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# aquacultureNEWS

I have fully settled into my role as Director of the Institute now and am delighted to introduce this edition of Aquaculture News, which showcases the impact that much of our work has had in the wider world. In these testing financial times, with increased pressure on researchers to compete for ever decreasing sources of funding, it is reassuring to see that the work of everyone in the Institute continues to support development of the aquaculture sector in a practical way, whilst also being recognised as world class in its own right.

As ever the Institute remains a dynamic research environment, with a wide range of nationalities represented, particularly amongst our post-graduate students. On our MSc programmes this year we have welcomed students from 22 different countries. Our record in recruiting and training scientists around the world continues to grow, as evidenced by the award winners in our 2<sup>nd</sup> PhD research conference. I am grateful to all involved in organising and running that event, not least the many sectoral partners who sponsored the event.

The University of Stirling is currently undergoing structural change and the Institute of Aquaculture will become part of a wider School of Natural Sciences. Our focus on post-graduate research and teaching will further support the growth of the School as a whole and we look forward to the increased opportunities for collaboration this new structure should bring. You will see from many of the articles included here that collaboration is second nature to us as scientists aiming to support an increasingly important food production sector. This edition includes detail of the history and current status of our research into sea lice control; research to ensure controlled reproduction for improved productivity; our key partnerships with industry to develop effective vaccines; our pioneering research on fish welfare; our intercontinental collaborations supporting international aquaculture trade; and an example of how our PhD students go on to develop effective businesses further supporting growth of the sector.

I hope this edition makes a suitable splash!

PROFESSOR BRIAN AUSTIN, DIRECTOR

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FRONT COVER: Brown trout making a splash  
Image courtesy of Denny Conway  
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 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 at [a.j.immink@stir.ac.uk](mailto:a.j.immink@stir.ac.uk) - with 'Aquaculture News' in the title.

# More than scratching the surface: sea lice research impact

Christina Sommerville

**Sea lice** (*Lepeophtheirus salmonis* and *Caligus elongatus*) research in Stirling originated at the time of the first outbreaks in the early days of salmon mariculture development. However, the first funded project was jointly awarded to Chris Sommerville and Rod Wootten whilst Rod still worked at DAFS (now Marine Scotland). Little was known at that time of the biology of the sea lice. The early research at Stirling described, amongst other things, a detailed life cycle of *L. salmonis* and generation times at different seasonal temperatures, information which was passed on directly to the fish farmers and which aided in the development of treatment schedules. A laminated illustration of the parasite and its life stages was made and soon found its way onto the wall of the site office at most farms. Further work established a suitable practical sampling regime for fish farmers and they were advised how to monitor the parasite development in cage systems. Farmers were trained through site visits and short courses at Stirling on how to recognise the different species of lice, *L. salmonis* and *C. elongatus*, how to identify each life stage and how to monitor the farm sites using our sampling regime; a technique which is basically still widely used today.

Clarification through experimentation showed that there were susceptible and non-susceptible stages to the then current treatment with dichlorvos and showed that it was important to treat at the most appropriate time to maximise efficacy and to minimise cost in labour and in environmental terms. This data, together with the epidemiological data (see below) determined the optimum time for treatments to deliver a) a more effective treatment, b) a reduced need to treat, and c) better long term disease management. A system was setup which invited farmers to send samples to Stirling for identification and for counting.

A further study on the method of application of treatment chemical, compared full, partial and no-enclosure of cages during treatment and made recommendations for their use. Treating a whole site simultaneously was stressed to be important in delaying the development of resistance. However, detection of

reduced sensitivity of lice to dichlorvos was observed as early as 1989 and at about the same time we developed an assay for cholinesterase activity and the effects of dichlorvos treatment on fish acetyl cholinesterase activity was assessed. Experiments which looked at repeat treatments at 3 and 6 day intervals showed that there was a cumulative effect of treatments with short intervals which resulted in incremental reductions in acetyl cholinesterase activity. Farmers were advised on the minimal interval to achieve between treatments as treatments were becoming more frequent with the spread of reduced sensitivity in farm lice populations.

A study carried out on behalf of the Scottish Salmon Growers investigated the sensitivity to dichlorvos in farms across Scotland using a specifically designed bioassay. The published results established for the first time the extent of reduced sensitivity to dichlorvos, the only treatment available at that time. The industry very quickly moved towards a crisis with sea lice becoming very difficult to control.

In anticipation of there being some considerable time before a new product could be brought to license to replace dichlorvos, we set about finding non-chemical ways of minimising infection. Working again very closely with the industry we determined the epidemiological pattern of infection in relation to a number of factors associated with farm husbandry and management. As a result of this study we were able to show that lice built up on farms over the growing cycle and the clearly infected 2+ fish were acting as a major source of infection of newly stocked vulnerable smolts. The first major recommendation was, therefore, for single year class stocking. We also showed that a fallowing period was highly beneficial in reducing the build up of lice epizootics at any one farm site.

Parallel studies on the biology of lice took an in depth look at the copepodid stage, which is the transmission stage which develops from the free living nauplius to become the stage infective to fish. This then moults into the first chalimus stage after forming a



An infestation of sea lice

filament attachment. We described the mode of attachment of the copepodid and the process of the filament formation in the chalimus I stage and their distribution on the salmon host. A histopathological study showed the feeding behaviour of the copepodid and the chalimus stages and concluded that there were minimal pathological effects on the epidermis of the host and a rapid healing of the lesion. This information was able to lead farmers to concentrate on the control of the mobile stages. A study of the functional morphology of the alimentary canal laid the basis for the subsequent major attempts at vaccine development which sought to find antigens which, taken in orally, would disrupt digestion. We have also described the morphology of the cuticle and its associated glands, also a target for future vaccine attack

A study of the eye structure and studies on the copepodid's responses to light were taken up in the anticipation of finding treatment or control methods using phototactic responses. Of particular interest was the strong positive response to light of certain wavelengths, which led to the invention of light lures. Unfortunately, a successful light lure has yet to be designed.

In the early 1990s we embarked on a long term programme developing alternative chemotherapies which continues to this day. Our approach has always been to work with pharmaceutical companies for whom we have screened many potential products over many years.

We have subsequently contributed to a number of new products for sea lice control which have reached the market. For example, the preliminary work on pyrethrins was published as long ago as 1993. We successfully tested and completed trials for licence for hydrogen peroxide treatments with Solvay Interlox and the organophosphate azimethiphos, marketed as Salmosan, with Ciba Geigy. The most successful product tested was emamectin benzoate (SLICE) for Schering Plough. We then carried out trials sponsored by Schering Plough to obtain a licence for this product. This was an innovative oral treatment and had, for the first time, a long duration of efficacy. SLICE has been the most effective and successful chemotherapeutant for lice to date and is used globally.

