of the North Sea and in North America. Although this sounds alarming, it has been difficult to provide the evidence that pollution is the cause of such ill-effects, or that it is the cause of any declines in population numbers. This is because we know very little about the biological effects of complex mixtures of chemicals, and estuaries, particularly, are highly dynamic and highly exploited environments in which population numbers fluctuate for many reasons not related to pollution. However, over recent years it has become apparent that particular types of chemicals can have dramatic and measurable effects on individuals. For example, the effect of chemicals which mimic the natural hormone, estradiol, can induce a protein, vitellogenin, many hundred-fold levels above normal in wild, exposed fish. Similarly PCBs can induce a protein, CYP1A, to very high levels. These proteins and their genes have been proposed as 'biomarkers' of pollution, meaning that their measurement in wild flounder, or another animal, can provide evidence that those individuals have been

exposed to a particular type of chemical.

Such biomarkers have been widely applied, and have been shown to be useful in situations where pollutants



Flounder

are derived from a point source, such as a sewage outfall, or in a spill situation, such an oil spill. In situations where fish are exposed long-term to complex mixtures of chemicals, biomarkers have proved less effective. This may not be so surprising because most biomarkers are discovered by acute, short-term, laboratory exposures to single chemicals, a situation quite unlike most environmental exposure scenarios. Very recently techniques for measuring the levels of many thousands of genes simultaneously in practically any organism have been developed [see article on page 2 of this edition]. We have applied this technology in an experimental situation to discover what effects long-term exposure to real polluted estuarine sediments have in flounder. Funded by NERC and in collaboration with Marine Scotland,

Aberdeen, we established mesocosms, large tanks, containing sediments collected from polluted UK estuaries and from an unpolluted estuary. We also collected live flounder from the unpolluted estuary and then put them into the mesocosms for seven months.

Thus, the only difference between the mesocosm systems was due to the pollutant status of the sediments. All other natural variables found in estuaries, such as genetic background, salinity, temperature, food source etc were eliminated. Following this exposure we measured the expression of thousands of genes in these flounders. When polluted flounder were compared to clean it was clear that none of the commonly used biomarker genes were affected, but that the expression of groups of genes with functions in the immune system and in apoptosis was increased. Apoptosis is a cellular process which brings about the self-destruction of a cell following either an extrinsic signal, or the intrinsic sensing of unsustainable cellular damage. The genes we have found point to the involvement of intracellular chemical damage, which in turn induces the intrinsic apoptotic pathway. Thus, this study points to a mechanism by which long-term exposure to complex chemical mixtures can adversely affect fish, and also provides new candidate biomarkers for these situations.



Unpolluted estuary (Ythan, Aberdeenshire)



Polluted estuary (Forth at Grangemouth)

**Marine aquaculture** relies heavily on fish meal (FM) and fish oil (FO) from wild stocks for the production of fish feeds. However, this practice is ecologically unsustainable given the worldwide reduction in fisheries landings, together with the growing production from aquaculture and formulation of more energy-dense diets (containing higher lipid levels). Indeed, recent estimates indicate that by 2010 aquaculture will utilize more than 85% of the world FO production. Therefore, the lack of FO supply may seriously limit aquaculture growth and the future of this activity strongly depends on the reduction of the dependency on FO and its replacement with alternative oils, while maintaining fish welfare and health benefits for the human consumer. Vegetable oils (VO) present a high potential in this respect. Nonetheless, VO, which can be rich in C18 polyunsaturated fatty acids (PUFA), are devoid of the n-3 highly unsaturated fatty acids (n-3HUFA) eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, which are abundant in FO. Thus, growth of fish on VO results in lower levels of n-3HUFA (also known as *Omega3* fatty acids) in their flesh, compromising their nutritional value and health-promoting effects to the human consumer.

Atlantic salmon (*Salmo salar*), are capable of producing HUFA from C18 PUFA in VO and so must express all the enzyme activities necessary for this biosynthetic pathway, even if their activity is low and unable to compensate for the reduction in dietary pre-formed n-3HUFA supply. Selective breeding programmes for traits of commercial importance are becoming common practice in aquaculture. Combining genetic selection with changes in commercial diet formulations (i.e., higher levels of inclusion of VO) might be a viable strategy to meet worldwide growing demands for aquaculture products, without any loss in fish welfare or nutritional value.

Therefore, given the unavoidability of drastic changes in current commercial feeding practices, there is a pressing need to identify strains of fish with the ability to utilize high fat diets, but with lower dietary requirements or higher ability to retain/biosynthesise n-3HUFA. To enable this, large-scale in depth studies exploring genotype-nutrient interactions are essential. A recent European Project named AQUAMAX - sustainable aquafeeds to maximise the health benefits of farmed fish for consumers (FP6 IP, 016249-2), in which the Lipid Nutrition Group at the Institute of Aquaculture is a major partner, has started to address these questions [see Aquaculture News 33 page 7 and Aquaculture News 34 page 6].

Substantial research has recently focused on the molecular and physiological effects of dietary FO replacement by VO and how metabolic pathways may be affected by dietary lipid composition. More recent work, as part of AQUAMAX, has started to analyse how the phenotype "fat deposition in muscle" (fat vs. lean families) affects liver and intestine transcriptome in response to complete FO replacement by a VO blend in Atlantic salmon diets. In this study, two groups of Atlantic salmon smolts, drawn from predicted "fat" or "lean" families (Landcatch Natural

Selection, Scotland) were fed experimental diets containing 25% fish meal and 44% vegetable meals and either 100% FO or 100% VO. The effect of these diets on gene expression in the liver and intestine (pyloric caeca) of both families was examined using the TRAITS/SGP salmon 17k cDNA microarray.

This study revealed that the two families reacted quite differently, in terms of expression of lipid metabolism-related genes, both in the intestine and liver. For instance, in the intestine, a significant "diet"-family interaction was observed for many genes, the majority of them involved in metabolism. In particular, a strong significant interaction was observed for genes that are involved in HUFA biosynthesis from C18 fatty acid precursors, which are abundant in VO. This was the case for  $\Delta 6$ - and  $\Delta 5$ -desaturases and elongase (elov12), which are widely known to be up-regulated in response to dietary FO replacement by VO in Atlantic salmon, as well as in other fish species. However, in this study, the response depended on the family, with "lean" fish responding atypically, showing no regulation of these HUFA biosynthesis genes, while in the "fat" family we observed the typical response of previously studied commercial lines of Atlantic salmon (Figure 1).

