Institution: University of Stirling

Unit of Assessment: A6: Agriculture, Veterinary and Food Science

Title of case study: Improved parasite control in the global Atlantic salmon farming industry

1. Summary of the impact

Sea lice are the principal disease constraint for world Atlantic salmon culture and cost >€33m yearly in the UK and >€305m globally in terms of control measures and lost production. Research conducted by the University of Stirling's Institute of Aquaculture (IoA) has provided tools and strategies for sea louse control in farmed salmon worldwide. Impacts have been delivered through an integrated pest management approach which involves

(1) introduction of management tools including fallowing, single year-class stocking and area management
(2) screening, development, licensing and monitoring of veterinary medicines
(3) development of alternative strategies such as use of cleaner fish (wrasse) and sea louse resistant salmon
(4) incorporation of integrated pest management principles into public policy and legislation.

These tools and approaches are now being used by the U.K. and global Atlantic salmon industries.

2. Underpinning research

Sea lice are ectoparasitic copepods causing major economic losses for the global Atlantic salmon aquaculture industry. Use of an integrated pest management strategy for sea lice control was first proposed by IoA's Professor Christina Sommerville at the Scottish industry's annual conference in 1994. 'Integrated pest management' is a term encompassing the co-ordinated use of a wide variety of tools and approaches for the effective management of crop and livestock pathogens, particularly to reduce drug resistance development, and this strategy is key to IoA's underpinning research.

Farm management approaches

Appropriate use of management tools is a critical aspect of integrated pest management for sea lice. Industry-funded work at IoA (Ref. 1) was the first to provide evidence to recommend fallowing and use of single-year-class stocking policies to assist sea louse control and to examine the effects of treatment through a farm cycle. In the late 1990s the use of Area Management Agreements to co-ordinate farm management within defined areas was developed from this research, ensuring appropriate fallowing after harvesting, synchronisation of treatments across Farm Management Areas and use of effective communication strategies. In addition to farm management approaches, integrated pest management strategies require the development and deployment of a wide range of complementary tools for pest control including use of medicines and biological controls.

Veterinary medicines

Following development of sea louse drug resistance to the organophosphate dichlorvos (~1992), the IoA, through its Marine Environmental Research Laboratory (MERL), has increasingly played a central role in the identification, development, licensing, deployment and monitoring of the vast majority of medicines used against sea lice worldwide (1993-2013). In collaboration with industry (including Pfizer, MSD Animal Health, Novartis, Solvay Interox; feed companies Skretting (Nutreco), Ewos and BioMar and producers Marine Harvest), the IoA has helped to develop hydrogen peroxide (research from 1993-2013), azamethiphos (from 1993), teflubenzuron (from 1994-2013), diflubenzuron (from 1996) and the most widely used and successful medicine to date, emamectin benzoate (SLICE®, 1993-2013) see Refs 2-4. Research has included *in vitro* and *in vivo* screening of candidate products, efficacy studies to identify dose and treatment regimen, farm-scale trials, regulatory studies to evaluate safety, pharmacokinetics, metabolism and residue depletion and bioaccumulation by filter feeders. Work to develop new drugs is supported by ecotoxicology risk assessments, environmental impact assessments and modelling of environmental concentrations of drugs (Ref. 5) conducted by IoA for industry and government, and incorporated into Scottish Environmental Protection Agency (SEPA) guidelines. Monitoring drug resistance is central to integrated pest management and IoA has been a world leader in industry-funded (Ciba Agriculture, Scottish Salmon Growers' Association Ltd, Schering-Plough Animal Health / MSD Animal Health) research to investigate the molecular mechanisms involved in reduced susceptibility to drug treatment and techniques to manage resistance (1993-2013).

Non-chemotherapeutant controls

Since 1993, industry-funded research efforts at IoA have increasingly included non-chemotherapeutant control methods including vaccines (£227,372, 1993-2010), functional / immunostimulant feeds (£209,057, 2008-2013) and trials to help develop genetically resistant salmon stocks. Work conducted with Landcatch Natural Selection (part of Hendrix Genetics) as part of a Defra LINK project (2009-2010) demonstrated substantial scope for the breeding of resistant salmon (Ref. 6).

Key IoA staff working on sea lice (1993-2013): Professor C Sommerville, Professor R Richards, Dr R Wootten, Dr W Roy, Professor J Bron, Dr A Shinn, Dr T Telfer, Dr M Roth, Dr J Stone, Professor H Migaud and Dr A Sturm.

3. References to the research (Stirling researchers in bold)

1. **Bron, J.E.**, **Sommerville, C**., **Wootten, R**. and Rae, G.H. (1993). Fallowing of marine Atlantic salmon, *Salmo salar* L., farms as a method for the control of sea lice *Lepeophtheirus salmonis* (Krøyer, 1837). *Journal of Fish Diseases,* 16, 487-493.
2. **Roth M**., **Richards R.H**., Dobson D.P., Rae G.H. (1996). Field trials on the efficacy of the organophosphate azamethiphos for the control of sea lice (Copepoda: Caligidae) infestations of farmed Atlantic salmon (*Salmo salar*). *Aquaculture,* 140, 217-239.
3. **McAndrew K.J**. **Sommerville C**., **Wootten R**.and **Bron J.E**. (1998). The effects of hydrogen peroxide treatment on different life-cycle stages of the salmon louse, *Lepeophtheirus salmonis* (Krøyer 1837). *Journal of Fish Diseases* 21(3), 221-228.
4. **Stone, J**., Sutherland, I., **Sommerville, C.**, **Richards, R.H**. and Varma, K.J. (1999) The efficacy of emamectin benzoate as an oral treatment of sea lice, *Lepeophtheirus salmonis* (Krøyer), infestations in Atlantic salmon, *Salmo salar*. *Journal of Fish Diseases* 22 (4), 261-270.
5. **Telfer, T. C**., **Baird, D. J**., McHenery, J. G., **Stone, J**., Sutherland, I., and Wislocki, P. (2006). Environmental effects of the anti-sea lice (Copepoda: Caligidae) therapeutant emamectin benzoate under commercial use conditions in the marine environment. *Aquaculture,* 260(1-4), 163-180.
6. Gharbi, K., Stear, M., Matthews, L. and **Bron, J.E**. (2011). LINK project LK0691 final report “An experimental and modelling framework for breeding Atlantic salmon for resistance to sea lice”..