The ability to use a variety of treatment products made it possible to slow the development of resistance so, alongside the development of new chemotherapies, we developed and promoted the concept of an IPM (Integrated Pest Management) strategy. An IPM strategy involves many non-chemical approaches such as facets of management, husbandry techniques and biological control and only finally chemotherapies used in rotation. We were the first to promote the idea of an IPM for management and control of sea lice which we presented through conferences and trade press. We have also pursued aspects of biological control to be used in an IPM. The most familiar biocontrol method is the use of cleaner wrasse, first developed in Norway where wild, captured wrasse are used to stock salmon cages. We looked at their pathogens to see if any were likely to act as vectors of disease to salmon and showed that they were susceptible to two important viral diseases - IPN and pancreas disease. Thus we were able to warn farmers that infected wrasse on site could create a situation where restriction orders may be imposed on a salmon site under the Diseases of Fish Act.

Other programmes on-going will define the genes which cause the development of resistance and may even produce a lice resistant salmon population for the industry. Population studies using a variety of molecular techniques helped understand the dispersion of lice around the Scottish coast and contributed information to the wild versus farmed debate by comparing the two populations. We found that there was more variation between individuals than between the wild and farmed populations. Microsatellite analysis indicated a high level of gene flow around the Scottish coast with implications for the spread of resistance.

Current research at IOA and Machrihanish Marine Environmental Laboratory involves the screening of further possible treatment compounds and the development and use of a bioassay method for determining the sensitivity of lice to therapeutants. The latter is now used by industry to screen lice populations on farms. Further research involves studies on the mechanisms of resistance in lice, a very significant issue currently.

## Scotland needs to flex its mussels

**A Scottish** Government report produced by Stirling Aquaculture has identified significant scope for growth in Scotland's shellfish industry, with mussel farming identified as an area that Scottish producers should place more focus on.

Researchers here analysed the prospects and opportunities of farming mussels, oysters and scallops. Despite Scotland's marine environment offering good opportunities for cultivating shellfish, production remains low compared to other parts of Europe.

Environment Minister Roseanna Cunningham said "There is fantastic potential for shellfish farming in Scotland, as our clean waters offer the right conditions for cultivation in what is an eco-neutral industry. As this study shows, there is significant scope to increase our productivity and the volume of shellfish, particularly mussels. The shellfish industry is an excellent example of sustainable development, supporting vital employment opportunities in remote parts of Scotland."



Walter Speirs of the Association of Scottish Shellfish Growers said: "The Association of Scottish Shellfish Growers very much welcomed this study, which was an attempt to explore the future for our sector. The input from Stirling University was unbelievably thorough, leaving no areas unexplored. It is very reassuring for our members to know that Scottish Ministers support our industry, and our attempts to expand it."

You can view the report at:  
<http://www.scotland.gov.uk/Topics/marine/science/Publications/publications/latest/other/prospectandopportunities>

# Not just keeping your fingers crossed: reproduction research impact

Herve Migaud, John Taylor and Andrew Davie

**The farming** of reproductively competent fish is a major concern in the aquaculture industry due to the potential impact on wild stocks through interbreeding with farmed escapees, potential welfare impacts and reduced productivity on farm. Over the years, the Reproduction Group at the Institute of Aquaculture has carried out research to tackle this problem using a range of management strategies including: photoperiod manipulations to delay reproduction outside the harvest window; production of monosex populations where sexual dimorphic growth allows harvesting prior to maturation; and ultimately sterility by means of triploidisation. The knowledge gained has led to the implementation of protocols, guidelines and practices within the industry that significantly improve the sustainability of the sector, generate growth and increase profitability. As an example, photoperiod regimes have been optimised and standardised in the salmon on-growing industry, significantly reducing the prevalence of early maturation during the first year at sea. Meanwhile monosex production has been implemented in the portion-size rainbow trout industry in combination with sterility to remove any problems associated with stock maturation. Similar work has been carried out in a number of other commercially important species in Scotland including Atlantic cod, halibut and haddock. However a lot of research, development and knowledge exchange remains to be done to help this sector to secure sustainable growth. In this article, we describe two ongoing research priorities that are being investigated in collaboration with a number of industrial partners.

## Triploid developments in the salmon and brown trout industries

Although not a new concept in itself, triploid induction is the only commercially acceptable method at present to ensure sterility. Originally tested in the late-80's/early 90's as a means to prevent pre-harvest maturation, triploidisation of salmon was abandoned as triploid stocks showed reduced performance and a high occurrence of deformity. However, with rapid expansion of the industry in recent years and increasing public concern of the potential impacts

of farmed escapees the industry is keen to re-explore the potential of triploidy to ensure reproductive containment. The Reproduction Group is now leading an FP7 EC funded project SALMOTRIP (221115, [www.salmotrip.stir.ac.uk](http://www.salmotrip.stir.ac.uk)) involving key R&D and SME partners from Scotland, Norway, France and the Netherlands to perform a full-scale feasibility study of commercial triploid salmon production. Results so far indicate that triploids can outperform their diploid siblings (up to 30% bigger) and there is a clear need for triploid specific breeding programmes. Out-of-season (S0+) triploid smolts were also produced for the first time; a significant development for the industry which heavily relies on inputs of S0+ fish to ensure year round supply of salmon. Key risk areas in current salmon diets and environmental conditions have been identified and dietary deficiencies in triploids must be investigated. We have explored thermal, oxygen, and nutritional thresholds, and related these to functional performance (e.g. exercise, growth and feed utilisation).



Diploid/triploid Atlantic salmon

This has increased our understanding of cellular physiology and will help in identifying sites where triploids should not be grown. Finally, a large scale customer perception analysis of triploid products is ongoing with the view to identify marketing strategies.



Brown trout

On another perspective, sport fishing for trout within the UK in both still and running waters has an estimated value exceeding £500 million per annum. However, most trout fisheries rely partly or, in many cases, entirely on stocking to maintain catches. Farmed trout often differ genetically from their wild counterparts. In this respect wild trout are at risk from interbreeding with farmed fish. As a preventative measure the Environment Agency has recently implemented the "National Trout and Grayling Strategy", which will only give consent to the stocking of rivers with non-fertile, all-female triploid brown trout. However, little information on triploid brown trout performance is available to date. The Reproduction Group initiated research to explore the production and assessment of triploid brown trout. This research will create and optimise induction protocols which will be directly transferable to farmers. Knowledge on culture performance (including growth, deformity prevalence and optimised environmental requirements) and behavioural interactions under natural/semi-natural conditions (feeding location, habitat choice, swimming ability and thermal tolerance) will be developed through a series of experimental and commercial field trials. Overall this area of research aims to transfer working protocols and guidance to the UK farming and sports fishing industry.