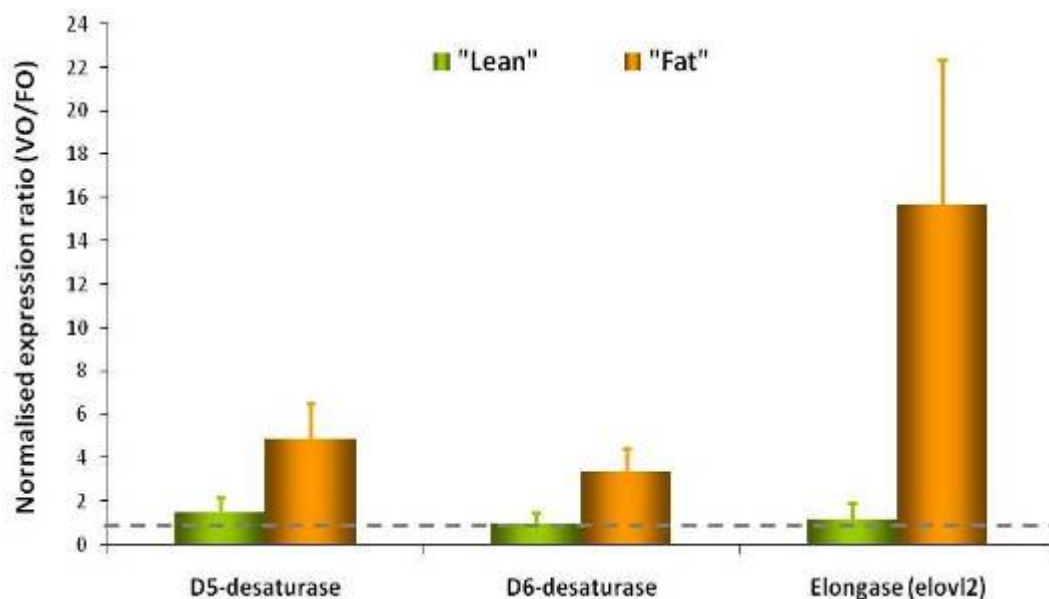
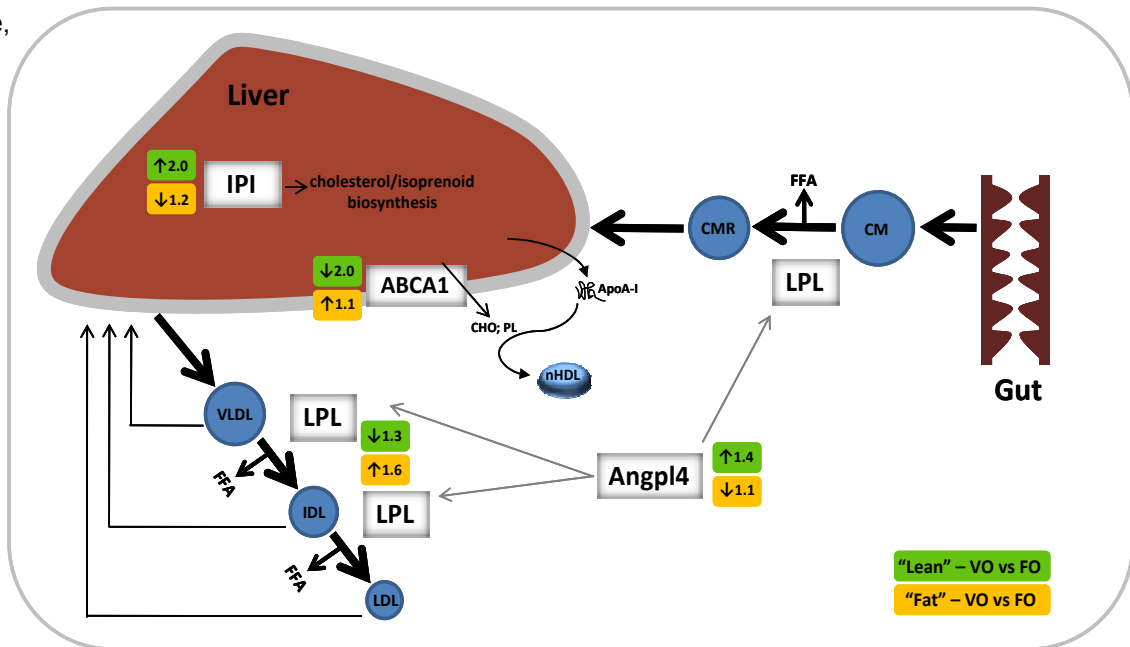


Figure 1. Relative expression of selected genes of HUFA biosynthesis, determined by quantitative real-time PCR, normalized by average expression of 3 reference genes, expressed as an expression ratio of VO/FO, in "lean" and "fat" Atlantic salmon families. Dashed line represents an expression ratio of 1, when there is no difference in expression between VO- and FO-fed fish.

As for the liver transcriptome, some considerable family-related differences were also observed. For instance, when comparing salmon fed the VO-diet in relation to the FO-diet, an opposite response (up-/down-regulation) was measured in the “lean” and “fat” families for several inter-related genes that participate in lipid and lipoprotein metabolism (Angptl4-angiopoietin-like 4; LPL-lipoprotein lipase), cholesterol transport/efflux (ABCA1-ATP-binding cassette transporter A1) and cholesterol/isoprenoid biosynthesis (IPI-Isopentenyl-diphosphate isomerase) (Figure 2).



