COMPLETED PROJECT CASE STUDY

# SCALING UP PRODUCTION AND IMPLEMENTATION OF FARMED CLEANER FISH IN THE SCOTTISH SALMON INDUSTRY

**PARTNERS**

*Institute of Aquaculture, University of Stirling | Mowi Scotland | Scottish Sea Farms | BioMar | Otter Ferry Seafish*

**PROJECT LEADS**

*Herve Migaud, Andrew Davie, Adam Brooker, Athina Papadopoulou, Antonios Chalaris, Thomas Cavrois-Rogacki*

## BACKGROUND

Annual economic impacts of sea lice to the global salmon sector are estimated to be £300 – 560m per annum, these estimates are derived from the efforts to mitigate sea lice and the direct losses associated with sea lice infections of farmed salmon. The Scottish aquaculture sector manages sea lice in accordance with the principles of integrated pathogen management. One of many measures used to control parasitic sea lice are cleaner fish, which are co-habited with salmon for their natural delousing behaviour. Cleaner fish, such as the Ballan wrasse and lumpfish, are cultured specifically for this purpose. Marine species of fish such as the Ballan wrasse are challenging to rear in captivity and research is required to optimise stock performances. This project brought together two of Scotland's leading producers of Atlantic salmon, Mowi Scotland and Scottish Sea Farms, a leading manufacturer of sustainable aquafeeds, BioMar, and the world-renowned Institute of Aquaculture based at the University of Stirling under the academic leadership of Prof Herve Migaud and Dr Andrew Davie. The partners completed four multidisciplinary work packages to investigate and improve many aspects in the rearing of Ballan wrasse.

It should be noted that this case study represents a very broad overview of a project with multiple trials and experiments. For more detailed insight and knowledge of cleaner fish production, the reader is encouraged to explore the excellent scientific manuscripts that were published during this project. The authors selected products to use in this study and they are mentioned; this is by no means an endorsement or recommendation.

This case study assumes some background knowledge of aquaculture, in particular cleaner fish. Readers unfamiliar with this topic are encouraged to consult the recently published review of cleaner fish farming and deployment. Figure 1 gives a brief overview of the farming of Ballan wrasse.

## AIMS

The overarching goal of the project was to scale up Ballan wrasse production by domesticating the species and optimising broodstock management, larval rearing, nutritional requirements, management of bacterial infections and post-deployment performance and welfare. The following sections briefly describe each work package in turn.


*Figure 1. Typical commercial production timelines for ballan wrasse and lumpfish. DD = degree days, DPH = days post-hatch. (Brooker et al., 2018)*

# BROODSTOCK MANAGEMENT WORK PACKAGE

Broodstock (the parent fish) management is essential to control fish reproduction in captivity and supply good quality eggs and larvae for optimal performance and health during the production cycle. Most marine fish species show strong seasonality in their spawning rhythm, but in farming, it is important to have fish at any time of year. There are many techniques to extend the spawning season and boost production of young fish: by altering photoperiod (day length) or temperature to change the fish's perception of the seasons; by induction of spawning via hormonal injection and using genetic information as first steps to improve the stock. Ballan wrasse are protogynous hermaphrodites, which means fish are first females and then can switch to males as the result of social interactions. The number of males is estimated to be only 10% in natural populations, and it can restrict production and genetic make-up of next generation fish. Ballan wrasse display structured spawning behaviour and co-habiting females make unequal contributions within the spawning group; this can also limit the performance of a hatchery. Key aims of this work package were to increase and control the number of males and provide genetic tools to begin selective breeding of the species. To achieve these aims a series of studies investigated the reproductive physiology, behaviour and performance of Ballan wrasse broodstock and develop new protocols to enhance egg productivity and quality. Furthermore, the researchers developed the tools to begin a breeding programme for this species.

## WORK DONE

Developed a method for sex induction using hormone therapy to obtain males, which gives the researcher/aquaculturist greater control over breeding.

The females spawn (lay) their eggs on a textured substrate (AstroTurf). A study investigated whether the mat's colour was important.

Tools for parental assignment and contribution to spawning developed using microsatellite panels to allow management of the stock and prevent inbreeding.

Investigated egg quality differences between wild Ballan wrasse and hatchery reared females, using fatty acid analysis.

