COMPLETED PROJECT CASE STUDY

SECURING SUSTAINABLE SUPPLY AND OPTIMAL DEPLOYMENT OF LUMPSUCKERS FOR SEA LICE CONTROL IN THE SCOTTISH SALMON SECTOR

PARTNERS

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PROJECT LEADS

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BACKGROUND

Sea lice are the single greatest challenge facing the modern salmon farming sector worldwide.

Management strategies must be effective, reliable, readily available, and broad in their scope to combat the great capacity of the parasites to adapt and build resistance. Within this toolkit, cleaner fish are a key solution. While in principle they can be very effective, their use must be sustainable, available to all that require it, and truly affordable at commercial scale in order to remain viable.

In the UK, an industry-led ballan wrasse research, development and production programme has been running successfully since 2012, boosting production numbers and animal quality, while reducing time required to reach deployment.

Lumpsucker production has only been initiated meaningfully in recent years. The first semi-commercial scale production began in spring 2014. With the basis for lumpsucker rearing and supply now established in the UK, it was an opportune time to initiate a strong, industry-led, research-driven programme dedicated to lumpsucker production.

This four-year project started in May 2016. It has been a true team effort with academic leads from Stirling University's Institute of Aquaculture joining forces with leading salmon businesses like Mowi and The Scottish Salmon Company, alongside other key players such as BioMar, Pharmaq and Otter Ferry Seafish.

AIMS

The overarching aim was to establish a secure and sustainable supply of lumpsuckers that are deployed effectively at salmon farms, while boosting numbers produced and raising quality standards.

This overall aim would be met by attaining the following three objectives:

1. Develop the knowledge and protocols required to support lumpsucker hatcheries and juvenile production facilities, through the implementation of sustainable, captive-bred broodstock management programmes.
2. Develop on-farm management strategies to maximise the delousing performance of lumpsuckers following cage deployment.
3. Develop tools to monitor and enhance our understanding of the lumpsucker's immune system, to inform the design and testing of vaccines that provide effective protection against known pathogens that are currently causing significant clinical losses in the species.

The project was structured around four work packages to study and develop tools for broodstock management, larvae/juvenile nutrition, cage deployment, and health management. Some of the work done during this project is summarised below.

> *The project has been very successful, as demonstrated by the new knowledge gained, tools and protocols developed and implemented in the industry, number of students trained, and extensive dissemination activities.*

1. BROODSTOCK MANAGEMENT

Project efforts focused on helping to close the production cycle, taking advantage of the relatively short generation time and rapid growth.

This work package included research on the optimal environmental conditions for spawning and the characterisation of reproductive traits of F1 stocks to reliably produce large numbers of good-quality eggs: establish methodologies and indicators of good gamete quality, natural vs. artificial spawning, stripping and artificial fertilisation methods, out-of-season spawning through photo-thermal manipulations, as well as developing a targeted set of genomic tools to assess genetic diversity to avoid "bottlenecking", and the mapping of markers associated with traits of commercial interest.

1.1. Characterisation of the reproductive physiology of F1 (+) stocks to reliably produce large numbers of good-quality eggs for a viable, self-sustaining hatchery

WORK DONE

This study was designed to explore the effects of temperature during the spawning season on captive broodstock performance. It aimed to describe the effects of rearing temperature on timing of spawning, egg productivity and quality across the observed thermal range associated with wild mature lumpfish and spawning in this species.

All fish used in the experiment were captive stock, reared from wild eggs caught in Norway and maintained at NOFIMA. Fish were maintained at ambient temperature, ranging from 5°C to 13°C, on a 24h low-intensity light photoperiod, and fed on Skretting Silk pellets. Individual morphometric, gender and stage of maturity were recorded, and fish were PIT tagged at 15 months old. Fish were randomly assigned to one of three treatment groups with a balanced sex ratio at 1 male : 2 female.