Therefore, this experiment clearly showed that two Atlantic salmon families with different fat deposition phenotypes adapt differently to 100% dietary substitution of FO with a VO blend. The relationship between genetic and biochemical mechanisms needs to be further investigated but results obtained so far open the possibility for lipid metabolism, in particular n-3HUFA and cholesterol biosynthetic pathways, to be also somewhat dependent on genetic factors, in addition to the fatty acid composition of the diet. Furthermore, previous biochemical studies at the Institute of Aquaculture showed that there was wide individual variability in the capacity of salmon to retain or biosynthesise n-3HUFA when fed VO diets. These results are the basis for a future experiment that will analyse the liver gene expression (again using microarrays) of different Atlantic salmon families phenotyped for flesh n-3 HUFA content, in response to a

Figure 2. Representation of some of the genes found in the liver transcriptome whose level of expression was significantly affected by an interaction between “diet” and “family”. These genes are involved in lipid and lipoprotein metabolism (Angptl4-angiopoietin-like 4; LPL-lipoprotein lipase), cholesterol transport/efflux (ABCA1-ATP-binding cassette transporter A1) and cholesterol/isoprenoid biosynthesis (IPI-Isopentenyl-diphosphate isomerase). CM-chylomicron; CMR- chylomicron remnant; VLDL- very low density lipoprotein; IDL- intermediate density lipoprotein; LDL- low density lipoprotein; nHDL- nascent high density lipoprotein; CHO- cholesterol; PL- phospholipids. Arrow represents either an up-regulation (↑) or down-regulation (↓) of gene expression in fish fed the VO-diet, compared to that fed the FO-diet, in the “lean” (green) and “fat” (yellow) families. The numeric value represents the expression ratio or fold-change.

100% VO diet, in order to investigate the physiological and molecular mechanisms involved in adaptation to diets containing low n-3HUFA levels and to establish whether this trait may be under genetic influence and thus amenable to selection.

In conclusion, a better understanding of the relationship between genetic and biochemical mechanisms influenced by radical substitutions of FO by VO in fish diets would aid the development of sustainable commerc-

ial feeding practices, while maintaining the health-promoting effects of high n-3HUFA levels for the human consumer. Therefore, the ultimate long-term objective is to identify candidate genes that correlate to this trait (flesh n-3HUFA content) and that might be heritable. If this goal is met, we could then begin to search for polymorphic QTL (quantitative trait loci) markers for the candidate genes of interest, which would enable developing tools for marker-assisted selection.

## What makes a salmon tick?

Andrew Davie, Elsbeth McStay and Hervé Migaud

How does a fish keep time? It has long been known that the daily change in light intensity and seasonal change in daylength commonly referred to as “photoperiod” entrains many physiological process in a wide range of commercially important fish species. However, in the absence of these environmental cues it appears that fish are able to maintain robust temporal order to a diverse range of processes including activity, appetite, spawning and migration. The question is, how can they do this?

These “biological rhythms” are a fundamental adaptation to life on this planet where most organisms exist in ~24 hour cycles. These rhythms are not simply a reaction to photoperiod but an internal endogenous response representing an organism’s capacity to anticipate predictable daily and seasonal alterations in external environmental conditions. At the core of these rhythms is the body clock which is a molecular chain of events (auto-regulatory feedback loop) which cycles with a period of about 24 hours.

In humans this system controls sleep wake cycles and is the route cause of jet lag; its regulatory roles in fish are only just beginning to become evident.

Work over the past few years within the Genetics and Reproduction group has focused on describing the role of the molecular clock mechanism in Atlantic salmon and rainbow trout. To achieve this, a number of salmon and trout homologues for known zebrafish and mammalian “clock” genes have been isolated, cloned and sequenced

to reveal that this basic molecular mechanism is highly conserved across the vertebrate phylum. In addition, quantitative real-time PCR assays have been established against a number of these targets which have subsequently been used to explore the clock's functional roles. Work in Atlantic salmon has demonstrated that cycling of these clock genes can not only provide a daily timing signal but can also provide seasonal timing information, suggesting that this system can act as a calendar as well as a clock (Davie et al. 2009). This supports recent findings in other laboratories that a number of these genes and, in particular, the one called Circadian Locomotor Output Cycle Kaput or "CLOCK", are directly related to the timing of seasonal cycles in reproduction and migration in salmonids. One suggestion is that allelic length variations in this gene could determine the period that the molecular clock cycles over and thus timing of events like spawning or smolt migration. Preliminary work in the group has revealed length variation in the salmon clock gene and therefore

we are continuing to explore the allele frequency distributions in relation to geographic location and life history trait in an attempt to isolate this functional link. Meanwhile work in rainbow trout has focused on describing the ontogeny of clock gene expression in embryos. This work is nearing its completion and amazingly it appears to show that a functioning clock rhythm is passed to oocytes via maternal mRNA. In essence it appears that the mother passes the time of day down to her oocytes to ensure all the embryos develop in synchrony.

Clearly this subject area is wide ranging and has great potential to reveal how strict temporal order is maintained in many important physiological traits. For this reason

PhD student Elsbeth McStay (pictured here) has joined the group to increase the research effort into this key regulatory system. She will be performing a comparative study of clock systems in Atlantic salmon and European sea bass in collaboration with Dr Javier Sanchez of the University of Murcia. As part of the work she will be tackling basic questions like: how does the expression of key clock genes vary with season and between tissue types? Where is the expression of *clock* mRNA localised within the brain? And how does the expression of clock genes vary over the life cycle? It is anticipated that such work will provide a significant advance in understanding of the body clock in these species which in time will be of significant help to the management of these species both for commercial culture as well as their conservation management.



## Kisspeptin leading the way

Hervé Migaud, Andrew Davie, Mairi Cowan and Rania Ismail

**The physiological** control of reproduction in fish is often described as a cascade of events running along the brain-pituitary-gonadal axis (BPG axis). As part of this cascade, recent research has suggested a critical role of the ligand kisspeptin and its receptor GPR54 (G protein-coupled receptor 54). In mammals, the kisspeptin/GPR54 system directly stimulates GnRH (gonadotrophin releasing hormone) neurons in the hypothalamus, GnRH is then released and stimulates the pituitary to release gonadotropins (i.e. LH, FSH) which in turn stimulate the gonad steroidogenic cells (follicular cells in females and Sertoli cells in males) to produce sex steroids that control gametogenesis. Kisspeptin may therefore be described as the master regulator of puberty. It is believed that the kisspeptin system is well conserved across vertebrates and could thus perform similar roles in fish. Furthermore, is kisspeptin the missing link between environmental stimulation (photoperiod mainly) and the initiation of puberty?