Grants for research underpinning impact include:

1. Industry-funded work to develop teflubenzuron, four contracts 1994-1999 £90,000
2. Industry-funded work to develop emamectin benzoate, 19 contracts 1993-2013 £570,864
3. Industry- and government-funded work on drug sensitivity of sea lice, development of bioassays and investigation of mechanisms of resistance, 12 contracts 1994-2013 £485,236
4. Defra LINK: The feasibility of breeding Atlantic salmon for resistance to sea Lice. Professor **Bron**, with Professor Stear and Dr Matthews, University of Glasgow, Dr Gharbi, University of Edinburgh and Professor Roberts, Landcatch Natural Selection Ltd. £94,419

4. Details of the impact

Integrated pest management, a concept introduced for sea lice control by the IoA, is now employed by all the major cultured salmon producing countries (see the 2013 Sea Lice Integrated Pest Management workshop http://aquafeed.com/read-article.php?id=4954). The impact of IoA’s sea louse research in the REF assessment period 2008-2013 is clearly demonstrated by

1) industry reliance upon integrated pest management strategies developed through IoA,
2) increasing levels of industry support and funding for sea lice research at IoA,
3) sustained growth in the Scottish and global Atlantic salmon aquaculture industry
4) welfare and economic impact through incorporation of IoA research findings into industry codes of practice and the involvement of IoA in formulation of Scottish Government policies for farmed fish health and welfare.

IoA sea louse research continues to have major impacts in controlling sea louse numbers infecting farmed fish in the UK and globally, reducing economic costs, supporting sustainability and protecting fish welfare.

Impacts on fish health and welfare:

IoA research was involved in the development of the majority of veterinary drugs used to control sea lice 2008-13. The Technical Director of the Scottish Salmon Producers' Organisation (which represents >95% UK producers) has stated that "Work carried out at IoA has been directly responsible for providing encouragement and support to international pharmaceutical companies in the identification, testing and licensing of new veterinary products for the treatment of sea lice" and that "In the course of the past decade, IoA staff have undertaken cutting edge scientific work that informs and supports those in the industry responsible for the control of lice and the welfare of fish". By facilitating new treatments, particularly emamectin benzoate (SLICE®), IoA has had a major impact on the control of sea lice worldwide. MSD (Merck) Animal Health confirm that, working with IoA "…we have conducted studies on efficacy and dosing to support the registration of Slice throughout the salmon farming world and recently conducted studies to assess the impact of the development of tolerance and ways to assess it and combat it."

Impacts on consumer health:

Improved growth and sustainability of the Atlantic salmon industry, resulting from IoA's impact in improved sea louse control, has impacts on consumer health by provision of affordable and sustainable healthy protein and essential fatty acids. The Managing Director of Marine Harvest (Scotland) Ltd. Comments that "IoA have increased the sustainability of salmon farming in rural communities and reduced environmental impacts through provision of effective assessment methodologies and improved consumer health through sustainable healthy food production as well as providing various essential services for government and industry."

Impacts on economy and commerce:

Over recent years, the strategy of UK and global salmon aquaculture has been one of integrated pest management. As recognised by the Managing Director of Marine Harvest (Scotland) Ltd. "IoA have contributed to improved economic sustainability of the salmon farming industry through development of novel chemotherapeutants, integrated pest management, selection for genetic resistance in salmon, involvement in public policy, industry codes of practice and legislative development through chairing and membership of national/international bodies". In summarising commercial impact, the SSPO states "…IoA has made a significant contribution towards better scientific understanding of this commercially important parasite, and that the application of this science by industry has, in a number of important respects, been transformational."

Impact on management practices:

The impact of IoA on management practices is confirmed by the Scottish Salmon Producers' Organisation: "The scientific work of the IoA has supported the salmon farming industry in developing a range of strategic and practical approaches focused on minimising impact. Prominent amongst these approaches is Area Management, which involves separating year classes within defined production areas, fallowing after year classes have been harvested, and synchronising and coordinating treatment with approved veterinary medicines across Management Areas. This approach was first developed in Scotland in the late 1990s on the back of new science and now forms the basis of good practice in commercial salmon farming internationally". This impact is also endorsed by Paul Wheelhouse, Scottish Minister for Environment and Climate Change who confirms "We recognise the value of the University's work to develop appropriate management approaches, which include fallowing, single-year class stocking, and the use of cleanerfish". Use of Management Areas, fallowing and single-year class stocking are key tenets in the newest Code of Good Practice for Scottish Finfish Aquaculture 2013 contributed to by IoA.