Investigated procedures for degumming, disinfecting and hatching synchronisation protocols.

Monitored a suite of phenotypic traits, including growth, age at maturation, deformities, disease resistance.

## OUTCOMES

Sex induction to achieve extra males was successful and the technical know-how was transferred immediately to industry. The authors found that methyl testosterone and fadrozole could be applied as a hormone therapy shortly after spawning to induce fish to become male the following spawning season.

Red or dark blue AstroTurf was found to be the best spawning substrate regardless of its position in the spawning tank.

Microsatellite markers for enhanced parentage assignment were developed and all broodstock genotyped. This allows for best practice management of broodstock to maintain genetic diversity (effective population size, He).

Hatchery produced egg quality of wrasse was shown to be similar to eggs from wild wrasse, therefore suggesting that nutrition in the broodstock is adequate for producing high quality fish larvae.

Egg handling studies showed spawning events occurred in cycles of approximately 12 days. Applying protease, e.g., 2% Alcalase®, for 1 h with gentle agitation was a suitable degumming procedure and this is best achieved within 24 h of egg collection, after which time embryo viability was reduced. Disinfection was achieved with bronopol.

During the project wrasse hatched in captivity spawned for the first time, representing the closure of the lifecycle in Ballan wrasse. All broodstock were chipped and genotyped, which accompanied with parentage assignment, allows targeted selection to begin to take place and the species to be improved for commercially important production traits. So far, the following traits are being recorded including growth, reduced deformity, age to puberty, disease resistance and importantly delousing ability.

# NUTRITIONAL REQUIREMENTS WORK PACKAGE

Feeding marine fish species can be a critical bottleneck in production. Marine larvae require live feed with specific nutrient requirements e.g., phospholipids. Live feeds, mainly rotifers and *Artemia* nauplii (brine shrimp), require culturing themselves and 'enrichment' to increase their nutritional value to marine larvae. Live feeds are given to marine larvae from after mouth opening until 'weaning', the transition from live feed to formulated feed. Larvae that do not get the right nutrition often have some deformities in their skeleton, it is thought by supplying exactly the right minerals at the right time that deformity can be minimised, and the welfare of the larvae/juvenile improved. This work package aimed to investigate aspects of wrasse nutrition to give juvenile wrasse the healthiest start in life and support optimal growth and health performance of juvenile fish. The work focussed on enrichment, weaning and on-growing protocols at wrasse hatcheries.

## WORK DONE

Screening and refinement of live feed enrichment protocols and use of probiotics and bronopol during live feeding stages.

Study of fish health status as per deformities (skeletal, cranial, jaw, swim bladder) and nephrocalcinosis (deposition of calcium salts in the kidneys) occurrence and development, causes and solutions.

Looking at fish quality (i.e., fin erosion, condition factor, overall condition)

Study of juvenile wrasse nutritional and environmental requirements (i.e., temperature, diet formulation and tank hygiene).

Study of the Ballan wrasse gut microbiota in relation to origin (farmed vs. wild, flow-through vs. recirculation).

## OUTCOMES

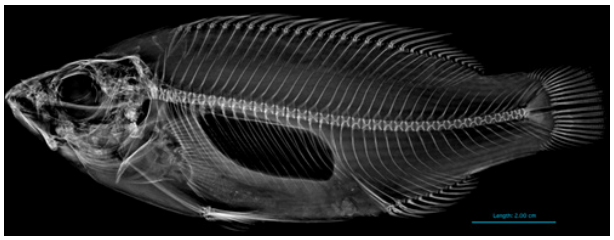

*Figure 2. X-ray of the Ballan wrasse skeleton.*

Among four commonly used commercial live feed enrichment products, Larviva Multigain resulted in the nauplii with the highest levels of DHA.

Marine lecithin is a good candidate to enrich *Artemia* nauplii in both phospholipid and long-chain polyunsaturated fatty acids.

Soya lecithin did not increase the long-chain polyunsaturated fatty acids content of the phopholipid fraction in comparison to standard enrichment.

Conjugated selenium (Se, presented with Amino acids) can be used to enrich targeted levels of Se in *Artemia* nauplii. The use of inorganic Se was not an effective strategy to enrich *Artemia* nauplii, even when it was delivered through phospholipid vesicles.