Two PhD projects within the Reproduction and Genetics Group (Mairi Cowan and Rania Ismail) are

presently investigating the kisspeptin system in two of the most commercially important fish species in Europe, Atlantic cod and European seabass. Their work aims to better understand the photoneuroendocrine regulation of reproduction in order to more precisely define the onset of sexual maturation and develop new husbandry practices. To do so, they have performed long term studies to monitor the expression of kisspeptin genes (specifically Kiss-1, 2 and GPR54 receptors) along a full reproductive cycle in key tissues along



the BPG axis (brain, pituitary and gonad). Regarding the cod, the maturing group of fish will also be

the BPG axis (brain, pituitary and gonad). Regarding the cod, the maturing group of fish will also be compared to a distinct group of immature fish (created through the use of continuous light from the summer solstice according to previous findings by the group). At present, partial sequences for key genes of interest have been constructed, primers designed and quantitative real time PCR validated. Full length coding sequences of the genes will be generated using 5' and 3' RACE methodologies. The time course expression data generated in these projects will provide the first clues as to how the kisspeptin system works in fish, whether it is conserved as in mammals and it is hoped to inform on the precise timing of puberty.

This work has clear benefits for the finfish aquaculture industry regarding the control of early maturation during on-growing and the management of broodstock spawning. Importantly, the results will also be relevant for most commercially important fish species in Europe which all have similar problems of reproduction. Firstly, definitions of puberty onset and

recruitment into maturation in cod will enable the aquaculture industry to refine management models for the manipulation of cod puberty during on growing. Indeed, early reproduction is one of the main bottlenecks faced by the cod industry leading to reduced growth and flesh quality, mortalities and potential genetic interaction with wild stock through broadcast spawning. Lighting regimes have been designed to suppress maturation in cod and also in halibut. However, to date, confusion remains as to when to apply these regimes especially with out of season stocks. The characterisation of kisspeptin as an assay for puberty could prove to be essential in defining windows of decision (onset of puberty) and optimising the timing of application which will significantly improve profitability of the industry.



Rania Ismail is studying the role of kisspeptin in the European seabass



Mairi Cowan is studying the role of kisspeptin in cod

## Cut and paste genes

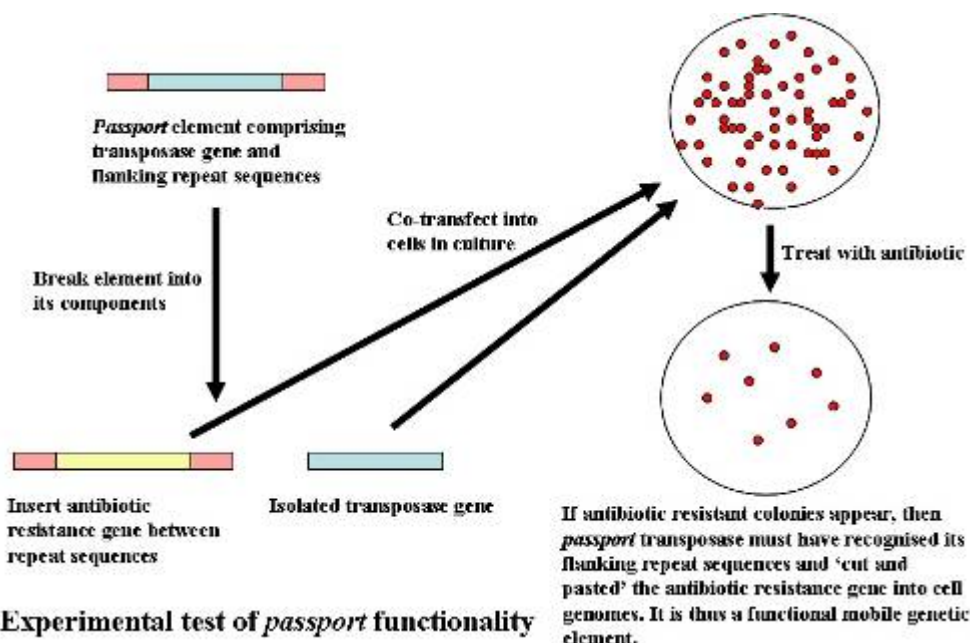
Michael Leaver

**Aquaculture fish** species comprise a phylogenetically diverse group with correspondingly diverse life histories, dietary requirements, disease susceptibility and, as a consequence, diverse culture conditions. This diversity is also reflected in their genomes which substantially differ in size and structure.

Studies at IoA and elsewhere have noted the presence of an unusually high number and diversity of mobile genetic elements (MGEs) in some fish genomes when compared with what might be expected from mammalian genome information. MGEs are DNA sequences encoding proteins capable

may be in generating genetic diversity through their ability to mobilise and colonise genomes, providing grist to the evolutionary mill of natural selection. One of the essential criteria in establishing MGEs as a significant evolutionary force in fish is to demonstrate that naturally occurring MGEs are capable of mobilising.

In collaboration with a US university, we have recently shown this to be the case with an MGE of the Tc1-like transposon class isolated from a flatfish genome, the first example of its type from a vertebrate genome. We have named this element *passport* and shown that *passport* encodes a functional transposase enzyme which



### Experimental test of *passport* functionality

of mobilising their cognate elements such that they can be “copied and pasted” or “cut and pasted” into new sites in the genome in an essentially random manner. This originally led to them being categorised as “junk” or “parasitic” DNA, and were regarded as a burden, requiring their hosts to expend more energy to replicate larger genomes, or causing harmful mutations when insertion caused disruption of critical genes. Although this may be true, it is also true that MGEs are components of the genomes of all organisms, including microbes and most probably have been for several hundreds of millions of years. Given their prevalence, MGEs may be integral components of the eukaryotic genome and thus should have a function related to the success of their hosts. Such a function

interacts with its flanking sequences to “cut and paste” the element from one location to another in genomic DNA. This has been demonstrated in mammalian and fish cell lines and has also been tested as a potential biotechnological tool for use in biomedical applications. Given that at least some MGEs present in fish genomes can mobilize, the question arises of whether they play a role in generating diversity on which natural selection can act. This question is the subject of ongoing studies comparing fish genes, genomes and their complement of MGEs.