Impacts on public policy and services:

IoA research outputs have had considerable impact upon public policy, industry codes of practice and new legislation. Richards has roles including SSPO veterinary advisor and chair of the Healthier Fish and Shellfish and Farmed Fish Health and Welfare Working Groups within the Ministerial Group on Aquaculture. He is facilitator of the Fish Health thematic area for the EU Aquaculture Technology Platform and Chair of the National Sea Lice Platform. Key elements of IoA integrated pest management strategies and research have led to policy impact through direct input into the Code of Good Practice for Scottish Finfish Aquaculture 2013, and the Aquaculture and

Fisheries (Scotland) Bill 2013 as well as input into the conditions to be attached to finfish business authorisations including delivery of single year class stocking of sites, sea lice treatment and fallowing of appropriate scale management areas.

Impact demonstrated through industry funding of IoA research:

During the REF assessment period 2008-2013, the IoA had demonstrable and increasing impact through industry funding of sea louse research targeting elements of an integrated pest management strategy. Over this period, industry funding for IoA and MERL includes 41 medicine-associated projects (£1,306,719), seven bioassay/sensitivity/resistance mechanism associated projects (£186,767) and eight functional feed associated projects (£271,340). There are three current Technology Strategy Board projects. The first of these, 2011-14 “Development of a high-density salmon SNP chip: a key tool for improving the competitiveness and sustainability of the UK salmon farming industry”, is a collaboration with Landcatch Natural Selection Ltd., Affymetrix UK Ltd., University of Glasgow and University of Edinburgh (£647,339) and follows from the underpinning research showing differential genetic resistance to sea lice in Scottish farmed salmon families. The first SNP chips produced by this project are now being used by the industrial partner to find breeding markers for salmon resistance to sea lice. The second project “Production and implementation of farmed wrasse in the Scottish Salmon industry” is a collaboration with Marine Harvest Scotland Ltd and Scottish Sea Farms Ltd., having a value of £2,139,000 for IoA and MERL, and seeks to provide a sustainable farmed source of wrasse to be used as biological controls (cleaner-fish) to remove lice from salmon. Impacts from this project are already being felt, with recent introduction of wrasse to a trial site in Scotland cutting drug treatments from 8x per farm cycle to none and giving direct savings of 7p per kilo (Scottish production is >158,000 tonnes p.a. and growing). The final project leverages IoA’s experience in sea lice and sea louse vaccine development, being part of TSB’s Sustainable Protein call. “Development of a novel sea louse vaccine: an environmentally friendly tool for increasing sustainability of protein production in UK salmon aquaculture” is an important collaboration with Pfizer (now Zoetis) and Moredun Research Institute (total £2,992,000) with impact confirmed by the Head of Pfizer/Zoetis Business Development and Global Alliances up to 2013.

5. Sources to corroborate the impact

1. Scottish Government Farmed Fish Health and Welfare Working Group http://www.scotland.gov.uk/Topics/marine/Fish-Shellfish/MGSA/Farmedfishhealthandwelfarewg

2. A Code of Good Practice for Scottish Finfish Aquaculture (Jan 2013) showing incorporation of IoA integrated pest management strategies especially section 3.5 Area management and Annex 11 National Strategy for Sea Lice Control http://www.thecodeofgoodpractice.co.uk/publish.

3. Scottish Government Healthier Fish and Shellfish website underlining contribution of Institute of Aquaculture personnel and research to policy implementation including “single year class stocking of sites, sea lice treatment and fallowing of appropriate scale management areas” http://www.scotland.gov.uk/Topics/marine/Fish-Shellfish/18364/18610

4. Scottish Aquaculture and Fisheries (Scotland) Bill 2013 having direct input from IoA through committee Chairs (Professor Richards) http://www.legislation.gov.uk/asp/2013/7/contents/enacted

5. Scottish Sea Farms wrasse video showing impact for industry of collaboration with IoA and its Marine Environmental Research Laboratory at Machrihanish http://www.youtube.com/watch?v=56aQgGpX3Ck

In addition, full written statements from individuals and organisations quoted above are available.

Institution: University of Stirling

Unit of Assessment: A6: Agriculture, Veterinary and Food Science

Title of case study: Management strategies to control salmon puberty: optimised productivity and sustainability of the fish farming sector

1. Summary of the impact

Salmon maturation prior to harvest constitutes an environmental, welfare and production bottleneck for the salmon aquaculture industry. Our research has reduced the number of fish that mature during the grow-out phase so they do not reallocate energy to develop gonads and display secondary sexual characteristics that reduce yield, harvest quality and increase disease susceptibility that can result in downgrading at processing and lost profitability. In addition, reproductively competent fish that escape from on-growing cages may breed with wild stocks, leading to potential introgression. This has a major impact on public perception of farmed salmon and it limits the expansion of the industry. The IoA Reproduction team has undertaken a comprehensive body of work since 1993 to address this critical production bottleneck through an array of management strategies. This work culminated in the REF period by the demonstration that salmon puberty can be reduced to <3% by the use of standardised lighting regimes (2008) followed by the first commercial production of sterile salmon (2012-13).