Enriching *Artemia* nauplii with 12mg of conjugated Se per litre for 4h prior to a 24h enrichment with long-chain polyunsaturated fatty acid rich commercial diets produces *Artemia* with Se contents similar to those found in copepods.

Repeated bronopol treatment with probiotic addition from the yolk-sac stage resulted in increased larvae survival.

Use of probiotic alone or clay alone (control) had no positive impact on larvae survival however best growth was obtained in the control treatment (clay only).

Scottish production of farmed Ballan wrasse is affected by vertebrae malformations, jaw/operculum malformation and nephrocalcinosis.

All deformities were observed at both post-weaning and pre-deployment, suggesting they occur in early development.

First description on the Ballan wrasse skeleton and proposes key to characterise it (Figure 2).

Benchmarking of a suitable on-growing diet with inclusion of specific raw materials, which improves by 23% growth compared to other fish meal-based diets and without compromising intestinal health or digestive functions. Thus, the currently required 20 – 24 month period to reach deployment size of 45 g could potentially be reduced to 16 – 19 months.

Diet based on polychaetes elicited reduced growth and suppressed intestinal digestive enzymes and fatty acid liver retention, possibly due to presence of anti-nutritional factors.

Increased dietary moisture level (16 % moisture) in Ballan wrasse decreased FCR by 21% compared to the standard 'dry' diet (5% moisture). Growth was not influenced by moisture level, implying better assimilation of feed.

Fish condition was maintained using agar blocks although feed intake varied with agar inclusion rate, confirming their potential use as a post-deployment diet.

Growth and feeding efficiency of Ballan wrasse juveniles were significantly improved in fish reared at a constant temperature of 16°C compared to 13 and 10°C. The production period could potentially be shortened by four months using water warmed to 16°C.

The protein content (i.e., 51%) of two of the most commonly used Ballan wrasse on-growing diets was questioned and may not fulfil the species requirements.

The partial replacement of fishmeal by plant-based ingredients, namely soy protein concentrate and pea protein, in the formulation of diets for Ballan wrasse juveniles is possible without compromising growth, feed efficiency and health.

# HEALTH MANAGEMENT WORK PACKAGE

Furunculosis caused by atypical strains of *Aeromonas salmonicida* is a major cause of disease and mortality in Ballan wrasse. Two types of the bacterium (V and VI) have been reported in diseased Ballan wrasse in Scotland. This work package set out to screen for bacterial pathogens during disease outbreaks, characterise atypical *A. salmonicida* isolates and understand the disease caused by these isolates. In addition, an autogenous vaccine was also developed and optimised to manage atypical *A. salmonicida* and the efficacy of the vaccine was tested.

Ballan wrasse eggs in the wild adhere to nests or rocks covered with algae with the assistance of an adhesive gum layer that is coating the external surface of the egg. The gum layer is associated with bacterial communities (microbiota), comprising of both beneficial and pathogenic bacteria. However, eggs are often degummed and disinfected to reduce the levels of bacteria in the hatchery. To understand and manage vertical transmission (broodstock to eggs) of atypical *A. salmonicida* we described the microbiota and investigated the impact of removing the gum layer.

Key definition: an autogenous vaccine is a vaccine prepared from isolates of pathogens obtained from the field.

## WORK DONE

Isolate and identify bacterial pathogens in Ballan wrasse and characterise the predominant pathogen (atypical *A. salmonicida*) from isolates collected in Scottish farms (2016 – 2018).

Development of an assay for the detection of atypical *A. salmonicida* type V and VI in Ballan wrasse.

Characterise the egg microbiota prior and upon disinfection regimes during incubation to determine the effectiveness of disinfection. Establish the potential vertical transmission of atypical *A. salmonicida*.

Investigate the potential to cause disease and the severity of disease of routinely recovered bacteria species from diseased Ballan wrasse by immersion and injection.

Develop an autogenous vaccine for *A. salmonicida* and assess the protection and immune response elicited by immersion and injection methods and optimise vaccination strategies (injection).

Investigate juvenile Ballan wrasse immune competence.

## OUTCOMES

A range of bacteria species found in the sampling programme, atypical *A. salmonicida* was the predominant bacterial pathogen isolated during disease outbreaks at sea cage and hatchery sites. Samples were screened for atypical variants of *A. salmonicida* and predominantly type V was found (as opposed to type VI). Other bacterial species (*Vibrionaceae* and *Photobacterium indicum*) were also identified.