Temperature was slowly increased for each group and kept constant throughout the experimental period at 5.9°C, 9.2°C and 14.3°C. Ovarian development of the females was assessed by external observation of swelling and ultrasound scanning, and then classified using a five-point scale (immature-1, immature-2, maturing, spawning, and spent).

On four subsequent samplings various individuals from each treatment were culled, with females in the late maturing/spawning category being selected based on ultrasound screening. Individual weight and total length were recorded, and gonads dissected and sampled for later image analysis of oocyte size and development. Milt samples were analysed by spectrophotometric assessment of sperm density.

OUTCOMES

Histological analysis confirmed that oocyte development was typical for a marine teleost with the primary growth oocytes ranging in size between 82 µm and 216 µm, secondary growth oocytes between 370 µm and 529 µm, and oocyte maturation between 624 µm and 1398 µm.

Analysis of four independent samples extracted from six pre-treatment females confirmed comparable development in all, with no difference in total oocyte distribution or lead cohort diameter oocytes and a mean relative fecundity estimated to be 40,440 ± 12,434 oocytes per Kg of body weight.

Analysis of individuals after temperature treatment showed that the length of the spawning season appeared to be inversely related to holding temperature; lasting 11, 28 and 72 days for the high, medium and low temperature treatments respectively. Total productivity in number of naturally spawned batches was comparable in the low and medium temperature treatments, but notably reduced in the high temperature. Mean batch weight was comparable in the low and medium temperature treatment, but significantly reduced (>50% reduction) in the high temperature treatment.

In the low and medium temperature treatments, 84.7% of females were spawning individuals against only 12% in the high temperature treatment with 68% maturing but not spawning, with no significant difference in size observed between spawning and not spawning females.

There was a significant effect of temperature treatment on egg quality, with no egg batches from the high temperature treatment reaching the eyed stage.

With respect to the individual milt quality tests, no eggs from the high temperature treatment were viable. For low temperature milt vs. low temperature eggs, eyeing rate ranged from 22.1 ± 15.8 % and 43.0 ± 9.6 % for medium temperature milt vs. medium temperature eggs.

1.2. Broodstock methodologies that ensure hatchery productivity, including indicators of good gamete quality, natural vs. artificial spawning, stripping and artificial fertilisation methods, and out-of-season spawning through photo-thermal manipulations

WORK DONE

On two separate dates (October 2018 and May 2019), ten females and three males caught at Averøy (Norway) were randomly stripped and sampled for eggs and milt.

Milt was tested for motility using a light microscope, and for density using a spectrophotometer, at the NOFIMA cleaner fish unit at Sunndalsøra. Milt samples showing reduced motility or low sperm density were rejected. The rest were mixed into a pool and quality-checked, ensuring a minimum of 300,000 sperm per egg.

To assess fertilisation rate, from each individual female three replicate batches of eggs from the stripped egg masses were used to make small pancakes 1-2 eggs thick. These were then fertilised and sampled after 15 minutes. Samples were placed on petri dishes and flattened, sterilised seawater added and incubated at 6°C, as well as the egg masses.

Another study was done using 17-month-old F1 broodstock reared at Otter Ferry Seafish. These individuals were derived from broodstock captured on the west coast of Scotland. Fish started maturing in December 2018 with natural spawning taking place in mid-January 2019, monitored by ultrasound every three days to assess suitability for stripping. Ten females were stripped between January and March 2019. Seven males were dissected, with milt sampled and quality assessed.

For fertilisation assessment, the same protocol as previously described for wild-caught individuals was followed.

Fertilisation rate was not assessed in the wild-caught October batches due to lipid globules impeding visibility within the egg. Fertilisation rates were calculated from the eggs incubated in petri dishes for the wild-caught May and F1 stocks. At 24 hours post fertilisation, the fertilisation rate was assessed by counting the number of fertilised eggs and the number of unfertilised eggs and providing a total number of eggs and a fertilisation percentage.

Wild-stock petri dish eggs were monitored until the eyed egg stage at 150-degree days (DD), with each petri dish photographed, and total eggs, eyed eggs and non-eyed eggs counted.