#### Original reference

Clark KJ, Carlson DF, Leaver MJ, Foster LK, Fahrenkrug SC (2009). *Passport*, a native Tc1 transposon from flatfish, is functionally active in vertebrate cells. *Nucleic Acids Research* 37, 1239-1247.

This work is supported by SARF, Genesis Faraday, BMFA and the Egyptian Government.

**Fish corticosteroid** receptors are critical physiological regulators of adaptation to different water salinities and the stress response, and are the subject of ongoing studies at the Institute of Aquaculture.

Tetrapods, four-legged animals including human and birds, have two corticosteroid hormones, the mineralocorticoid aldosterone and the glucocorticoid cortisol (corticosterone in some groups). As the name suggests, mineralocorticoids are involved in the regulation of salt retention in the colon and kidney. Glucocorticoids have roles in the regulation of carbohydrate metabolism, for which they were named, and take part in coordinating many other physiological processes including development, cell cycle, immune function, the stress response, central nervous functions, growth and reproduction. The effects of corticosteroids are mediated through corticosteroid receptors expressed in the different target tissues of the hormones.

Corticosteroid signalling in teleost (bony) fish shows a number of peculiarities. Firstly, while tetrapods have one glucocorticoid (GR) and one mineralocorticoid receptor (MR), many teleosts have two GR and one MR. Secondly, teleosts lack the mineralocorticoid hormone aldosterone. Hence, the three teleost corticosteroid receptors mediate the effects of one hormone, cortisol. Cortisol has both glucocorticoid and mineralocorticoid functions in teleosts, making its physiological roles more diverse than in mammals. Thus fish possess multiple receptors enabling them to differentially regulate these functions.

Corticosteroid receptors reside in the cytoplasm in association with chaperone proteins. When cortisol binds to the receptor, the resulting complex dissociates from the chaperone proteins and translocates into the nucleus. Here, it binds to the regulatory region of cortisol target genes, switching their expression on or off. Different cell types have different amounts of corticosteroid receptors and receptor-specific

cofactors required for gene expression. Moreover, some cortisol target genes may be permanently deactivated in some cell types. In this way, a hormone can have distinct effects at different time points and sites within the body.

Teleosts are a large, diverse group, comprising fish living in practically all aquatic habitats of the planet. Some fish are restricted to freshwater or marine environments, while others can cope with both. Some teleosts perform migrations during their reproductive cycle. Anadromous species, such as Atlantic salmon, live mostly in the ocean and breed in fresh water, while catadromous fish such as eel live mostly in fresh water and breed in the sea. In salmon, cortisol is

least sensitive receptor GR1. A recent study in cooperation with Dr Nic Bury, King's College London, has elucidated the molecular basis for the low hormone sensitivity of the trout GR1 receptor. This GR has an unusual carboxy-terminal sequence, deviating from a consensus sequence present most other known GRs, which is one of several structural determinants of the low sensitivity. Bioinformatic analyses revealed that similar unusual sequences are present in GRs from salmon and brown trout, suggesting this unusual receptor might have evolved in salmonids. Ongoing research is focusing on the physiological roles of this receptor.

In addition to their roles in critical physiological transitions in some important aquaculture species, corticosteroid receptors and receptors for other hormones may also be abnormally affected by interacting with environmental pollutants. European flounder are euryhaline fish, i.e. can live in fresh- or sea water. With a studentship funded by the Natural Environment Research Council, and under the supervision of Dr Michael Leaver (pictured left) and Dr Armin Sturm (right), Louise Colliar (pictured on the bench) is investigating the role of corticosteroid receptors and peroxisome proliferator-activated receptors (PPARs, another type of hormone receptor) in normal physiology, and as targets of environmental chemicals. Using molecular cloning methods such as degenerate PCR (polymerase chain reaction) and RACE (rapid amplification of cDNA ends), Louise has isolated gene sequences for two GRs and one MR in flounder. Characterisation of the receptors *in vitro* is ongoing and has shown that some environmental chemicals may be potent antagonists of PPAR function. In future Louise will extend her studies to experimenting with these receptors *in vivo*. The results of this work will provide new information regarding adaptation to stress, salinity changes and the consequences of chemical pollution in fish.



one of the hormones involved in orchestrating the complex physiological changes that occur during the transformation of juvenile salmon between the freshwater-adapted parr and the migrating smolt. Less is known about the roles of cortisol in osmoregulation in other species.

A. Sturm has previously been involved in the initial molecular cloning and characterisation of teleost corticosteroid receptors, using rainbow trout as a model species. The different corticosteroid receptors of trout show distinct sensitivities to cortisol, with MR being about 50 times more sensitive to the hormone than the

## Recent microarray publications from the Institute of Aquaculture

Chipman, J.K., George, S.G., Williams, T.D., Diab, A.M., Sabine, V., Ortega, F. and Falciani, F., 2006. A high density flounder (*Platichthys flesus*) cDNA microarray as a tool in the identification of expression changes in gene sets predictive of exposure to pollutants. *Toxicology Letters*. 164, S42.

Diab, A.M., Williams, T.D., Sabine, V., Chipman, J.K. and George, S.G. 2008 The GENIPOL European flounder *Platichthys flesus* L. toxicogenomics microarray: application for investigation of the response to furunculosis vaccination. *Journal of Fish Biology*. 72, 2154-2169

Falciani, F., Diab, A.M., Sabine, V., Williams, T.D., Ortega, F., George, S.G. and Chipman, J.K., 2008. Hepatic transcriptomic profiles of European flounder (*Platichthys flesus*) from field sites and computational approaches to predict site from stress gene responses following exposure to model toxicants. *Aquatic Toxicology*. 90, 92-101.

Leaver, M.J., Villeneuve, L.A.N., Obach, A., Jensen, L., Bron, J.E., Tocher, D.R. and Taggart, J.B., 2008. Functional genomics reveals increases in cholesterol biosynthetic genes and highly unsaturated fatty acid biosynthesis after dietary substitution of fish oil with vegetable oils in Atlantic salmon (*Salmo salar*). *BMC Genomics*. 9.