Atypical *A. salmonicida* was the most pathogenic bacterium when administered both by immersion and injection, and this led to the development of a bath challenge model for atypical *A. salmonicida* for the first time and the establishment of a successful i.p. infection method with the same bacterium in juvenile Ballan wrasse.

The ontogeny of Ballan wrasse adaptive immune elements (MHC II – CD74 and IgM) reported at 35 and 90 days post hatch, respectively.

A multivalent autogenous vaccine was produced to protect against atypical *A. salmonicida*, injection and immersion challenge models were developed using atypical *A. salmonicida* and used to assess the efficacy of vaccination. Injection vaccination offered good protection against atypical *A. salmonicida* in pre-deployment Ballan wrasse (>25g) and the vaccine elicited specific antibody responses (IgM) in the fish.

The same autogenous vaccine did not offer protection in juvenile Ballan wrasse (0.5 or 1.5g) when used as an immersion vaccine. Although genes involved in adaptive immunity were expressed (MHC II and IgM) at this stage, it is unknown whether the immune system is fully competent. Further studies should investigate immersion vaccination at a later stage of development (>2g).

Ballan wrasse eggs microbiota was described, atypial *A. salmonicida* detected on egg samples but vertical transmission of the pathogen has not been confirmed yet and the Alcalase® treatment to degum eggs cleared most of the microbiota associated with the eggs.

In summary, the results of this work package have provided essential information on the pathogenicity and fish susceptibility to routinely recovered bacteria from diseased Ballan wrasse in hatcheries and cages sites in Scotland. This work also established that the available autogenous vaccine is highly protective against homologous and heterologous strains of atypical *A. salmonicida* when administrated through injection, while immersion vaccination was not successful with the experimental conditions used in this study.

## POST DEPLOYMENT PERFORMANCES WORK PACKAGE

Wrasse are produced to exploit their natural cleaning behaviour, which is referred to as delousing. In order to delouse as effectively as possible, the aquaculturist must understand this behaviour in detail and provide Ballan wrasse with an optimum environment. In direct collaboration with the commercial partners, Stirling scientists studied and described best management practices for the use, deployment, management and feeding of Ballan wrasse. This part of the project also involved comparing Ballan wrasse behavioural patterns to those of the lumpfish. Passive-acoustic telemetry (PAT) was used to study cleaner fish behaviour in salmon pens; this technique uses hydrophones and acoustic transmitters implanted to the fish to track their movements. Four experiments using PAT to track fish positions were carried out to study cleaner fish behaviour at commercial salmon sites in Scotland.

### WORK DONE

Cultured wrasse and lumpfish were PAT acoustic tagged and their behaviour tracked and monitored to compare the two species.

Compared the behaviour of farmed Ballan wrasse and wild Ballan wrasse using PAT.

Investigated the impact of pre-deployment and post-deployment acclimation on Ballan wrasse behaviour using PAT.

Protocols for optimum stocking ratios and time of deployment, standardisation of salmon hides, positioning and size in relation to the number of cleaner fish, feed type and ingredients, feeders type, design and positioning and general cage-management practices were established by the commercial partners.

### OUTCOMES

In the PAT studies, both wrasse and lumpfish were active during the day. Wrasse spent 60% (± 2%) at or below 15m but rested at shallower depths (night). Wrasse made infrequent use of hides. In comparison, lumpfish spent 80% of the time at depths shallower than 10m and made extensive use of hides, especially during night. These differing behaviours support the combined use of lumpfish and wrasse.

Ballan wrasse were more active during the day than at night. The main difference between farmed and wild wrasse was that farmed wrasse spent more time at depth than wild wrasse. Wild Ballan wrasse also exhibited larger 'home ranges' and higher activity (swimming speed) than non-acclimatised farmed wrasse, indicating more use of the pen volume.

Pre-deployment acclimation involved tank furniture, introducing natural photoperiod (light dark cycles) and providing feed blocks to farmed Ballan wrasse. This did not fully alter their behaviour at sea and only a single fish clearly displayed day-night activity rhythms as seen in wild fish.