For the F1 stocks, fertilised eggs were transferred to a 48 micro-well plate and eyeing rate calculated at 150 degree days and assessed as previously described.

Samples were taken from all batches for composition analysis: lipids, fatty acids, minerals, pigments.

Wild-caught broodstock were larger than the F1 stocks, but not significantly larger than other individuals of their respective wild populations.

OUTCOMES

Stripped egg volumes from the wild stocks were significantly larger than those from the F1 stock. However, the number of eggs per ml was significantly higher in the F1 broodstock, as egg diameter was significantly lower.

There was no significant difference in estimated batch fecundity or sperm densities between stocks.

Wild-derived eggs performed significantly better in all three measured quality stages: fertilisation, eyeing, and hatching rates. There was no significant difference in quality between wild eggs derived from fish caught in October and those from May.

Analysis of total lipid content between wild and F1-derived eggs shows a 39% reduction in total lipid levels within the F1 eggs compared to the October and May wild-caught samples, which were comparable.

F1 eggs were significantly lower in absolute levels of fatty acids than wild caught, with the total being 53% and 58% lower than that of the wild derived October and May respectively, and no significant difference between wild populations.

The relative proportion of all fatty acid groups showed no difference between wild October and May samples, however the wild samples consistently differed from the F1 eggs. Absolute levels of total saturates and monosaturates were significantly higher in the wild October and wild May stocks than the F1 stock. Despite this, there was no significant difference in proportional levels of monosaturates between the three stocks.

Although there was no significant difference in total lipid content at any point through development, there was a significant reduction in total saturates (28%), total monosaturates (33%), and total PUFA (40%) throughout embryonic development, between the point of stripping and hatched larvae.

Within the total PUFAs there were significant reductions between the 0 DD and hatched larvae, despite no significant change in DHA:EPA ratio over development. However, there was a significant increase in the ARA:EPA ratio between developmental stages and non-fed hatched juveniles.

Mineral levels within the F1 stocks were consistently different to those in the wild stocks, with few differences between wild stocks. The levels of major minerals within the F1 stocks were significantly lower than the two wild stocks. On the other hand, within the minor minerals there was less obvious difference between the three stocks. Of the 14 minerals analysed, only four – Ni, Cu, Ca and Co – were identified as having a positive association with hatch rate.

Changes in the composition in major minerals showed significant increases from stripping point in comparison with 200DD levels, except for phosphorus, which was significantly higher in larvae compared to all other developmental stages. Mg increased 16-fold, Ca sixfold, and K levels doubled.

For the minor minerals there were no significant changes through development for Ni, Cu, Zn, Cr and Se. There were significant increases in Fe, Co and V where levels increase throughout development. Fe levels were 25 times higher in hatched juveniles than at the point of stripping.

There were five pigments identified within lumpfish eggs at the point of stripping, which were consistently present in all sample sources: canthaxanthin, astacene, astaxanthin, echinenone and lutein. Total pigment levels in the wild samples were threefold higher than observed in the F1 samples.

β-carotene was identified in all samples post stripping, with a threefold increase in levels of between 24hr and hatching. There was a significant reduction in the levels of canthaxanthin, astacene and Echinenone between stripping (0 DD) and 24hr, with levels remaining constant thereafter.

The compositional profiles of F1 eggs were significantly lower in key elements in all three major nutritional components measured, suggesting that nutrition was not optimal. This shows relationships between hatching rate and mineral concentrations as well as potential mineral uptake by the developing egg.

This work has provided the first analysis of egg biochemical composition for this species and demonstrated that, whilst broadly comparable to other marine species, there are some unique aspects. Importantly, eggs from F1 stock appeared to be deficient in a number of micronutrients suggesting that broodstock diets should be refined in the species.

1.3. Short-term cold storage and sperm density assessment of lumpsucker

WORK DONE

A total of 17 sexually mature males were sampled from a F1 broodstock held at Otter Ferry Seafish, with holding temperature not exceeding 10°C to ensure good gamete quality, and a low-intensity 24h photoperiod. Sexual maturation initiated at 17 months old, during January 2019, with a mean weight of 638grs.