Martin, S.A.M., Taggart, J.B., Seear, P.J., Bron, J.E., Talbot, R., Teale, A.J., Sweeney, G.E., Høyheim, B., Houlihan, D.F., Tocher, D.R., Zou, J. and Secombes, C.J. 2007. Interferon type I and type II responses in an Atlantic salmon (*Salmo salar*) SHK-1 cell line using the salmon TRAILS/SGP microarray. *Physiological Genomics* 32: 33-44

Seear, P.J., Carmichael, S.N., Talbot, R., Taggart, J.B., Bron, J.E. and Sweeney, G.E. 2009 Differential gene expression during smoltification of Atlantic salmon (*Salmo salar* L.): a first large-scale microarray study. *Marine Biotechnology* DOI 10.1007/s10126-009-9218-x

Taggart, J.B., Bron, J.E., Martin, S.A.M., Seear, P.J., Høyheim, B., Talbot, R., Carmichael, S.N., Villeneuve, L.A.N., Sweeney, G.E., Houlihan, D.F., Secombes, C.J., Tocher, D.R. and Teale, A.J., 2008. A description of the origins, design and performance of the TRAILS/SGP Atlantic salmon *Salmo salar* L. cDNA microarray. *Journal of Fish Biology*. 72, 2071-2094.

## Native oyster hunt

Janet Brown and Liz Ashton

The native oyster *Ostrea edulis* (shown right), also known as the flat oyster and the European oyster, has significantly declined in abundance and distribution since the 19<sup>th</sup> century, mainly as a result of over-exploitation. Calls have been made for a decent sized effort at restoration and the Scottish Aquaculture Research Forum (SARF) is to be congratulated for putting forward funds for preparation of such a project proposal. Support for native oyster restoration is evidenced by the fact it is a UK Biodiversity Action Plan Priority Species. The restoration of a sustainable native oyster fishery can bring social and economic benefits. Moreover research from USA has also highlighted the important ecological roles oysters play in filtering the water column, contributing to the recycling of nutrients and organic material and increasing biodiversity by providing a viable habitat and food source for many other species.



required, and researching potential funding bodies, their requirements and time frames.

Our aim is very much for this project to be participatory as significant stakeholder cooperation and will is needed to get the level of funding required for a pilot scale re-establishment of native oysters. Protection of stocks and public ownership are also crucial for the long term sustainable future of this valuable species for both biodiversity protection and commercial potential.

To get the necessary inputs from all stakeholders takes time and no one can afford to invest the necessary amount of time without some sort of support. Thus in July 2008 SARF put forward a proposal to fund a project specifically to “develop and deliver a funding application to re-establish, on a pilot scale, a native oyster population in Scotland, with a view to assessing the potential to derive a sustainable harvest from the re-established population”. Dr Janet Brown and her team consisting of Dr Liz Ashton and David Scott are currently undertaking this work, with support from SARF, Crown Estates, Scottish Natural Heritage and the University of Stirling, via the input of Janet’s time.

The work has involved field trips to the Firth of Forth, West coast, Shetland, Skye and North Uist to identify potential sites and collaborators, all of which have been fruitful. The work has also involved collating and updating information on oyster restoration work, reviewing the technical requirements, approaches and regulatory framework

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# From devil to white fish

Lindsay Ross and Carlos Martinez Palacios

**Dr Carlos** Martinez Palacios is currently spending part of his sabbatical year working at the Institute with long-term collaborator Prof. Lindsay Ross, where they are concluding one set of major projects on an alien catfish invasion but initiating a new set of projects on an endangered whitefish native to the Mexican altiplano lakes.

Carlos and Lindsay and their respective research groups are finishing a two year project with the so-called “devil fish” (*Pterigoplychthys disjunctivus*) from the Amazon river which was introduced to México and



Fig. 1. Pez Diablo – the “devil fish” in a Brazilian fish market.

which has had a massive negative impact on the local fisheries and markets. The fish was released accidentally into the upper tributaries of the Rio Balsas catchment, probably from aquaculture for the aquarium trade, in 1995. At that time it was a relative rarity and even the subject of some competition between fishermen to land a specimen. However, the fish took an unoccupied niche and in recent years the population has increased to such an extent that 80% of every catch now consists of this alien species. There is no tradition of utilization of this fish in the region and hence, up to now it has been considered as a by-catch, albeit a massive one, from what is essentially a tilapia fishery. Concern has also grown because the fish damages gill nets and the spiny scales and fins make the animal difficult to remove from the mesh, as well as being painful to handle.

Our projects have focused on finding solutions to this invasion and population explosion. One proposal from many ecologists has been that of complete removal – but this is virtually impossible and totally impractical. A better immediate term solution is to intensively exploit products from this new fishery and in the last two year we have demonstrated several exploitation methods which are now being followed up by local communities and government agencies.

The species is consumed fresh in Brazil, Colombia and other countries.

This fishing activity and natural predation contribute to keeping the populations under control. In fact, in parts of Brazil the fishery is now controlled to avoid over-exploitation. Our trials in Mexico show that with proper training high quality fillets can be recovered from the species (Fig. 2). Simple preparation has resulted in a product which has high nutritional value and which has been well accepted in taste trials with the local communities. Trials with artisanal smokers have also been very successful. The smoked tail fillets have a good flavour and quality and also give a product with added value. The main impediment to this approach is in developing a market.



Fig. 3. Farmed juvenile pescado blanco, *Menidia estor*.

Once the flesh has been recovered from the species, the head, guts and skin can be ensiled to produce a proteinaceous product for addition to animal feeds. Preliminary trials at the kilogram scale have shown that a stable, semi-moist product can be fed as partial substitute in feeds for pigs, sheep, cattle and chickens. The alimentary tract of these fish contains a range of powerful enzymes for dealing with the periphyton on which they principally feed. These enzymes can be extracted, concentrated, freeze dried and used for digestion of sewage and polluted waters to reduce environmental impacts.



Fig. 2. The fresh product tastes really good!