## Monosex production is essential to ensure profitability of the halibut sector

The marine aquaculture sector is dominated by salmon on-growing, however, there is an increasing realisation in the sector that the integration of alternative species (ranging from finfish to macroalgae) will optimise the use of available resources, increase productivity and ultimately lead to a more sustainable industry. One such alternative species is Atlantic halibut which has been farmed in the UK for almost 20 years. However, a key bottleneck restricting the productivity of halibut farming is that patterns of growth and sexual maturation are different between sexes. Male halibut reach sexual maturity approximately 2-3 years earlier than females at a size that is below the optimal harvest weight while females reach harvest size prior to any sexual development. Thus there is a clear advantage to rear monosex (all female) stocks over mixed sex populations. With the support of the Scottish Aquaculture Research Forum, the Reproduction group has been examining two

methodologies that could potentially allow the commercial production of all-female halibut stocks. Firstly we explored the use of flow cytometry to sort halibut semen based on cellular DNA content, a technique employed increasingly in terrestrial agriculture. However, as we were unable to demonstrate any measureable difference in DNA content in sperma-

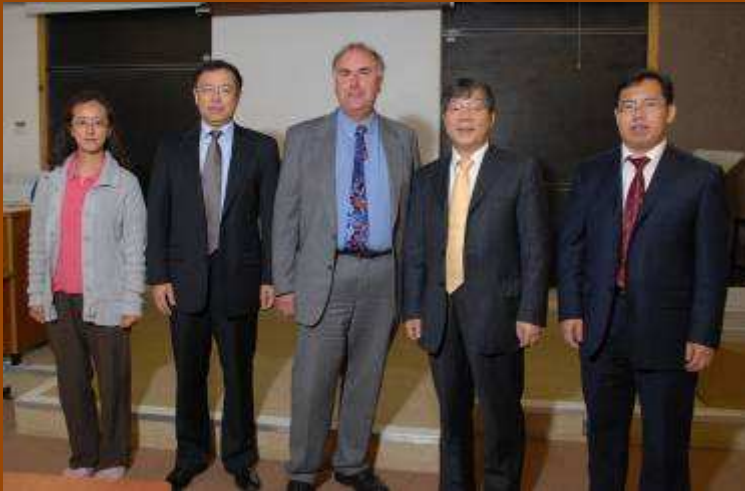


### Halibut sex reversed

tozoa in halibut (or any other commercially important fish species we tested) this technique is of no use to the sector until some other form of sex linked marker is identified. In parallel to this work we have also refined a protocol to produce neomale halibut broodstock. In this process juvenile halibut were briefly fed with steroid treated feed during sexual differentiation. Following treatment

97% of the population was phenotypically male although the 50:50% male to female genotype ratio was unaffected. When they reach sexual maturity the normal male (male phenotype and genotype) will produce mixed sex offspring while the neomales (male phenotype and female genotype) will naturally produce all-female offspring. This is a long term investment as it has taken 3 years for the first fish to reach sexual maturity so work is now in the pilot phase of validating methodologies to confirm neomale status and thus guarantee all female progeny production in the coming years. The collaboration, now in its 4<sup>th</sup> year, has helped the sector realise a dream of producing all-female halibut, however, new opportunities are being sought to continue the partnership and help upscale the neomale identification and ensure that the industry can make all-female production a commercial reality in the coming years. Ultimately it is hoped this work will improve the competitiveness of the UK industry and may help return investor confidence in the British marine finfish farming sector as a whole.

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/>



A delegation from Ocean University visited the Institute to discuss partnership options, including research and teaching collaborations that could see a significant increase in the number of Chinese students attending the Masters courses.

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# This isn't going to hurt: aquatic vaccines and diagnostic tools

Sandra Adams

**Disease is still** regarded as a major constraint to aquaculture production globally. Control of disease is complex and relies heavily on a combination of pathogen detection, disease diagnosis, treatment, prevention and general health management. Rapid disease diagnosis and vaccination play a crucial part in this and the team from the Aquatic Vaccine Unit, Institute of Aquaculture, led by Professor Sandra Adams have made a significant contribution to both of these areas over the last twenty years.

Research in the Aquatic Vaccine Unit focuses on fish disease control through the development of novel rapid diagnostic tests (both molecular and antibody based) and vaccines for both fresh water and marine cultured fish species. In addition, basic research focuses on the elucidation of host pathogen interactions and the immune system of fish. Numerous vaccines, antibodies and kits have been/are being commercialised from research projects performed at Stirling. The Aquatic Vaccine Unit is also an active partner in the Scottish Fish Immunology Centre with partners in Aberdeen (Aberdeen University and Marine Scotland immunology laboratories), promoting the training of immunologists. Moreover, it has close links with local schools, taking Nuffield Bursary placements every year.

## Fish Vaccines

Vaccination has made a huge impact in reducing the risk of furunculosis in salmon in Scotland and Norway. This in turn led to a reduction in the use of antibiotics that has been sustained, and an increase in productivity as a result of vaccination. Vaccines developed in the Aquatic Vaccine Unit are part of this success with a furunculosis vaccine being developed in a joint programme between Marine Scotland in Aberdeen and Stirling. Other vaccines developed at Stirling in the late 1980's and 1990's include enteric redmouth (ERM), vibriosis and pasteurella vaccines, with many others in the pipeline over recent years.

Vaccination has been extremely successful in reducing the disease risk in fish, however, biological, scientific

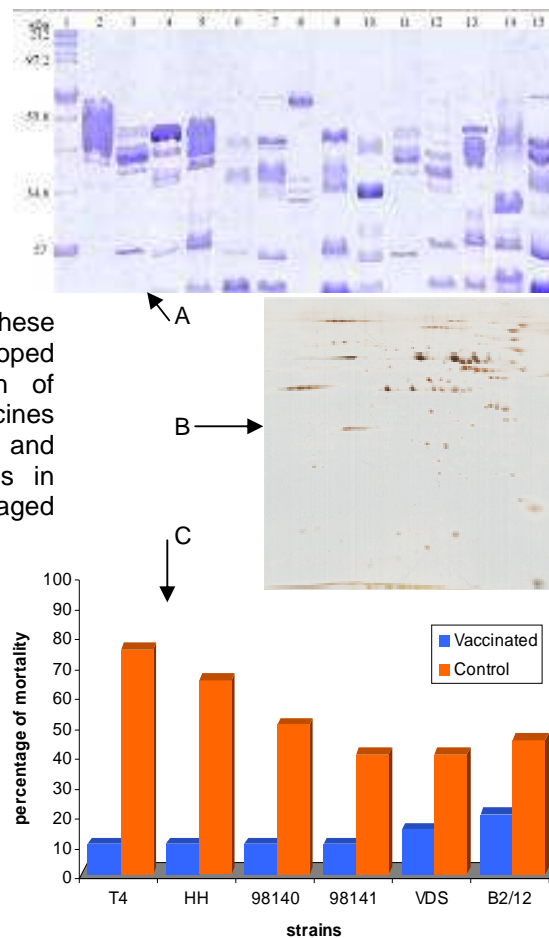
and technical restrictions still prevent the production of commercial vaccines for most economically significant fish diseases. Knowledge of the sequence of pathogen genomes, gene function and derived products now allows innovative approaches for vaccine development using genetic, immunology and chemistry/physiology-based technologies. These are the kind of approaches now being taken by Stirling for vaccine development. In addition, novel vaccine antigen identification methods are being devised using combinations of various techniques such as genomics, proteomics, knockout technologies and epitope mapping. In other words fish vaccine development has become much more sophisticated in recent years. These novel vaccines are being developed because the simpler approach of using inactivated whole cell vaccines does not always succeed, and attempts at attenuated vaccines in general have not been encouraged from a safety point of view.