Testes were dissected out, weighed and gonadosomatic index (GSI) calculated before milt was strained out, volume measured and spermatocrit calculated. A 1:1000 dilution of milt was made using a commercial milt extender and three replicate counts made in a haemocytometer.

For the cold storage experiment, five different milt extender solutions were tested: Modified Turbot Extender (MTE), Herring Ringers Solution (HRS), Mounib's solution, Mounib's 1% BSA inclusion and Spermcoat. Milt was obtained from seven males and 1:5 stock dilution was created for each.

A standardised activation test was performed in triplicate using a dilution of 1 milt:1000 activating solution (seawater +1% BSA). Swimming activity was observed under a microscope and activation time (duration of sperm motility) measured. Activation tests were conducted every seven days until milt was determined as non-activating at 21 days.

OUTCOMES

The males processed displayed a mean GSI of 3.5 ± 1.1% and a mean volume of 5.1 ± 3.6 ml of milt. Sperm concentration was 12.37×109 ± 2.41×109 sperm/ml with an average spermatocrit of 87.8 ± 5.7%. A significant positive relationship between sperm concentration and spermatocrit was observed.

However, no relationship was observed between either sperm concentration or spermatocrit and GSI or volume of milt. Similarly, there was no relationship between GSI and volume of milt recovered.

Following activation, fresh lumpfish spermatozoa remained active for 03:00 ± 00:20 (hh:mm) and no significant difference was found between fresh milt and milt diluted in extenders on the day of stripping.

Following seven days of cold storage, spermatozoa stored in Mounib's solutions were not motile following activation and for the other three extenders (MTE, HRS and Spermcoat) spermatozoa displayed active swimming response. However, activation time decreased significantly: MTE 26.8 %, HRS 50.5 % and Spermcoat 54.4 % reduced. Activation time was higher for milt stored in MTE than Spermcoat.

Following 14 days in the extenders the MTE, HRS and Spermcoat treatments were statistically comparable, with all showing a further significant reduction in activation time.

At 21 days, sperm could not be activated for any of the extender solutions.

Lumpfish milt can therefore be effectively stored using extender solutions for up to two weeks. The most effective storage medium found in this study was MTE at 1:5 milt to extender ratio. Sperm concentration can be estimated confidently directly on fresh milt samples using spermatocrit.

1.4. Impacts of post-ovulatory ageing on egg quality

The applied goal of this research was to provide information regarding the stripping of mature broodstock to minimise the negative impacts of post-ovulatory ageing.

WORK DONE

Eggs from ten wild broodstock females from Norway and F1 Scottish broodstock from Otter Ferry Seafish (low 24h light, <10°C) were compared. Fish started maturing in December 2018 with natural spawning taking place in mid-January 2019. Fish were stripped between January and March 2019 when hydrated oocytes were observed by ultrasound.

Fertilisation assessments at 24h post-fertilisation, eyeing and hatching assessments were also completed. The full materials and methods for all analyses can be found in Poutney et al. 2020.

OUTCOMES

The over-ripening of eggs through mismanagement of broodstock can have a significant detrimental effect on egg quality.

No significant differences between stocks, wild vs F1, in terms of eggs per ml or stripped egg volume were observed in this study. However, batch fecundity was significantly higher (+ 83%) within the wild stocks compared to F1. Likewise, there was significantly lower batch success within the F1 stocks, with only 11% of all batches producing hatched juveniles.

A significant effect of time post-stripping on the quality of lumpfish eggs was observed. Fertilisation rates started at 97.5 ± 0.3% for the wild stocks and 77.6 ± 1.6% for the F1 stocks, decreasing to 18.6 ± 5.8% and 2.9 ± 0.8% at 120h post-stripping for wild and F1 stocks respectively. Within the wild stocks there was no significant difference in fertilisation rate until 48h post-stripping. Within the F1 stocks there was a significant decrease in fertilisation rate at 24h post-stripping.