As there was no established use for the species the practice was to simply discard the product. This had environmental consequences as well as being a waste of a resource. The problem became so serious that the national biodiversity agency (CONABIO) expressed concern and National and State governments supported our projects to resolve the issue. This involved not only technical research to develop exploitation methods but also essential work to raise the value of the species so as to promote its exploitation and commercialization of viable products. A fully illustrated recipe book showing how the fish can be used has been a superb example of the community level work achieved through the project.

Lindsay and Carlos are immediately commencing a new, very large project (one million pounds sterling over 2 years) to demonstrate the feasibility of the culture of the atherinopsid fish, pescado blanco (*Menidia estor*) a native and high value fish from central México (Fig. 3). This is the result of almost 10 years of collaborative joint research, funded principally by CONACyT (the National Council of Science and Technology) and the local government of Michoacán state, as well as input from the Darwin Initiative. This work established a substantial technical background which has enabled pilot aquaculture of the species.



The new project is a major

Fig. 4. The popularity of pescado blanco in tourist restaurants has ensured its ever-increasing price and declining population.

development involving creation of a new autonomous centre which will demonstrate the feasibility of the commercial culture of pescado blanco. The main objective will be to develop and promote the culture of species of atherinopsid fish from the Mexican altiplano. It is intended that the products will be accredited with a *denomination of origin* which we hope will help to ensure that aquaculture takes place within the natural biogeographic area of the species. This will promote conservation of members of the species flock, many of which are currently endangered. The new centre will be created during the next year and will represent a major transfer of technology from our research to a wide range of social and private groups who are already interested in developing businesses based on the commercial culture on these species.

## More in STORRE

Clare Allan, University Library

**STORRE** is the University's Open Access repository; its aim is to make Stirling's research more widely and easily available.

See <http://storre.stir.ac.uk>

It holds the full text of the University's theses from September 2006 onwards; covering PhDs and Masters by Research. A small collection of our older theses is also included, and will be added to, due to ongoing digitisation work and our involvement in the British Library's new thesis digitisation service (<http://ethos.bl.uk>).

STORRE also holds a growing collection of other research publications – see the Institute of Aquaculture collection at: <http://storre.stir.ac.uk/dspace/handle/1893/12/browse-date>.

Recent theses submitted to STORRE:

Adamidou, Styliani, 2008  
Effect of extrusion on the nutritional value of peas (*Pisum sativum*), chickpeas (*Cicer arietinum*) and faba beans (*Vicia faba*) and inclusion in feeds for European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*)  
<http://hdl.handle.net/1893/615>

Agbo, Nelson W., 2008  
Oilseed meals as dietary protein sources for juvenile Nile tilapia (*Oreochromis niloticus* L.)  
<http://hdl.handle.net/1893/984>

Asmah, Ruby, 2008  
Development potential and financial viability of fish farming in Ghana  
<http://hdl.handle.net/1893/461>

Bundit, Jatuporn, 2008  
The nutrition and feeding of a native Thai species, the marble goby (*Oxyeleotris marmoratus*), involving on-farm and experimental studies  
<http://hdl.handle.net/1893/256>

Butterfield, Gareth Melgalvis, 2008  
Genetic variation for disease resistance in rainbow trout (*Oncorhynchus mykiss*)  
<http://hdl.handle.net/1893/391>

Gratacap, Remi M. L., 2008  
Characterisation of *Vibrio anguillarum* for the development of vaccine in cod (*Gadus morhua*)  
<http://hdl.handle.net/1893/1142>

MacIntyre, Craig Mackenzie, 2008  
Water quality and welfare assessment on United Kingdom trout farms  
<http://hdl.handle.net/1893/434>

Madalla, Nazael, 2008  
Novel feed ingredients for Nile tilapia (*Oreochromis niloticus* L.)  
<http://hdl.handle.net/1893/795>

Manji, Farah, 2008  
Development of methods to determine prevalence of *Flavobacterium psychrophilum* in farm systems  
<http://hdl.handle.net/1893/1127>

Martinez Chavez, Carlos Cristian, 2008  
Photic entrainment and onset of puberty in Nile tilapia (*Oreochromis niloticus*)  
<http://hdl.handle.net/1893/354>

Tildesley, Andrew Saul, 2008  
Investigations into *Ergasilus sieboldi* (Nordmann 1832) (Copepoda: Poecilostomatoida), in a large reservoir rainbow trout fishery in the UK  
<http://hdl.handle.net/1893/1261>

Walton, Keith, 2008  
Aspects of the Atlantic salmon immune response during infection with the salmon louse, *Lepeophtheirus salmonis* (Krøyer, 1837)  
<http://hdl.handle.net/1893/1313>

Del-Pozo Gonzalez, Jorge, 2009  
A study of the aetiology and control of rainbow trout gastroenteritis  
<http://hdl.handle.net/1893/1081>

Minghetti, Matteo, 2009  
Characterisation and expression of copper homeostasis genes in sea bream (*Sparus aurata*)  
<http://hdl.handle.net/1893/1113>

Ureta Schmidt, José P., 2009  
Selective improvement of rainbow trout: assessment of potential in UK strains  
<http://hdl.handle.net/1893/1317>

We're also digitising older paper theses that are requested a lot in the Library – so we've recently added James Bron's PhD to STORRE:

Bron, James Emmanuel, 1993  
A study of the biology and behaviour of the copepodid larva of the salmon louse *Lepeophtheirus salmonis* (Kroyer, 1837) (Copepoda; Caligidae)  
<http://hdl.handle.net/1893/625>

## Catch up

You can now keep in touch with other alumni of the Institute via a Google Group:

<http://groups.google.co.uk/group/institute-of-aquaculture-alumni>



20 officers from United Kingdom Trade and Investment (UKT&I) missions around the world visited the Institute in July as part of a tour of UK agricultural expertise. Here James Bron shows some of them the 3-D delights of the confocal microscope.



The Howietoun barbecue was re-born this summer. Everyone enjoyed excellent food and a bit of light competition! Thanks to Iain Semple and the organising team.



It is with great sadness that we must report on the untimely loss of two Institute alumni, both of whom contributed significantly to the sector.