In order to develop an effective vaccine the protective antigens need to be identified and their protective response confirmed in the host species. Research in the Aquatic Vaccine Unit has been focusing on improving methods to identify which serotypes to include in traditional whole cell vaccines, as well as developing new methods to identify potential vaccine antigens. Four examples are presented here where vaccine antigens have been successfully identified at Stirling using different technologies: rainbow trout fry syndrome (RTFS), vibriosis in cod, *Aeromonas hydrophila* in carp and betanodavirus in sea bass. Such methods can be used for the development of many other vaccines.

### RTFS vaccine development - a traditional whole cell approach

Many of the current fish bacterial vaccines are whole cell vaccines where the isolates included are simply collected from disease outbreaks and are incorporated on that basis.

Inclusion of all serotypic variants is important but in many cases serotyping systems are not robust. Thus, a new serotyping system was developed for *Flavobacterium psychrophilum* so that representative isolates could be selected from each serotype. The *F. psychrophilum* vaccine developed by Dr Alison Morgan, Kim Thompson and Sandra Adams is currently on field trial with a view to commercialisation.



**Fig. 1 A. hydrophila vaccine development:**  
A. Antigenic diversity of outer membrane profiles of *A. hydrophila* isolates by SDS polyacrylamide gel electrophoresis (PAGE)  
B. Western blotting sera from infected carp on to 2D SDS gels identified potential vaccine antigens  
C. Vaccination with the recombinant vaccine protects against a variety of field isolates of *A. hydrophila* to different extents

### *Aeromonas hydrophila* vaccine development - using in vivo expression technology (IVET) and immunoproteomics to identify protective antigens

Farmers have found it difficult to treat *A. hydrophila* infections in fish due to the resistance of this pathogen to a number of antibiotics. Thus, researchers have been trying to

develop a vaccine but this has proven difficult, mainly because of the differences in the antigenic (bacterial components) expression between strains of *A. hydrophila*. A new approach using immunoproteomics was used to develop a recombinant vaccine that would protect against a wide range of *A. hydrophila* strains.

Salmon vaccines from the past are based on inactivated (whole cell) cultures of the pathogenic organism grown *in vitro*. Some of the vaccines gave good protection, however, many pathogens have been shown to 'switch off' important protective antigens when cultured *in vitro* and so many of these vaccines failed. In such cases alternative methods of culture (e.g. the inclusion of iron chelating agents) were required so that expression of the important 'protective' antigens were induced, e.g. in the development of a furunculosis vaccine. This was accomplished by Saravanne Poobalane, Kim Thompson and Sandra Adams who implanted live *A. hydrophila* into fish (enclosed in sealed chambers), i.e. IVET. Application of sera from fish (that had been infected with *A. hydrophila* and then recovered) in Western blot analysis on one and two-dimensional gel electrophoresis (immunoproteomics) pinpointed potential vaccine candidates and a recombinant vaccine against *A. hydrophila* was produced and shown to be protective in carp (*Cyprinus carpio*) (Fig. 2). This vaccine will be taken forward to commercialisation. Recombinant and DNA vaccine technologies are powerful tools for vaccine development as these enable the isolation of potential protective antigens from suppressive ones.

**Betanodavirus vaccine development- identifying vaccine antigens by epitope mapping**

Epitope mapping is a useful technology for the identification of protective antigens, particularly for viruses. The Pepscan procedure was used by Janina Costa, James Bron, Kim Thompson, William Starkey, Randolph Richards and Sandra Adams to identify betanodavirus B-cell epitopes recognized by neutralizing mouse monoclonal antibodies (MAbs) and serum samples obtained from European sea bass, *Dicentrarchus labrax*, naturally infected with betanodavirus. Pepscan was performed with a panel of thirty-four 12-mer synthetic peptides that

mimicked the entire betanodavirus capsid protein. Sea bass serum

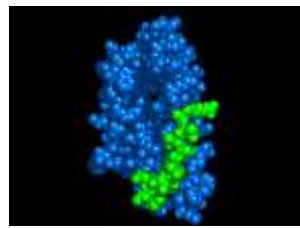


Figure 2. Detection of immunogenic epitopes of betanodavirus by epitope mapping

samples reacted strongly with three regions of the capsid protein and one of these regions was also recognized by neutralizing MAbs and therefore could potentially be used towards the development of nodavirus vaccines.

**RAPID DIAGNOSTIC TOOLS**

Rapid, accurate diagnosis of disease is essential for effective outbreak control. Once the causative agent has been identified appropriate advice can be given to neighbouring farmers/trading partners. Prompt action in the early stages of any disease problem can have an enormous impact on the scale of the outbreaks. Rapid diagnostic methods therefore provide powerful tools during emergency management.

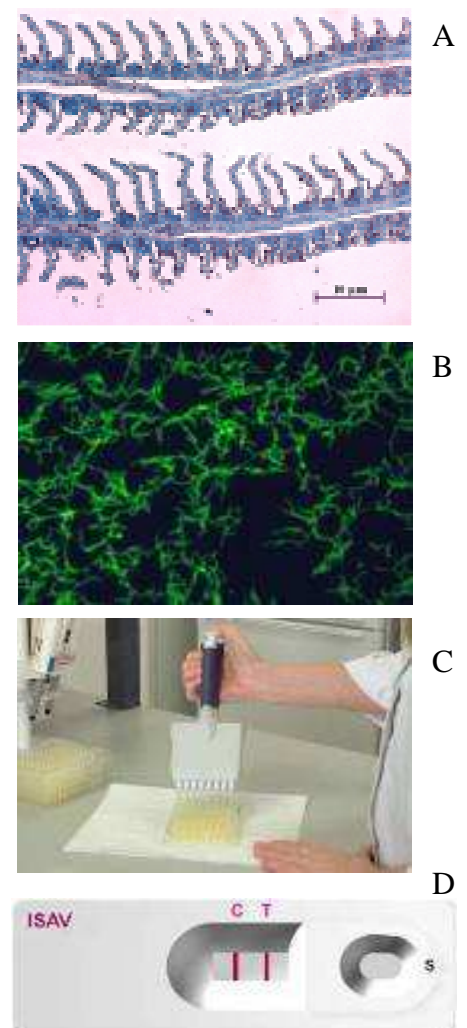
A variety of methods are available to detect pathogens in both fish and in the aquatic environment. These include traditional diagnostic methods and a diverse range of immunological and molecular methods. A variety of novel rapid diagnostic methods (immuno and molecular diagnostics) have been developed for use in aquaculture over the last 20 years in the Aquatic Vaccine Unit. The availability of standardised reagents (e.g. antibodies for immunodiagnostics) is crucial if comparable tests are to be run in different laboratories. The Aquatic Vaccine Unit was instrumental in developing a wide range of monoclonal antibodies (mAbs) for this purpose and a spin-out company, Aquatic Diagnostics Ltd ([www.aquaticdiagnostics.com](http://www.aquaticdiagnostics.com)), was set up in 2001 by Kim Thompson and Sandra Adams following a SMART Biotechnology award to market these reagents worldwide.