2. Underpinning research

The fish farming industry has expanded at a fast pace in the last 30 years and Atlantic salmon has reached a production of 1.2 million tonnes a year in Europe (>10% increase/year). With the radical change of scale in production came the necessity to standardise/optimise rearing protocols to ensure reliable, year round, consistent supply of quality fish to a growing market and minimise welfare and environmental impacts. To achieve this it is critical that the industry controls the sexual maturation of its fish in the production phase. Sexually maturing fish will result in losses of growth, increased agonistic behaviour and disease susceptibility, which are major welfare issues and product downgrading because of reduced flesh quality and secondary sexual attributes. In addition, farming of reproductively competent stock can have detrimental effects on the fitness of wild stocks if they escape and interbreed. This is negatively perceived by consumers and wild fisheries managers. Research in the reproduction group over the last 20 years has focused on the understanding of salmon reproductive physiology and the development of stock management strategies to control puberty in farmed fish, especially photoperiod signalling and sterility through triploidy. This has led to new knowledge and tools in:

- fish reproductive physiology and sexual dimorphism (from 1993 to present);
- light sensitivity and seasonal control of puberty in farmed fish through detailed studies of the photoneuroendocrine system (PNES) at endocrine (melatonin pathway, Migaud et al., 2007; Vera et al., 2010) and molecular (clock mechanisms, Davie et al., 2009) levels from in vitro, tank based to full scale commercial testing (from 2002 to now);
- new lighting technologies (LED, narrow bandwidth light, cold cathode) that are more energy and biologically efficient (from 2007 to now, Leclercq et al., 2011);
- triploidy protocols to induce sterility in salmonids and optimisation of production traits from egg to harvest in freshwater and seawater through studies on nutritional requirements, environmental sensitivity and selection (from 2008 to now, Leclercq et al., 2011; Taylor et al., 2013a and b);
- transfer of triploid induction technology to leading salmon breeding companies (Aquagen in Norway and Hendrix Genetics in the UK);
- consumer perception studies and perceived risk-benefit of triploidy leading to marketing strategies. The work aimed to study intrinsic and extrinsic associations and beliefs with respect to triploid salmon products and production and develop appropriate communications means (http://cordis.europa.eu/documents/documentlibrary/117787031EN6.pdf).

The research that has delivered these findings/insights has been a combination of near-market, applied science and fundamental studies including the application of molecular and genomic technologies that provided the basic science underpinning our understanding of the molecular, biochemical, and physiological control of puberty and light sensing, leading to direct commercial applications.

This work has been coordinated by Professor Migaud (2002-present) and colleagues in the Reproduction and Genetic group including McAndrew, Penman, Taggart and Davie, bioinformatician Bekaert and key post-doctoral fellows including Taylor and Vera.

3. References to the research

The results of these studies have been extensively disseminated since 1993 through over 80 papers in peer reviewed scientific journals, numerous international and national conferences/meetings and, importantly, several articles and presentations each year in/at industrial/trade forums/meetings and trade/popular press. The combined impact of a very substantial and comprehensive 'body of work' has been highly influential. These six references offer glimpse of the range and depth of the research.

1. Migaud H., Davie A., Martinez Chavez C.C., Al-Khamees S., (2007). Evidence for differential photic regulation of pineal melatonin synthesis in teleosts. *Journal of Pineal Research* 43 (4), 327-335.
2. Davie A. Minghetti M., Migaud H., (2009). Seasonal Variations in Clock-Gene Expression in Atlantic Salmon (*Salmo salar*). *Chronobiology International,* 26, 379-395.
3. Leclercq, E., Taylor, J.F., Sprague, M. and Migaud, H., (2011). The potential of alternative lighting-systems to suppress pre-harvest sexual maturation of 1+ Atlantic salmon (*Salmo salar*) post-smolts reared in commercial sea cages. *Aquaculture Engineering* 44 (2), 35-47.
4. Leclercq, E., Taylor, J.F., Fison D., Fjelldal P.G., Diez-Padrisa M., Hansen T. and Migaud, H. (2011). Comparative seawater performance and deformity prevalence in out-of-season diploid and triploid Atlantic salmon (*Salmo salar*) post-smolts. *Comparative Biochemistry and Physiology Part A*. 158, 116-125.
5. Taylor J.F., Sambraus F., Mota-Velasco J., Guy D., Hamilton A., Hunter D., Corrigan D., Migaud H., (2013a). Ploidy and family effects on Atlantic salmon (*Salmo salar*) growth, deformity and harvest quality during a full commercial production cycle. *Aquaculture* 410–411, 41-50.

Grants for research underpinning impact include:

1. 2013-16 SALMOTRIP+, funded by the world's largest salmon producer, Marine Harvest, and feed manufacturer, Biomar 'Impacts of triploidy on production traits in Atlantic salmon' (PI Migaud, £535k).
2. 2013-16 FISHLIGHT-TECH funded by Philips Lighting, "Biological efficiency of light in commercially important fish species" (PI Migaud, £100k).
3. 2012-16 EU FP7 ARRAINA "Aquaculture feeds and fish nutrition: paving the way to the development of efficient and tailored sustainable feeds for European farmed fish" (PIs Bell/ Tocher/Migaud), £5M, IoA budget £460k.
4. 2012 (Jan-Dec) funded by Biomar "Effect of phosphorous supplementation on triploid deformity prevalence" (PIs Taylor/Migaud, £43k).
5. 2011-15 BBSRC Case Award, BB/J500835/1 "Mechanisms underlying the impacts of triploidy on production traits in Atlantic salmon" (PI Migaud, £94k).
6. 2008-11 EC FP7 SALMOTRIP "Feasibility study of triploid salmon production"(PI and coordinator, Migaud): £830k, IoA budget £380k.
7. 2006-09 BBSRC Case Award BBS/S/M/2006/13133 "Characterisation of false maturation and development of diagnostic tools in Atlantic salmon" (PI Migaud, £58k).
8. 2006-09 Norwegian Research Council Contract 174231 "Narrow bandwidth lighting technology in fish farming and effects on performance from early stages to adult fish" (PI Migaud), IoA budget £120k.
9. 2002-06 EC FP6 PUBERTIMING QLRT-2001-01801 "Photoperiod control of puberty in farmed fish: Development of new techniques and research into underlying physiological mechanisms" (PIs Bromage/Migaud), IoA budget £160k.