Post-deployment acclimation involved temporary maintenance of the Ballan wrasse separate from the salmon in a keep net, and artificial kelp and feed blocks were provided. The effect of both pre- and post-deployment acclimation was that the acclimated Ballan wrasse displayed behaviour more similar to wild fish; the fish were active at shallower depths and more quickly established larger home ranges than non-acclimated fish.

Ballan wrasse can be deployed at 40-50g, approximately 18 months after hatching. The stocking ratio can be 2.5 – 5% Ballan wrasse : salmon. Sea deployment during spring.

Lumpfish are stocked at 20 – 30g, approximately 5 months after hatching. The stocking ratio can be 10 – 15% lumpfish : salmon. Sea deployment during autumn.

Hide design evolved during the study and a curtain fake-kelp design is currently preferred to maximise hide space and encourage interactions with the salmon. Two hide-cleaning strategies are used: regular removal and pressure washing or have two hides with one in the water and the other air dried to manage biofouling.

Although cleaner fish are encouraged to feed on lice, providing a feed block is necessary to promote good condition and welfare, and these are suspended close to the hides. Pelleted feeds are also available.

Nets should be cleaned or changed frequently to minimise cleaner fish grazing on biofouling rather than sea lice.

> *"Multiple improvements to Ballan wrasse farming were made through this industry–academic collaboration. This development supports salmon farmers worldwide to utilise a sustainable intervention against sea lice."*

## IMPACT

The project was very successful and fully achieved its goals. *Scaling up the production of Ballan wrasse* mainly focussed on developing the rearing processes for this species to increase production but also acclimatise farmed Ballan wrasse for effective delousing. Seven processes were developed or optimised:

- Sex induction to provide males
- New vaccine and efficacy confirmed
- Genetic marker panel for genotyping
- Egg management by degumming and disinfecting
- Deployment protocols for natural behaviour with diurnal rhythmicity
- Enhanced live feed enrichments
- Husbandry conditions for enhanced growth performances

Parental assignment developed as a service that will allow the genetic improvement of the species through selective breeding. Two products were improved:

- Further validation of wrasse feed blocks developed by Stirling academics
- Wrasse hides and furnishings.

During the project first generation (F1) broodstock were bred in captivity, which is a world first for the species. This opens many possibilities for the future, especially the improvement of the species traits by selection. The project was awarded the Applied Research Award at the Aquaculture Awards 2019.

Speaking on the value of the project to the academic team, Prof. Herve Migaud said:

> *"This project helped IoA scientists involved to become and remain the leading scientists working on cleaner fish research and overall on integrated sea lice pest management strategies and work closely with the leading salmon industry partners in the UK."*

Three PhD projects and ten MSc theses were authored as part of this project by students at the Institute of Aquaculture, University of Stirling.

Reporting on the project's legacy, Dougie Hunter, Technical Director at Mowi Scotland, said:

> *"The project has brought a plethora of results that can ultimately be used within the production hatcheries to improve fish health and welfare. Techniques developed by the various researchers in association with farm staff will improve fish husbandry and allow further innovation under production conditions."*

## ADDITIONAL INFORMATION

**PHD THESES DURING THE PROJECT (3)**

Thomas Cavrois-Rogacki (2019). Optimisation of the hatchery production of Ballan wrasse (*Labrus bergylta*) with an emphasis on nutritional and environmental requirements

Athina Papadopoulou (2019). Health screening and autogenous vaccination strategies for atypical Aeromonas salmonicida in farmed Ballan wrasse (*Labrus bergylta*, Ascanius)

Antonios Chalaris (2018). Research and development to optimise hatchery production of Ballan wrasse (*Labrus bergylta*): bacterial control and nutritional aspects

**MSC. THESES DURING THE PROJECT (10)**

Andrea Bertini (2019). Characterisation of the spawning performance of Ballan wrasse (*Labrus bergylta*) broodstock and development of egg degumming using enzyme Alcalase®.

Motsatsi Patience Selotole (2019). Survey of external and internal deformities in cultured juvenile Ballan wrasse (*Labrus bergylta*) during hatchery production.

Silvia Viale (2019). Investigation of the early immune defence in Ballan wrasse (*Labrus bergylta*).

Lucia Drabikova (2019). Large-scale screening for vertebral deformities in pre-deployment stages of farmed and wild Ballan wrasse (*Labrus bergylta*).