Figure 3. Detection of Koi herpesvirus (KHV) using immunohistochemistry (IHC) [A]; detection of *F. psychrophilum* by indirect antibody test (IFAT) [B]; measuring antibody response following vaccination by ELISA [C]; and detection of infectious salmon anaemia virus (ISAV) using Lateral Flow technology [D].

The Vaccine Unit has close links with local schools, taking Nuffield Bursary placements every year. This year two pupils from Dollar Academy (see below), supervised by Kim Thompson, James Bron and Sandra Adams went on to win the UK Science, Technology, Engineering and Maths (STEM) Senior Gold Prize for their project using fish macrophages as an *in vitro* model to study tuberculosis (TB).



Rebecca Chroston and Emma Donoghue (Form VI Dollar Academy) won the UK team STEM Senior Gold Prize for their six week research project hosted in the Aquatic Vaccine Unit (Image courtesy Jan van der Merwe)



# Squeeze or spread: does it matter how many fish we put in a cage?

Jimmy Turnbull

**The number** and size of fish in cages or ponds has been an aspect of fish farming that has attracted a considerable amount of criticism. However, much of this criticism has been ill informed. In this article I will attempt to explain the issues relating to the density of fish and their health and welfare. I will also explain how research has generated understanding of the issues and how that understanding has been turned into productive farming practices.

Many people's opinions of animal welfare are related to images of chickens crammed into small cages and some people extrapolated from their feelings about conditions in terrestrial farming systems to fish farming. However, any extrapolation from terrestrial farming systems to fish farms has to be approached with a great deal of caution.

Farmed terrestrial animals, even birds, live in a 2 dimensional space. Therefore you can estimate the density as number of animals (or birds) per square meter or weight (biomass) per square meter. This is often referred to as the stocking density. Fish occupy 3 dimensional spaces and therefore any estimate of density, whether number of animals or biomass, has to be related to the volume not area and is therefore measured in cubic meters. Even this is not simple since the animals or fish may not be evenly distributed, especially in large volumes.

Generally farmed fish will shoal or school together, however, under some circumstances they may change their behaviour and start individually defending territories. These territories may be related to structures in the tank or cage or to the source of food. In either case this can result in increased aggression and reduced access to feed, for at least some fish. There are many aspects of the fish's environment that affect the change from territorial to shoaling behaviour including, the species and life stage, stocking density, the water velocity, water temperature and feeding system. However, in simple terms there may well be a lower limit to safe stocking density. Fish health, welfare and productivity will suffer below certain stocking densities.

Even when fish shoal the area they use may represent a greater or smaller proportion of the available space. In very cold weather some fish such as fresh water Atlantic salmon parr tend to gravitate to the bottom of tanks and remain there relatively inactive. Other factors also affect shoaling, such as light levels and the number of fish in cages. There is some evidence that high numbers of individuals and higher light levels may result in more dense shoals of fish. In addition tidal currents may reduce the volume of a cage available to fish and the flow through the cage will be reduced by the growth of organisms on the mesh, making estimated volumes inaccurate.

Because fish do not usually occupy all the available space overall stocking density (number of animals or biomass per unit volume) is not necessarily a good indication of what the fish experience. For this reason people have devised other measures of crowding or loading of the system. For tanks with water flowing through them these include carrying capacity (kg of fish per litre of water per minute) or flow index (kg of fish per litre per minute per cm) and others (Ellis *et al.* 2002).

All this information demonstrates that "stocking density" is neither a simple nor necessarily a meaningful term. If we then attempt to look at how stocking density affects the health, welfare and productivity of fish things get even more complex. Just putting more fish in a cage or tank does not cause direct harm or damage; most detrimental effects occur through social interactions and water quality (MacIntyre *et al.* 2008). For example, social interactions include aggression resulting in physical damage or reduced access to feed, and water quality could be related to lower levels of oxygen in some parts of the cage or increased levels of ammonia.

Therefore stocking density does not necessarily affect the experience of the fish and any affect is indirect. For this reason there is substantial evidence (Turnbull *et al.* 2008) that stocking density is not a good way of accurately predicting productivity or welfare.

*Does stocking density mean anything?* It does, but as a general indication of conditions, not a specific accurate measurement that can be used to control farming practices.



**Fish can have good or bad health and welfare at high and low stocking densities depending on local conditions**

Over a wide range of stocking densities you can get good or bad health, production and fish welfare depending on the quantity and quality of the water and many other factors such as the feeding system. Despite this complexity and uncertainty, if you keep putting more and more fish into the water eventually you will get to a stage where things will go wrong and problems will occur.

On most farms, experienced fish keepers will know how much their system can hold, if they exceed this level (number or weight of fish) then they have to be very careful or problems may occur. The acceptable level will be lower or higher depending on the local conditions and farming system. Even when things do start to go wrong they do not necessarily result in a collapse with catastrophic losses but may first appear as relatively mild damage to the fins. Although you cannot say exactly when problems will occur it is still possible to estimate when the probability of things going wrong increases.



**A net covered in a variety of organisms that can affect the flow of water through the net**

Despite the complexity, careful statistical analysis can identify stocking densities when the risk of things going wrong starts to increase. A project funded by the LINK Aquaculture scheme looked at stocking density in marine Atlantic salmon cages and found that the chances of poorer welfare started to increase at stocking densities above 20-25 kg/m<sup>3</sup> (Turnbull *et al.* 2005). This was the result of a great deal of data collection and very detailed and careful analysis. However, this does not mean that fish will inevitably suffer above this density and it also does not mean that fish will invariably be healthy and productive below this density. It means only that there is a

greater risk of having problems above this density.

*If experienced farmers can deal with stocking density why do we need scientific analysis?* Many people who do not understand the complexity of the social and physical environment of fish have campaigned for lower stocking densities. A sustainable aquaculture industry has to be ethical but also environmentally and economically sustainable. Unnecessary reductions in the number of fish farmers can grow may render their business unprofitable and as explained above, low stocking densities can also be bad for fish. What the aquaculture industry requires is a set of guidelines that allows the development of a sustainable industry, is good for their fish and is also acceptable to processors, retailers and consumers.