### **Ibrahim Okumus PhD Aquaculture, 1993**

Ibrahim carried out his doctoral research under Dr Hadrian Stirling, working on concepts that are now widely promoted as "Integrated Multi-Trophic Aquaculture" (IMTA) systems, particularly the uptake of salmon farm wastes by mussels. After the award of his doctorate, Ibrahim returned to his native Turkey and a post in teaching and research at Karadeniz Technical University, Trabzon. He quickly attained both national and international recognition, initiating local and regional projects, editing national journals and actively participating in wider EC projects and FAO consultancies. He was an active participant in the EC funded AQUA-TNET network, helping to build links with other institutions and had most recently made Karadeniz Technical University a partner in the PESCALEX projects to help promote language skills and translate specific training materials into Turkish. Despite declining health he remained professionally active, which made his death in December 2008 a great shock to the aquaculture community. Ibrahim will be missed by all who knew him and our condolences go to his wife and daughters.

He was clearly very well loved and respected by all the people he worked with and trained. Comments from his own colleagues sum up his importance to Turkish and international aquaculture research and development 'We are proud of his achievements. He was a man full of love of country, representing the values of society. He was a spirit to his students, colleagues and scientific community. His loss will be felt everywhere.'



Ibrahim, welcoming visitors to a conference



Louise, enjoying the great outdoors, as usual

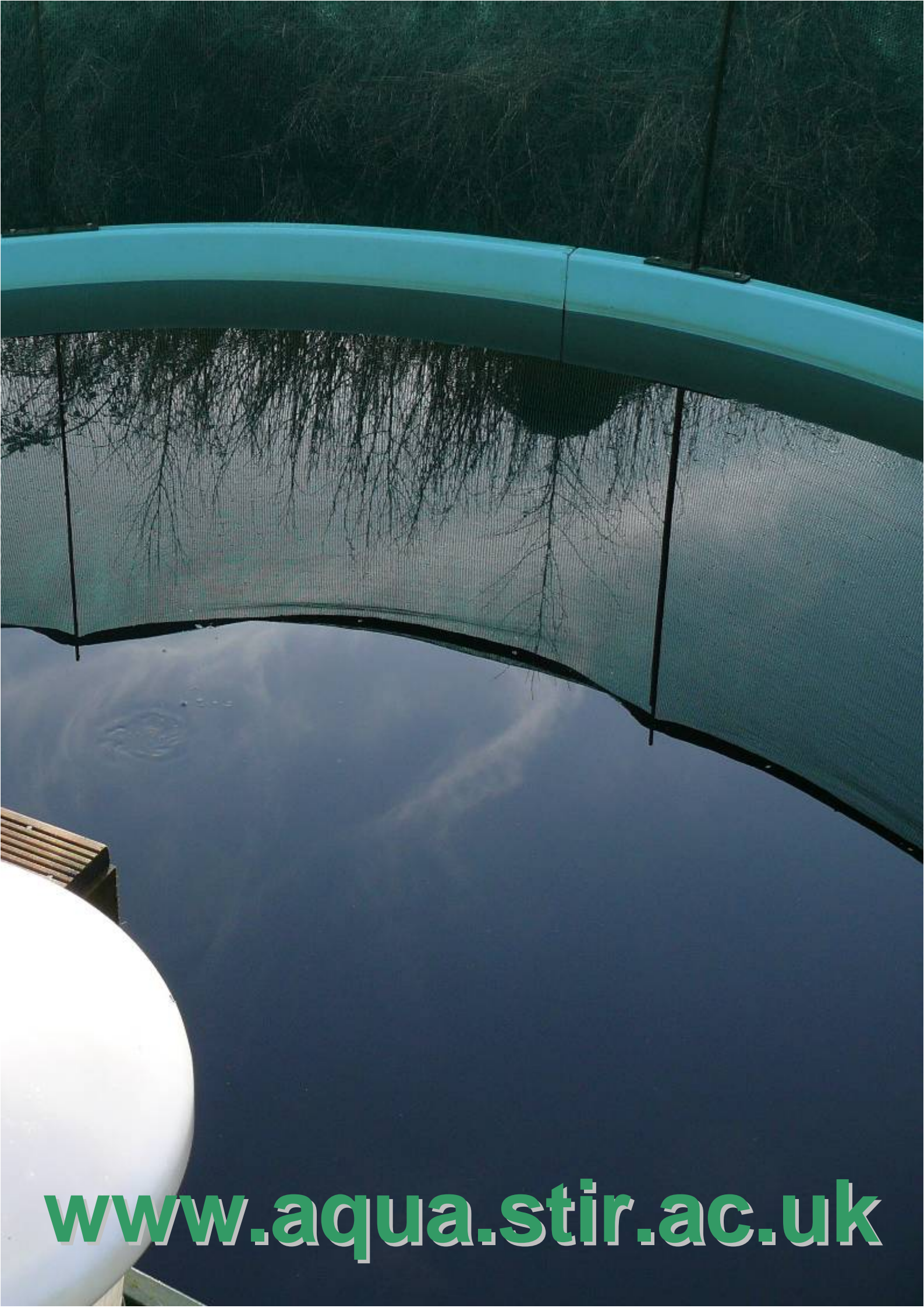
### **Rachel Louise Andrews MSc, Aquatic Vet Studies, 2007**

Louise had a background in agriculture and a passion for animal welfare. She came to Stirling after developing a stronger interest in fish, but returned to everyday veterinary practices, where she was especially involved with small animals, after completing her MSc in Aquatic Veterinary Studies in 2007.

Finally, the urge to set up a practice specialising in diseases of fish became paramount and she moved to Coggeshall, Essex, where she established Andrews Veterinary Services. She attracted clients across the country, dealing with domestic exotic species, as well as being called upon by owners of fish farms and other corporate clients. No distance seemed too far for her to travel, but she frequently kept in touch with clients by email where a problem could be dealt with remotely.

But it was not to last. Louise was fatally injured in a tragic accident on a farm track whilst inspecting fish ponds during the evening of Friday June 12<sup>th</sup> 2009. She will be deeply missed by the many friends, sporting colleagues and clients she acquired during her life and of course by her family.

We shall miss them both.



[www.aqua.stir.ac.uk](http://www.aqua.stir.ac.uk)