A similar pattern was observed for eyeing and hatching rates between both stocks.

There appeared to be a significant difference in the performance of the wild and F1 eggs at all time points. However, the rate of degradation followed a similar pattern. This suggests that there is a small window during which these benefits can be lost because of mismanagement of ovulation and stripping.

Intensive management of broodstock with daily assessment is required to identify ovulating individuals for stripping, with a short window (24h) post-ovulation before a significant reduction in quality happens.

The ovarian fluid of wild lumpfish was identified as containing several potential biomarkers for egg quality

and ageing. Future work is needed to clarify the role of some of these proteins.

1.5. Development of genomic tools to assess genetic variability in F1 stocks

This section aimed to develop a panel of microsatellite markers that could be used to assess genetic diversity and parentage and to assess the diversity of current F1-reared lumpfish with respect to wild caught, with a view to informing broodstock establishment.

When producing new F1 stocks of lumpfish, these fish need to be diverse enough to respond to disease challenges and variable environmental conditions.

Lumpfish have a quick generation time (reaching maturity in two years) and for this reason there is potential to not only establishing F1 stocks with a closed lifecycle, but also selecting for traits of commercial interest such as disease resistance and delousing efficacy.

WORK DONE

A microsatellite panel was developed from sequence data and the resulting markers used to assess a snapshot of genetic diversity of a wild-caught broodstock in comparison with other five hatchery stocks.

An enriched microsatellite library was created using ddRAD in accordance with Janson et al. (2017). From this, putative microsatellite markers were identified, and an iterative process of primer design test and optimisation was performed.

Following optimisation, a panel of 16 fluorescently labelled primers were selected and used to amplify genomic DNA. All PCR reactions followed a standard protocol. Results were analysed using Genemarker V. 1.85. Automatic scoring was run based on a pre-designed binning panel. All results were manually verified and adjusted as required. Only samples with at least 12/16 markers amplified were retained for further analysis.

OUTCOMES

The microsatellite markers that were developed demonstrated high variability and can be used to assess genetic diversity, and potentially parentage, should the application be required. The panel designed and tested is cost effective and shows enhanced performance compared to that which has previously been published.

Using this panel to assess stock structure and genetic relatedness demonstrated that, in general, 'discrete production batches' are highly related with low genetic diversity. All F1 stocks demonstrated high levels of relatedness, reduced genetic diversity, and therefore, heightened risk for rapid escalation of inbreeding. The effective population size, assuming random mating, was found to be 69.

If lumpfish are to be F1 bred, the deployed stocks should be from multiple families to maximise genetic diversity and thus genetic robustness of the stocks. It is recommended that broodstock should be genotyped/stripped and crossed with unrelated individuals.

1.6. Screening/mapping of markers associated with traits of commercial interest

To complement the microsatellite panel developed and to make further advances in the development and application of larger volumes, single nucleotide polymorphism (SNP) marker panels were explored. This could enable more advanced selective improvement of the broodstock in key traits such as sex, growth, disease resistance, and delousing behaviour.

WORK DONE

Three different studies were completed:

Study 1 – Pilot study of SNP marker development for gender: tested the application of restriction site associated DNA sequencing analysis (RAD seq) for identification of markers associated with gender as well as geographic origin. A panel of 95 fish was used (40 male & 55 female) from four geographic locations.

Study 2 – Large-scale, family-based study of growth rate in lumpfish: completed a simultaneous rearing of multiple lumpfish families in standardised environmental conditions. This provided a controlled study of phenotypic variation in growth rate both within and between families, and also enabled the desegregation of growth performance between genders. This was set up at the NOFIMA Cleanerfish Unit in Norway. Ten lumpfish families were created from wild-caught adults sourced from Averøy. Each parent was individually phenotyped and materials preserved for genetic analysis before gametes were stripped. At the point of hatch the six best families (highest eyeing and apparent survival rate) were retained. At 1g the best four families were identified (based on survival rate estimates), and 7,000 fish from each family were selected for rearing up to end point. Weight and length of 300 individuals per family were recorded.