For this reason both the salmon and trout industries in the UK approached us to try and develop guidelines for stocking density. This has resulted in scientific publications (Turnbull *et al.* 2005; North *et al.* 2006; Berrill *et al.* 2009;), but has also been incorporated in industry standards, welfare organisation fish farm standards and European guidelines. This research has not been restricted to collaboration with fish farmers and we have had a variety of dialogues with other stakeholders, including retailers and welfare pressure groups to seek their opinion and inform them (Berrill *et al.* 2010). We are still working in this area and currently have funding from the RSPCA Freedom Food to work with salmon farming companies to examine stocking density in fresh water salmon. All of this has contributed the profitability and ethical nature of farmed fish production.



**How fish respond to various stocking densities depends on many factors including the feeding system, above is an example of a demand feeder on a trout farm**

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# Showing off: successful 2<sup>nd</sup> PhD Research Conference

Richard Corner and Andrew Shinn

**How can** 18 months have passed so quickly? This was a feeling when we started to organise the 2<sup>nd</sup> PhD Research Conference, and to consider the enormity of once again attracting funding, publicising the event, encouraging our PhD students to provide presentations and posters, organising guest speakers and all that a conference entails.

The 2<sup>nd</sup> PhD Research Conference was held on Monday 28<sup>th</sup> June, opened by our Director Professor Brian Austin, who welcomed over 160 people, including students, staff and external guests, who made up 1/3 of the audience. As in 2008 external participation was vital and we had attendees from farm companies, feed companies, therapeutant manufactu-

Peter's talk was followed by 3 oral sessions in which 9 PhD students presented their research results and importantly how this research is liable to impact the development in relevant sectors of the industry. Identifying research impact is an increasing requirement from funding bodies, as they ensure that money is spent wisely and has as large an impact as possible. The international nature of the student body in Stirling was once again on show, the 9 presentations given by 8 nationalities; from Bangladesh, Egypt, France, Germany, Holland, Malaysia, Spain and the UK. The research areas covered equally diverse: Disease, Reproduction, GIS, Nutrition, Environment/Ecotoxicology, Genetics and Welfare and International Development.

tough challenge. Silvia Soares (Portugal, left) was the deserved winner, for a very effective poster evaluating mortality loss in Atlantic salmon culture. Second and third prizes went to Laura Martinez (Spain) and Victor Peredo-Alvarez (Mexico). Well done to them too.

In a short space of time our biennial conference has become an important focus for the presentation of research results to a wide audience. The international nature and focus of our students, their ability to rise to a challenge and their commitment to achieving high standards are second to none. A fantastic day.

<http://www.aqua.stir.ac.uk/research-conference/2010/>



ers, trade bodies and others. These companies (listed on the right) also provided £6,500 towards organisation of the event, and we are grateful for their generosity.

In a change this time we welcomed Aquaculture Champion Mr Peter Hajjpeiris, Chief Technical, Sustainability & External Affairs Officer at Birds Eye Iglo. His thought provoking talk focused on the positive; on attributes the aquaculture sector has in meeting food demand, its place in maintaining food security in the long term, the global nature of the aquaculture sector and the need to maintain high quality branding in areas of retail, manufacturing and research. There was agreement between delegates that Peter had highlighted the key issues in our collective ability to meet food demand, and on issues of food security and aquaculture sustainability.

All the talks were of a high quality but judges had to choose what they considered the best. First prize went to a delighted Mohammed Khan (Bangladesh, below) for an exceptionally good talk on the genetics and production of all male tilapia to overcome environmental and fish growth performance. Second prize went to Jan Heumann (Germany) and third



prize to Louise Colliar (UK). Well done to you and all our presenters.

The oral sessions were once again well supported by posters, with 41 PhD students (and who emanate from 22 countries) displaying their work very effectively in The Crush Hall. Once again judges had a

Thank you to all the organisations who supported the conference:

- Stirling Graduate Research School
- MASTS Graduate School
- Intervet Schering Plough
- Birds Eye Iglo
- Novartis Aquatic Animal Health
- Scottish Aquaculture Research Forum
- Scottish Salmon Producers Organisation
- EWOS Innovation
- Skretting Ltd
- Biomar Ltd
- Lighthouse Caledonia Ltd
- Marine Harvest (Scotland) Ltd
- Wiley Blackwell
- Alpha Laboratories
- Scientific Laboratory Supplies
- Sarstedt
- VWR
- Fisher Scientific



# Take your SEAT please: Sustaining Ethical Aquaculture Trade

Francis Murray, Dave Little, Iain Gatward

SEAT is an innovative project involving large-scale international collaborative research, funded through the EU 7<sup>th</sup> framework programme (FP7) and managed by the Institute of Aquaculture, University of Stirling. The project title 'Sustaining Ethical Aquaculture Trade' indicates the scale that this project is working at, with work packages (WPs) addressing all levels of this highly complex industry from small-scale producers in Asia through to supermarket chains and consumers in Europe. It epitomises the 'farm-to-fork' model and the final outcomes of the project will have an impact throughout this value chain.

The project is exploring the sustainability of the trade in aquaculture products originating from Asia, on which the EU has become increasingly dependant. Entire value chains are being considered for tilapia, catfish, prawns and shrimp extending from China, Vietnam, Thailand and Bangladesh to the European Union.

Country	Major Species	Minor Species
Bangladesh	Prawn	Shrimp
Thailand	Shrimp	Tilapia
Vietnam	Pangasius	Shrimp
China	Tilapia	Shrimp

By strengthening the knowledge base around seafood trade between Asia and the EU, SEAT will provide the evidence required to support further expansion of the industry, while ensuring a fair deal for producers who

are meeting appropriate social and environmental goals and offering a safe and sustainable product for consumers.

There are four key objectives to be addressed by SEAT, as highlighted below:

- Develop improved and transparent measures of sustainability for target aquatic food systems through creation of an **Ethical Aquaculture Food Index (EAFI)**
- Gain and disseminate an in depth understanding of emergent Asian aquatic food production / market chains through inter-disciplinary effort
- Enhance the sustainability and ethical 'values' of four major aquatic food commodities including examination of environmental services, economic efficiency, social justice, food quality and safety and animal welfare
- Enhance farmed aquatic food, scientific, business and policy linkages between Asia and Europe

A large focus of the project involves the engagement of Small and Medium Enterprises (SMEs), as they play a significant part in these value chains and their sustainability. Through regular consultations and discussions with SMEs, SEAT will assist in enhancing their capacity through

provision of improved understanding of stakeholder perceptions and trade / policy related information.