4. Details of the impact

The basic understanding of fish reproduction acquired by the IoA reproduction group led to the development of protocols for the salmon farming industry to control early maturation. Key strategies have been identified and researched to tackle this problem, e.g. reduction in the prevalence of early maturing fish prior to harvest either using photoperiodic treatment or sterility using chromosome manipulation, a technique unique to aquatic fish and shellfish among farmed animals. The knowledge gained by the group in these areas has led to the implementation of protocols, guidelines and practices within the industry that have significantly improved the sustainability of the sector, generating growth and increased profitability.

Optimised photoperiod regimes developed by the reproduction group in collaboration with leading light manufacturers (BGB Engineering, Akva and recently Philips Lighting) have led to a considerable reduction in the prevalence of early maturation during the first year of salmon on growing at sea from >40% in the late 1990`s to <3% (from 2008 onwards) and reduced energy usage through a combination of timing and duration of the light exposure window as well as the adoption of new lighting technologies and standardised light intensity. This has been implemented globally by the salmon farming industry with companies that operate in all salmon production countries (Marine Harvest, Scottish Seafarms) through knowledge transfer activities (workshop, consultancies, training) and contributed very significantly to reduce costs and improve fish welfare. This knowledge base has subsequently helped us pioneer similar light regimes for Atlantic cod incorporating the use of specialist surface nets to shade ambient sunlight, these were implemented commercially in Scotland and Norway. However, such light regimes do not address the potential environmental concerns associated with salmon escapees interacting with wild salmonid stocks (in Scotland alone, 1.9 million farmed salmon escaped into the natural environment between 2002 and 2009 due to human error, storm damage or holes in nets). Therefore, there is an increasing pressure from retailers, fish welfare standards and accreditation bodies, NGOs and governments towards the development and implementation of sterile stocks. While other strategies delay or limit the prevalence of early maturation in culture, sterility fully suppresses puberty in females and by doing so removes any introgression concerns for wild stocks in the event of escapees.

Work done by the reproduction group is at the forefront of the research on sterility through chromosome manipulation (induced triploidy) since 2008. A suite of projects (Salmotrip, 2008-11, BBSRC, 2011-15 and Salmotrip+, 2013-16) coordinated by Prof. Migaud in collaboration with the world leaders in salmon production, breeding and genetic enhancement (Marine Harvest, Aquagen, and Hendrix Genetics) investigated the commercial feasibility of producing sterile salmon with a focus on breeding and selection, fish performances, nutritional requirements, fish welfare and deformity, and consumer perception towards such a new product. Research performed by the group on triploid salmon led to the first introduction of triploid salmon on the market in 2012 by Aquagen and the first commercial scale farming in Norway (2.5 million triploid salmon eggs produced in 2012) and Scotland (forecast of 1 million triploid eggs produced in November 2013). Research on nutritional requirements of triploid salmon (2010-13) led to the development of triploid diets that reduce skeletal deformities by as much as 50% and completely abolished cataract prevalence (patent pending). These dietary formulations are now being commercially produced by one of the largest feed manufacturer in aquaculture (Biomar) and supplied to farms on growing triploid salmon in UK, Norway and Tasmania. Knowledge transfer activities performed by the group since 2008 had a very significant impact, not only on the fish farming sector (producers, breeders, processors) but also on retailers, non-governmental organisations (NASCO), governmental bodies and the general public at national and international levels. Project results have been disseminated widely through workshops organised during the European Aquaculture Society meetings in 2010 and 2011, trade journal articles and scientific papers in peer-reviewed journals. Importantly, data generated is playing an important role in legislative decision-making regarding future aquaculture policies and the use of triploidy within the salmonid industries (rainbow and brown trout also) and fisheries. A demonstration of this is the introduction by the Norwegian coastal and fisheries ministry of ‘green licences’ for salmon farming where green standards must be met through technological and operational solutions to reduce environmental challenge including a substantial reduction in the negative impact of escapes on wild stocks through genetic (i.e. triploidy) and physical containment. Also, the UK Environment Agency (EA) has introduced new legislation to protect wild brown trout in England and Wales and by 2015 all trout stocked into all but totally enclosed waters with no significant natural brown trout populations will be with sterile all-female brown trout. This was made possible by research done in the group which standardised triploidy induction technique and develop monitoring tools for confirmation of triploidy status.

The impact of our research into the control of puberty in farmed fish, as demonstrated above in salmon, cannot be underestimated as it is a key milestone in the expansion of a sustainable aquaculture industry in Scotland and worldwide.

5. Sources to corroborate the impact

1. Patent (No. 13183966.1 – 1357) "Composition comprising phosphorous for use in preventing deformities in triploid fish" with Biomar on triploid salmon specific diets.