Study 3 – Comprehensive QTL analysis of growth rate and gender in lumpfish: leveraged the outputs of the previous studies to perform a high-resolution RADseq study across multiple families. This identified focused qualitative trait loci (QTLs) for an array of growth traits as well as gender and then mapped these QTLs to physical locations of the genome. A panel of 536 individuals was selected to include 125 individuals from each of the four families, and replicated samples of each of the parents used within the study. For each family, 50 individuals from the top 10% in growth performance and 50 individuals from the lower 10% of growth performance were included, plus 25 randomly selected individuals.

OUTCOMES

Study 1: After the removal of low-quality and incomplete reads, a total of 34,695 unique loci were identified that covered 50% of each population with a minimum allele frequency of 0.01. No statistically significant loci could be determined associated with gender. A quality assurance and re-verification programme was run to confirm results. It was concluded at this stage that either there was no discrete genetic regulation of sex in the species or that if such

a location did exist it was masked by the randomising noise of recombination.

Study 2: At the conclusion of the study there was a striking difference in growth performance between and within families, despite being produced on the same day and reared under comparable environmental conditions.

Study 3: High throughput sequencing of these 536 individuals produced over 3 million paired end reads in total. After the removal of low-quality and incomplete reads, 78.9% of the total raw reads were retained. To identify robust genetic markers, these were filtered to show only those with at least two alleles. A total of 10,630 markers were identified and used in subsequent analyses. Individuals were clustered based on their genetic distance, grouping individuals of the same origins together (families). These markers were used to conduct a quantitative trait locus (QTL) analysis for both gender and morphometric ratio association. One single major QTL was identified for sex determination and a total of eight QTLs for weight, standard length, total length and Fulton's condition factor, with some QTLs being shared amongst various characteristics.

The SNP markers defining the QTLs for weight and standard length were further investigated to provide a small subset of markers, fit for a quick SNP assay. This approach produced a robust combined subset of 26 SNP markers, which can be used for a range of broodstock management applications.

This study represented the first comprehensive study of genetic association with traits of commercial interest in lumpfish, generating a discrete panel of SNP markers that have been demonstrated to explain commercially significant variation in growth rate. Uniquely, these markers could be deployed within lumpfish aquaculture to select and generate slower growing families.

> *The value for the aquaculture industry has been a vast array of new knowledge to help decision-making and improve protocols to enhance performance, robustness, welfare and ultimately delousing abilities. It has also supported investment into this sector in the form of cleaner fish staff, new facilities, and a vision for the future.*

2. LARVAL REARING AND JUVENILE GROW-OUT

The large lumpfish larval size allows direct weaning onto formulated feeds. However, it is not clear if past practices for wild-derived larvae are appropriate for F1 derived stocks, which will have a different nutritional history.

Research was undertaken to examine the physiological drivers and underlying genetic factors associated with rapid growth. This was with an aim to optimise rearing environments and handling protocols, alongside feeding strategies to assure larval quality and performance, and optimisation of the juvenile grow-out phase to ensure enhanced productivity, quality and welfare of the farmed stocks.

2.1. Optimised early larval feeds and rearing conditions to enhance growth, survival and robustness

Lumpfish grow extremely fast, meaning production time is a short six months from hatching to deployment. However, lumpfish are the only aquaculture species where one of the aims is to slow down growth. Lumpfish are ready to vaccinate at approximately 8-10g and there is a period of 500DD before the vaccine becomes effective. In this time, lumpfish are still growing exceptionally fast and are sometimes ready to deploy before the vaccine is effective. Also, smaller lumpfish are preferred in the sea cages, as studies have shown that there is a negative relationship between lice grazing and lumpfish size.

There is very little information published on lumpfish growth and very few publications on lumpfish' specific nutritional requirements.

The aim of this trial was to compare the performance of various diets and provide benchmarking nutritional data.