Funding for SEAT commenced in August 2009 and work began in earnest in October with PhD students from each of the four Asian countries spending 3 months at the University of Stirling's Institute of Aquaculture (IoA). The four Asian institutes partnered in this project are Bangladesh Agricultural University (BAU), Bangladesh; Shanghai Ocean University (SOU), China; Can Tho University (CTU), Vietnam; and Kasetsart University (KU), Thailand. Visits were also made to Stirling by WP coordinators to introduce their respective work plans, which are as follows:

- WP1:** Project Management (University of Stirling, Scotland)
- WP2:** Scoping and systems overview (University of Stirling, Scotland)
- WP3:** Life cycle analysis (Leiden University, Netherlands)
- WP4:** Environmental models (University of Stirling, Scotland)
- WP5:** Social and economic dynamics (WorldFish Centre, Malaysia)
- WP6:** Food Safety and Public Health (University of Copenhagen, Denmark)
- WP7:** Contamination risks (Wageningen University, Netherlands)
- WP8:** Ethical framework (University of Bergen, Norway)
- WP9:** Action research (University of Stirling, Scotland)
- WP10:** Improving transparency of trade (FAO, Rome)
- WP11:** Policy development (CEFAS, England)



**WP12:** Dissemination (University of Stirling, Scotland)

Other partners working on the project include WWF in Vietnam and the Danish Institute for International Studies (DIIS) in Denmark. More than 50 individuals are directly associated with the project, including 13 PhD students throughout Europe and Asia.

In January 2010 the project inception meeting was held in Rayong, Thailand, with 42 participants representing each of the 14 project partners. This very productive meeting gave all partners the opportunity to introduce their work packages and skills / knowledge being brought to the table.

Since the inception meeting work has continued at a rapid pace to complete WP2 and the largely qualitative part of the project. This phase, managed by the IoA, has largely been around boundary setting, stakeholder analysis and defining the scope of work and research context for the 3 remaining years of SEAT. Over this time period extensive field work has been undertaken throughout the Asian countries to identify the key species and specific locations to be considered in subsequent WPs, particularly 3, 4, 5, 6 and 7. The results to-date are summarised in the table below, which indicates the major and minor focus species to be considered in each country ongoing.

The fieldwork and the scoping phase will be finished in September 2010, at which time an LCA training course is being held in Thailand for project participants. Following on from this training all project partners, including PhD students, will begin detailed data collection and assessment of their respective WPs.

The SEAT project will be running until July 2013 and we are keen to consult with industry as widely as possible throughout this period, particularly with SMEs in Europe and in Asia. If you are part of a relevant business or organisation and wish to find out more please contact us at the below addresses or go to the SEAT website for more information:

[www.seatglobal.eu](http://www.seatglobal.eu)



## A new metabolomics facility for environmental research – opportunities for research at the University of Birmingham

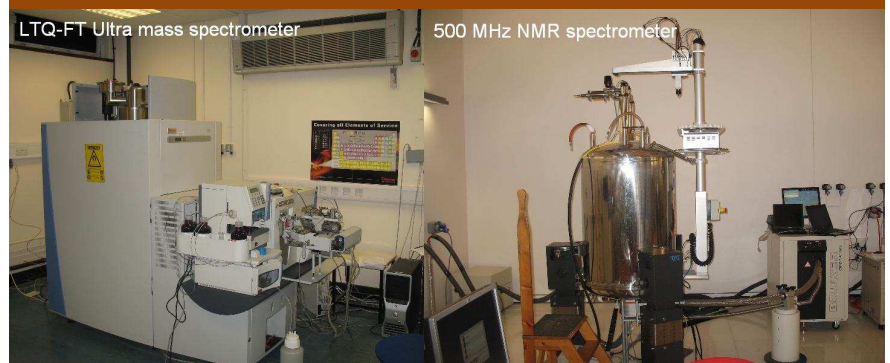
One of the current challenges for environmental scientists is to find robust measures to describe the influence of external pressures, for example climate change, on plants and animals in the wild. Traditional approaches, such as counting individuals in their natural habitat, are often inadequate as they are insensitive to detecting sub-lethal stresses that can degrade the health and fitness of organisms. Furthermore, measures such as “alive” and “dead” cannot provide any insight into the underlying causes of the stress.

Metabolomics describes the study of the entire composition of small molecule biochemicals (or metabolites) in a given cell, tissue, biofluid, or whole organism. Changes in the concentrations of these thousands of metabolites like glucose, cholesterol, urea, or ATP can be induced in response to a changing environment, for example by changes in water or air temperature, acidity, food supply, or by the influence of environmental pollutants. Traditionally, only certain subsets of the metabolome, like sugars or amino acids, or even single metabolites could be investigated in a targeted approach. Today it is possible to analyze a large proportion of the metabolome at once, in an untargeted approach, using sensitive, high-resolution techniques such as nuclear magnetic resonance (NMR) spectroscopy and FT-ICR mass spectrometry (MS).

The Natural Environment Research Council (NERC) has recently funded a new metabolomics facility at the University of Birmingham to facilitate just this approach. The facility represents one of five nodes within the NERC Biomolecular Analysis Facility (NBAF; [www.nbaf.nerc.ac.uk](http://www.nbaf.nerc.ac.uk)). We are using the University's world-class NMR and MS instrumentation as well as advanced computational approaches in order to obtain the high-quality datasets needed to identify the often subtle changes in the metabolome that are indicative of environmental stress. This “discovery driven” research can be used to generate novel hypothesis about the biochemical stress response mechanisms, which can be tested in subsequent targeted experiments.

Environmental scientists from across the UK who are conducting research within the NERC scientific remit (<http://www.nerc.ac.uk/funding/application/topics.asp>) and who meet principal investigator status (<http://www.nerc.ac.uk/funding/available/researchgrants/eligibility.asp>) are eligible to apply to NERC to gain access to this state-of-the-art metabolomics facility. Applications to conduct small-scale pilot projects are strongly encouraged.

For more information, including how to apply, please visit our website at <http://www.biosciences-labs.bham.ac.uk/nbaf-birmingham/>, or contact the Facility Director, Dr Mark Viant ([m.viant@bham.ac.uk](mailto:m.viant@bham.ac.uk)), or Facility Manager, Dr. Ulf Sommer ([u.sommer@bham.ac.uk](mailto:u.sommer@bham.ac.uk)).  
NERC Metabolomics Facility \* School of Biosciences \* University of Birmingham \* Birmingham \*  
B15 2TT \* United Kingdom \* Phone: +44-(0)121-414-8699



In 1997 Luca Montorio, an applied biology graduate from Turin made the original decision to travel to the most northerly part of the UK, Unst in the Shetland Isles, to work in the aquaculture industry. His move was much inspired by the offer of a job in a very uncommon and technically demanding salmon hatchery at Quoys, then part of the locally owned Sandisons Group, and run by an adventurous and innovative engineer, Stewart Owers. The Quoys hatchery was a remarkable training ground, with highly unusual water supplies associated with Unst's unique geology, large stocks of salmon smolts to produce, and a complex recycle system to develop and manage.