2. Report of the Meeting of the NASCO/North Atlantic Salmon Farming Industry Liaison Group http://www.nasco.int/pdf/2011%20papers/CNL(11)14.pdf

3. Report on sterile salmon production to the Norwegian Ministry of Fisheries published in 2012.

4. Consultation for the Scottish Aquaculture and Fisheries (Scotland) Bill 2013 http://www.scottish.parliament.uk/S4_RuralAffairsClimateChangeandEnvironmentCommittee/General%20Documents/2013.05.08_-_Letter_from_the_Minister_-_Aquaculture_and_Fisheries_(Scotland)_Bill_-_Stage_2_follow_up.pdf

5. Trade article on preventing reproductive development of farmed finfish (2012) http://www.thefishsite.com/articles/1339/preventing-reproductive-development-of-farmed-finfish

In addition, written statements corroborating the impacts are available from Marine Harvest Scotland, AquaGen, North Atlantic Salmon Conservation Organisation and the UK Environment Agency.

Institution: University of Stirling

Unit of Assessment: A6 Agriculture, Veterinary and Food Science

Title of case study: Improving sustainability of UK salmon farming through replacement of marine fish oil while ensuring nutritional quality is preserved through maintenance of omega-3 levels

1. Summary of the impact

Omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) are essential nutrients and have many beneficial effects on human health. Fish are the major source of omega-3 LC-PUFA in the human diet, and its level was maintained in farmed fish through the use of fish oil as a major component of extruded aquafeeds. Around 10 years ago it became clear that demand for fish oil would rapidly outstrip supply, limiting expansion of aquaculture activities, if fish oil use was not reduced. The challenge this presented was that alternatives to fish oil lack omega-3 LC-PUFA. However, replacement of fish oil with more sustainable alternatives is now standard practice in the industry. Research into fish oil replacement and omega-3 metabolism in the Nutrition Group, Institute of Aquaculture has been at the forefront of the scientific research in the UK and Europe that has ensured nutritional quality of farmed fish by developing alternative feed ingredients and feeding strategies that have maintained levels of omega-3 LC-PUFA despite radical changes to feed composition driven by sustainability and food security. This work culminated with recent demonstrations that farmed salmon can be net producers of marine protein (2010) and oil (2011).

2. Underpinning research

Fish are a unique and rich source of omega-3 LC-PUFA, essential nutrients that have well-established beneficial effects in a range of human pathologies including cardiovascular and inflammatory diseases, and neurological disorders. Thus it is crucial that the levels of these fatty acids should be maintained in farmed fish and seafood products. Over-exploitation of wild fisheries has meant that about 50% of fish and seafood for human consumption is now farmed and aquaculture is the fastest growing global food production system. Aquaculture has been highly dependent on dietary fish oil and fishmeal derived from marine fisheries, representing a production system that is, at best, at its sustainable limit. Continued expansion of aquaculture, which is essential to meet the global demand for fish, is only therefore possible by replacing marine-derived resources with alternative nutrient sources. The greatest challenge in replacing fish oil has been that suitable, sustainable alternatives do not contain the health promoting omega-3 LC-PUFA. Research in the Nutrition Group has:

a) produced a large and comprehensive data set on the effects of substitution of fish oil with alternatives including vegetable oils, other marine oils (krill etc) and single-cell oils (*Schizochytrium/C.Chonii,* etc) on growth performance and feed efficiency, composition and product and nutritional quality (esp. omega-3 LC-PUFA level) of farmed fish;
b) developed key criteria for the selection of alternatives to fish oil that will minimise impacts on product omega-3 levels;
c) been key to the development of feeding strategies to maximise substitution without affecting farming performances while minimising impacts on omega-3 LC-PUFA levels (Bell et al., 2003);
d) advanced knowledge of fish lipid and fatty acid metabolism and genetics that has enabled formulation of feeds specifically designed to match the lipid biochemistry and physiology of the fish (Morais et al., 2011);
e) provided heritability data and molecular markers to assist in future breeding programmes to select for high flesh omega-3 LC-PUFA levels (Leaver et al., 2011);
f) delivered molecular tools (e.g. genes of omega-3 biosynthesis) to enable highly strategic approaches including the development of engineered oilseed crops to produce omega-3 LC-PUFA tailored to the aquaculture industry, and the development of fish strains designed for increased endogenous production of omega-3 LC-PUFA (Monroig et al., 2010).

The research has been a combination of near-market, applied science supported by basic, fundamental studies. The applied research has involved the design of novel feed formulations and testing of alternative raw ingredients through trials with key farmed species including Atlantic salmon, rainbow trout, and Atlantic cod. The basic science has studied fundamental biochemical, molecular and genetic mechanisms involved in the control and regulation of lipid and fatty acid metabolism and, particularly, omega-3 LC-PUFA, 'EPA' and 'DHA'.

3. References to the research

The studies related to omega-3 LC-PUFA metabolism and nutrition have resulted in around 80 papers in scientific journals/books, 90 oral and poster presentations at International and national conferences/meetings and, importantly, 36 articles and presentations at industrial/trade forums/meetings and in the trade/popular press. The overall impact of the work on fish oil substitution has been highly influential. The six references here give just a glimpse of the range and depth of influence. Impact on commercial activities was advanced by publications in aquaculture journals, widely read by industry, with underpinning science published in 'high impact' journals. Bell and Tocher have h-indices of 47 and 52 and 12,000 unique citations (11,697; WoK, Sept 2013). Tocher is a highly cited researcher in Thomson Reuters Highly Cited list, globally the top 250 most-cited researchers in a defined discipline.