Konstantinos Zoas (2019). Variability in the essential fatty acids profiles of live prey in Ballan wrasse (*Labrus bergylta*) hatcheries.

Aileen Bone (2018) A preliminary investigation into the bacterial communities found on Ballan wrasse (*Labrus bergylta*) eggs before and after hatchery disinfection.

Kathryn Garvey (2018). Assessment of atypical Aeromonas salmonicida infection Ballan wrasse (*Labrus bergylta*) following experimental bath challenge using molecular screening and histopathology.

Edward King (2017). The optimization of *Artemia* enrichment targeting an enhanced nutritional profile for marine larvae.

Andrew Rolland (2017). Optimization of selenium enrichment of *Artemia* for enhanced nutritional profile of marine larvae.

Sébastien Esnault (2016). Investigation of the batch and impact of enrichment source on the nutritional quality of *Artemia* for Ballan wrasse (*Labrus bergylta*) production.

**SCIENTIFIC PUBLICATIONS (11)**

Brooker, A.J., Papadopoulou, A., Gutierrez, C., Rey, S., Davie, A. and Migaud, H., 2018. Sustainable production and use of cleaner fish for the biological control of sea lice: recent advances and current challenges. Veterinary Record, 183.

Brooker, A.J., Davie, A., Leclercq, E., Zerafa, B. and Migaud, H., 2020. Pre-deployment acclimatisation of farmed Ballan wrasse (*Labrus bergylta*) to sea-cage conditions promotes behaviour analogous to wild

conspecifics when used as cleaner fish in Atlantic salmon (*Salmo salar*) farms. Aquaculture, 520, p.734771.

Clark, W., Leclercq, E., Migaud, H., Nairn, J. and Davie, A., 2016. Isolation, identification and characterisation of Ballan wrasse Labrus bergylta plasma pigment. Journal of fish biology, 89(4), pp.2070-2084.

Grant, B., Davie, A., Taggart, J.B., Selly, S.L., Picchi, N., Bradley, C., Prodohl, P., Leclercq, E. and Migaud, H., 2016. Seasonal changes in broodstock spawning performance and egg quality in Ballan wrasse (Labrus bergylta). Aquaculture, 464, pp.505-514.

Grant, B., Picchi, N., Davie, A., Leclercq, E. and Migaud, H., 2016. Removal of the adhesive gum layer surrounding naturally fertilised Ballan wrasse (Labrus bergylta) eggs. *Aquaculture, 456*, pp.44-49.

Eric Leclercq, Benjamin Zerafa, Adam J. Brooker, Andrew Davie, Hervé Migaud, 2018. Application of passive-acoustic telemetry to explore the behaviour of Ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*) in commercial Scottish salmon sea-pens. Aquaculture 495, 1-12.

Papadopoulou, A., Wallis, T., Ramirez-Paredes, J.G., Monaghan, S.J., Davie, A., Migaud, H. and Adams, A., 2020. Atypical Aeromonas salmonicida vapA type V and Vibrio spp. are predominant bacteria recovered from Ballan wrasse Labrus bergylta in Scotland. Diseases of aquatic organisms, 140, pp.47-54.

Paredez, G.R., Verner-Jeffreys, D., Papadopoulou, A., Monaghan, S., Smith, L., Haydon, D., Wallis, T., Davie, A., Adams, A. and Migaud, H., 2020. A commercial autogenous injection vaccine protects Ballan wrasse (*Labrus bergylta*, Ascanius) against Aeromonas salmonicida vapA type V. bioRxiv.

Thomas Cavrois Rogacki, Andrew Davie, Hervé Migaud, Oscar Monroig. (2019) Short term lecithin enrichments can enhance the phospholipid and DHA contents of the polar lipid fraction of *Artemia* nauplii. Aquaculture 510, 122-130.

Thomas Cavrois Rogacki, Andrew Davie, Oscar Monroig, Hervé Migaud. (2019) Elevated temperature promotes growth and feed efficiency of farmed Ballan wrasse juveniles (*Labrus bergylta*). Aquaculture 511, 734237.

Cavrois-Rogacki, T., Rolland, A., Migaud, H., Davie, A. and Monroig, O., 2020. Enriching *Artemia* nauplii with selenium from different sources and interactions with essential fatty acid incorporation. Aquaculture, 520, p.734677.