During his initial work at Quoys hatchery, Luca found remarkable and superior biological performance from one of the main treatment units within the main recirculation system. Further investigation proved that specific and important advantages were given to the recirculation system by this filter media which was key to maintain stable water conditions at Quoys hatchery. Recognising Luca's interest and skills, and keen to retain technical and commercial advantages, Stewart encouraged Luca to focus his work round a PhD at the Institute, looking more closely at how to improve the filtration systems at Quoys, and in particular to explore the potential of novel filter media and materials. This was highly rewarding because by the end of the research Quoys hatchery production capacity was considerably increased while providing a more stable and secure water quality environment for rearing salmon. Luca also carried out a market review of the options for his system – primarily in aquaculture, particularly for recycle systems for smolts and other products.

As part of the restructuring of the salmon industry in Shetland, Sandisons decided to sell its interests in Quoys to focus on other business areas, but Luca and Stewart kept up their fascination for filters, further developing ideas and concepts, realising that as well as serving the aquaculture industry, better water treatment has far wider applications. This seeded the idea for the present technology system and the Italy/Shetland partnership story continues.

### THE TECHNOLOGY: OLIMPIO REACTIVE BIOCONTACT SYSTEM

The outcome of many years spent in developing the Quoys hatchery and researching water quality is the **Olimpio Reactive Biocontact system** which in basic terms allows improved and reliable removal of nitrite in biological filter systems. As such it has significant potential in aquaculture water treatment, as well as in other sectors. It can be seen as an ameliorative technology, applied in existing treatment systems without changing their components or configuration. Now patented, the technology involves a controlled coating process with a specific blend of minerals which can activate any conventional biological media (see picture). Together with this, a unique mineral **Regenerator**, containing a specific active ingredient, has also been developed. The **Regenerator**, conveniently in tablets or in bulk format is periodically fed into the filter to recharge the media to maintain their optimal performance.

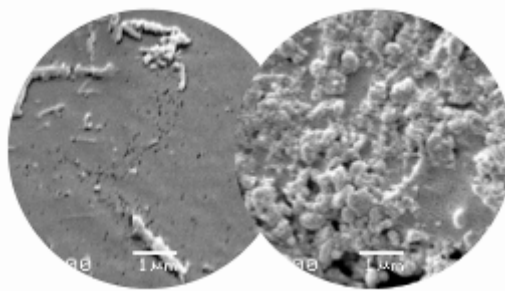


Figure 1: SEM view of a standard media surface before (left) and after mineral coating (right).

The **Olimpio** system doubles the rates of nitrite removal compared with conventional technology (see graph). It provides better stability to production systems, reducing the need for water replacement, and enabling far more reliable recycle system performance. Costs are very easily recovered, and better water quality brings wider benefits to stock quality and reduced potential for

disease losses. The system has been extensively tested in full scale aquaculture recirculating systems (from 10 to 1000 m<sup>3</sup>) and is available for comprehensive commercialization in the freshwater aquatic sector while further development is undergoing for marine systems. While it can easily be applied to re-engineer existing systems and improve performance and safety, purpose built systems are

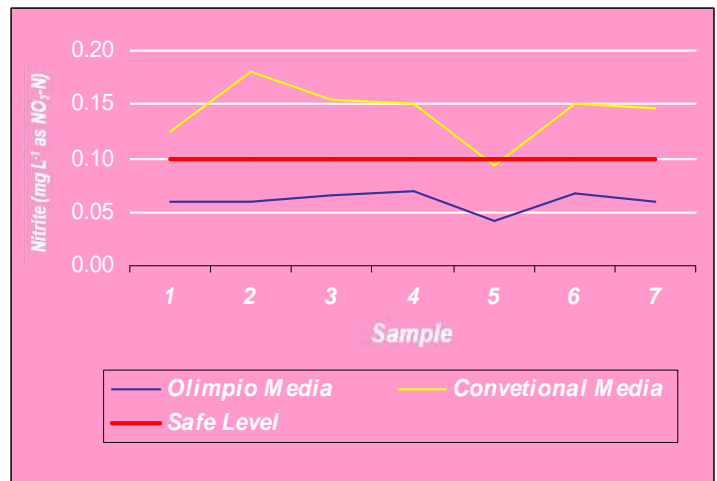


Figure 2: Comparative nitrite performance: Olimpio media had on average 10% extra ammonia oxidation.

also available, and Luca has developed and is testing a highly efficient treatment configuration built around this technology, with simpler operation and greatly improved performance over conventional systems.

### THE COMPANY: QUANTO LIMITED

Quanto Limited was established in 2009 by Luca and Stewart to manufacture, through constant development and innovation, a wide range of filtration media to fit different configurations and systems, in aquaculture, the aquarium sector and the water industry. Together with Stirling Aquaculture, Luca and Quanto are developing a number of recirculating aquaculture projects, primarily for salmon smolts, and in a separate initiative a range of aquarium products has been developed, now being launched under the Olimpio brand in collaboration with a major Italian manufacturer and distributor of ornamental equipment. Currently being introduced into the Italian, Swedish, Swiss and Greek markets, the products are expected to

enter the UK and other markets in the next 12 months.

As director of Sandisons (Unst) Ltd, operating diverse businesses from quarrying to manufacturing and merchandising, Stewart Owers' managerial experience is a key element in the company, and in setting up the manufacturing technology in Unst. With his PhD training and more than 10 years management experience on intensive aquaculture operation and production, Luca is responsible for market and technical development and is based in Stirling. This allows close collaborative contact with Stirling Aquaculture (STAQ), who will provide a research and commercial partnership, linking with a wide range of commercial aquaculture developments in the UK and internationally.

### THE FUTURE: NEW DEVELOPMENTS WITH STIRLING AQUACULTURE

The close link between STAQ and Quanto Limited built up during Luca's PhD training have been a valuable example of the synergy the Institute attempts to build with its students, commercial partners and academic research. Manager John Bostock and staff of the Environment Group have been particularly helpful in supporting the fledgling enterprise. A current area of joint innovation aims to prove a low-cost recirculating system design with potential to grow highly marketable products. This aims to provide a reliable, simple and cost effective production solution for people with low technical familiarity with aquaculture. A full scale system of approximately 20 m<sup>3</sup> has just been completed at Buckieburn, the Institute's freshwater facilities, and will be in operation from the next production cycle in December. Based on its outcomes, and options for wider technology applications, including links with other STAQ/Institute partners in aquaponics, and with its international research interests in integrated multitrophic aquaculture (IMTA), further collaboration agreements are expected.

## 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>.

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Musa, Nadirah, 2010  
Sperm activation in Nile tilapia *Oreochromis niloticus* and the effects of environmentally relevant pollutants on sperm fitness  
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Pratoomyot, Jarunan, 2010  
Investigating alternative raw materials and diet formulations on growth performance, lipid metabolism and gene expression in Atlantic salmon (*Salmo salar* L.)  
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Barker, Sarah E., 2009  
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Derayat, Amid, 2009  
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Effect of dietary lipid sources on the reproductive performance of Nile tilapia *Oreochromis niloticus*  
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