1. Bell JG, Tocher DR, Henderson RJ, Dick JR, Crampton VO. (2003). Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partly restored by a subsequent fish oil finishing diet. *J. Nutr*. 133, 2793-2801.
2. Torstensen BE, Bell JG, Sargent JR, Rosenlund G, Henderson RJ, Graff IE, Lie Ø, Tocher DR. (2005). Tailoring of Atlantic salmon (*Salmo salar* L.) flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend. *J. Agric. Food Chem*. 53, 10166-10178.
3. Monroig Ó, Zheng X, Morais S, Leaver MJ, Taggart JB, Tocher DR. (2010). Multiple fatty acyl desaturase (FAD) genes in Atlantic salmon: cloning and functional expression of cDNAs confirm presence of three Δ6 FADs. *Biochim. Biophys. Acta* 1801, 1072-1081.
4. Bell JG, Pratoomyot J, Strachan F, Henderson RJ, Fontanillas R, Hebard A, Guy DR, Hunter D, Tocher DR. (2010). Influence of genotype/phenotype of on effects of on replacement of dietary fish oil with vegetable oils in Atlantic salmon (*Salmo salar*) families/strains selected on the basis of flesh adiposity: growth, flesh proximate and fatty acid compositions. *Aquaculture* 306, 225-232.
5. Leaver MJ, Taggart JB, Villeneuve LAN, Bron JE, Guy DR. Bishop SC, Houston RD, Matika O, Tocher DR. (2011). Heritability and mechanisms of n-3 long chain polyunsaturated fatty acid deposition in the flesh of Atlantic salmon. *Comp. Biochem. Physiol*. 6D, 62-69.
6. Morais S, Pratoomyot J, Taggart JB, Bron JE, Guy, DR, Bell JG, Tocher DR. (2011). Genotype-specific responses in Atlantic salmon (*Salmo salar*) subject to dietary fish oil replacement by vegetable oil: a liver transcriptomic analysis. *BMC Genomics* 12, 255.

Key associated grants:

1. Aquaculture feeds and fish nutrition: paving the way to the development of efficient and tailored sustainable feeds for European farmed fish, ARRAINA. EU FP7 2012-16, £460K.
2. Evaluating novel plant oilseeds enriched in omega-3 long-chain polyunsaturated fatty acids to support sustainable development of aquaculture. BBSRC IPA, 2012-15, £400K.
3. Development of protein-rich and starch-rich fractions from faba beans for salmon and terrestrial animal production, respectively. Technology Strategy Board, 2012-15, £195K.
4. Oxidation, lipids, DNA and mitochondria. EU FP7-PEOPLE-2011-IEF Panel LIF 297964, 2012-14, £145K
5. Fish intestinal nutrigenomics in response to fish oil replacement in Atlantic salmon diets. EU FP7-PEOPLE-2007-2-1-IEF Panel LIF 219667, 2009-11, £130K
6. Sustainable aquafeeds to maximise the health benefits of farmed fish for consumers, AQUAMAX" EU FP6 IP 016249, 2006-10, £550K

4. Details of the impact

Principal objectives of our research were to develop feed formulations and feeding strategies for the replacement of fishmeal and fish oil in feeds for farmed fish without compromising production, fish health and, in particular, the levels of health-promoting n-3 LC-PUFA and the nutritional quality of the products to consumers. This work has resulted in farmed salmon now being potential net producers of marine protein and oil (2010-11). This is a highly successful outcome given the previous situation of salmon farming as the world's biggest consumer of fishmeal and fish oil.

Underpinning studies investigated the effects of various fish oil substitutes and blends, and the levels, duration and timing of substitution, in trials encompassing the entire growth cycle of salmon from first-feeding fry to market size (1998-2005). The knowledge generated informed industry on best practices for the substitution of fish oil with vegetable oil in aquafeeds, and directly influenced commercial salmon feed formulations that now show levels of fish oil substitution of between 25 and 70%, mainly with rapeseed oil. Different formulations and feeding strategies were devised for minimising negative effects on tissue omega-3 LC-PUFA compositions including partial replacement of fish oil over the entire growth cycle, or complete replacement followed by a pre-harvest 'finishing' phase using feeds with fish oil to restore omega-3 LC-PUFA levels.

The major impact of this work is that these strategies are now employed by the industry with the majority of production utilising partial replacement with oil blends, and the finishing feed strategy being employed to ensure that products comply with the high specifications of premium retailers and quality certification. Recently, it was demonstrated that feed formulations have enabled farmed salmon to be net producers, rather than consumers, of marine protein and oil (Crampton et al., 2010; Bendiksen et al., 2011). The impact of our research into the substitution of fish oil in aquafeeds cannot be underestimated as the global supply of fish oil would have been exceeded several years ago if substitution of fish oil, particularly in salmonid feeds, had not been researched and successfully implemented. Our contribution has not simply been to ensure the continued expansion of aquaculture in a more sustainable manner, but has specifically focussed on ensuring that the nutritional quality of the product was not compromised. Omega-3 LC-PUFA levels are critically low in Western diets and we cannot afford to allow levels in fish, the primary source of these essential nutrients in our diet, to decline significantly.

The applied research was supported by fundamental studies including the application of molecular and genomic technologies such as transcriptomics and proteomics that provided the basic science underpinning our understanding of molecular, biochemical, and physiological aspects of omega-3 LC-PUFA metabolism in fish (2006-12; Monroig et al., 2010; Morais et al., 2011). A major impact of these studies was that retention of omega-3 LC-PUFA in flesh was a heritable trait in Atlantic salmon and so could be enhanced by selective breeding (2009-11; Leaver et al., 2011). Another impact was fish genes cloned in our lab being utilised in studies to introduce the LC-PUFA biosynthesis trait into oilseed crops (Robert et al., 2005).

Six projects had international partners with the Nutrition Group being a core consortium partner in all these projects (coordinator of FOSIS and RAFOA) and the lead partner with respect to fish oil substitution and omega-3 LC-PUFA metabolism and nutrition. Uptake of these research findings has been through very direct pathways as all the research was performed in collaboration with the major global feed companies (BioMar, EWOS and Skretting), either as partners in major RCUK (FOSIS) or EU projects (RAFOA, FORM, AQUAMAX & ARRAINA), or through industry-led collaborative projects with BioMar and EWOS. In most cases the studies were also performed in collaboration with major fish producers, such as Marine Harvest, in their own facilities for semi-commercial scale trials using sea pen cages and fish grown to market size. Many projects included other key players in the aquaculture supply chain including oil producers (Croda, DSM, Technology Crops Inc), fish breeding companies (Landcatch Natural Selection), processors (Pinneys), retailers (Sainsburys), and trade organisations (Scottish Salmon Producers Organisation, Federation of European Aquaculture Producers). All results were disseminated widely in the scientific literature, at conferences and workshops, and in trade and technical reports and the national press. This led to the rapid application of the findings in the form of new commercial formulations and feeding strategy recommendations that were made widely available so that beneficiaries included the entire aquaculture sector from feed ingredients, feed manufacture, fish producers and processors, to retailers and consumers including SMEs. Key to this was the role of the Nutrition Group as principal partners in an EU FP5 Thematic Network (FORM, Fish oil and meal replacement) with a specific KT/KE remit focussed on exchange of information and an output consisting entirely of exploitation and dissemination activities primarily through four annual meetings/workshops where our results were presented to a wide audience of key stakeholders including the aquaculture industry, trade organisations, consumer groups and health and safety authorities.

The Nutrition Group has been at the heart of the consultations and committees defining aquaculture nutrition research not only in the UK and Europe but also globally. In the early phase of EU FP6, the Nutrition Group, along with our key collaborators in Norway (NIFES) and France (INRA), developed an Expression of Interest that was adopted by the EU and resulted in two new

Calls, providing research funding of almost €25 million to the SEAFOODplus and AQUAMAX projects that spearheaded EU research in aquaculture nutrition over the subsequent years (2006-2010). Bell was a key member of the European Aquaculture Technology and Innovation Platform (EATIP) (2008-2010) and was leader of Goal 1 tasked with a remit to "Strengthen sustainability of aquaculture by developing Future Fish Feeds based on a sound scientific basis". The EATIP programme was adopted in an EU FP7 call on aquafeeds that was answered by the ARRAINA project in which the Nutrition Group are members of the core consortium. Recently, Tocher was an appointed member of the US National Academies, National Research Council (NRC) Committee on Nutrient Requirements of Fish and Shrimp. This Committee was convened for the first time in 20 years with responsibility to completely update the NRC Bulletin on Nutrient Requirements of Fish, last produced in 1993. The NRC Report is universally regarded throughout the world as the "Gold Standard", and the new Report "Nutrient Requirements of Fish and Shrimp" was published in August 2011. All of the above contributed to the high regard the aquaculture industry have for the research of the Nutrition Group. This resulted in the Group being in great demand to carry out both contract work and analyses, and the establishment of the Nutrition Analytical Service (NAS), the highly successful commercial arm of the Nutrition Group with over 40 customers around the globe and an annual turnover of almost £0.45 million.

The impact of our work is readily attested by key industrial partners:

"The economic benefits have been far reaching both in reduction of overall production costs and in facilitating increased growth in this sector. As such the research activities of the Nutrition Group, IoA are greatly valued by the Aquaculture industry"; Global Research Director, BioMar Ltd.

"More importantly however has been the nutritional knowledge which has been generated by the IoA Nutrition Group to ensure that eating qualities and human health benefits of eating oily fish containing n-3 LC-PUFA remain in place"; Technical Services Manager, Marine Harvest Scotland.

"Skretting ARC are delighted to provide this endorsement of the outstanding and important contributions that the Nutrition Group, IoA have made in the development of sustainable aquafeeds and their major positive impact on the industry in the UK and globally". Head of Nutrition Research, Skretting Aquaculture Research Centre.

"The work of the Nutrition Group IoA has had a significant impact on our ability to replace fish oil in feeds for Atlantic salmon"; Principal Scientist, EWOS.

5. Sources to corroborate the impact

1. Fish Farmer – cited in CEFAS Report (page 36). IoA (RAFOA) was shortlisted in 2006 for 1st Fish Farmer Fit for the Future award recognising outstanding achievement and innovation in aquaculture.
   http://www.fishfarmer-magazine.com/news/fullstory.php/aid/368/Fish_Farmer__Fit_for_the_Future__awards_shortlist_announced.html

   and http://cefas.defra.gov.uk/publications/shellfishnews/sfn0506.pdf

2. Australian Northern Territory Government Technote – cites FOSIS and RAFOA projects http://www.nt.gov.au/d/Content/File/p/Technote/TN124.pdf

   In addition written statements of corroboration are available from Biomar Ltd, EWOS, Skretting, the Chair of US National Academies, NRC Fish and Shellfish Requirement Committee, the Director of Genetics, Hendrix/Landcatch Natural Selection and Marine Harvest Scotland.