WORK DONE

Three commercially available feeds were provided to triplicate tanks. For the first ten days of the trial, the lumpfish were co-fed Artemia alongside the various diets. Over the course of the final three days of co-feeding, Artemia numbers were lowered and by the end of the ten days they were fully weaned. Feeding regime of the dry diets was based on recommendations from the feed company.

An initial pooled sample was taken from the origin tank before the larvae, 22 days post hatch (DPH), were distributed to the trial tanks. At day 14, 28 and 49, three pools of ten fish were sacrificed from each tank. Larvae were sampled for dry weight and photographed for subsequent digital image analysis of external morphometrics, and mineral and fatty acid analysis. At day 63 the fish had reached the point at which grading is usually performed. Total length of 100 live fish per tank was taken to assess the population size spread and samples taken for subsequent analysis: moisture content, ash content, protein, lipid, fatty acid profile and mineral analysis.

OUTCOMES

Mortality was very low throughout the trial, with the highest cumulative mortality for the whole treatment seen in diet B at 9%. Interestingly, a rise in mortality was seen at around day 24 (46 DPH), which was just under two weeks after the final day of Artemia provision.

Growth profiles (dry weight, wet weight and total length), Specific Growth Rate (SGR) and Thermal Growth Coefficient (TGC) showed a generally consistent trend, with diet A higher than diet C, and diet B not significantly different to either. Lumpfish are unique in that slower growth is preferred and diet B and C, which are the feeds specifically marketed towards lumpfish, succeeded in somewhat restraining growth. This showed a commercially relevant difference in performance of the lumpfish that had been fed the three diets.

2.2. Test and optimise juvenile/adult feed formulations to support growth and normal development

Currently, there is very little basic growth data on lumpfish, making it difficult to understand if they adhere to traditional assumptions in terms of condition factor or growth curves.

K, a function of fish condition, is based on the assumption of isometric growth. Isometric growth in a species dictates that different body parts grow at similar ratios, meaning that the adult shape mirrors that of the juvenile form. Knowing the growth relationship is useful for targeting certain life stages of a species, to ensure the right nutrition at these crucial stages.

The primary aim was to compare the differences in growth performance of lumpfish reared on different commercial feeds in a typical hatchery environment, establishing a benchmark for which macronutrients may be important specifically to lumpfish, and hence supporting later formulation of species-specific feeds.

WORK DONE

This trial was completed at Otter Ferry Seafish. Fish were stocked in 80-litre conical tanks. The system was made up of 12 flow-through tanks, filtered and UV treated. Water temperature varied between 10-14ºC.

Experimental fish (mean weight = 0.89 g ± 0.29, mean total length 28.52 mm ± 3.15) were derived from a single common population. Fish were randomly netted from the holding tank and randomly distributed at 500 fish per tank. During the trial, as they grew, an equal number of randomly selected fish were removed from each tank to maintain the correct stocking density.

The trial tested four different diets: TROFI (AgloNorse 4), Otohime (B2, C1 and C2), BioMar (ProWean 500, 700 and 900), and Skretting (Gemma Micro and Gemma Diamond). All diets were relatively equal in protein content (57-60%); however, the sources of protein differed:

Daily feed was weighed out as a total tank weight percentage, according to the number of fish in each tank and the last weight sampled.

There were five sampling points throughout the experiment including initial and terminal sampling (day 0, 15, 24, 29 and 44). For all sampling points, 90 fish per tank were randomly selected and wet weighed. On the initial sampling (day 0), 18 fish were terminated, as well as six fish per tank on day 44, for x-ray morphological study.

OUTCOMES

Mortality was low for all four treatments (<3%), with no significant difference between feeds over the length of the trial.

There was no significant difference in body weight, length or condition factor between treatments on days 0 and 24. Terminal sampling on day 44 however, showed that the fish fed Otohime (12.28 ±0.3 grs) were significantly heavier than fish fed Gemma (10.19 ± 0.3 grs) or AgloNorse (10.53 ± 0.3 grs), while ProWean (11.07 ± 0.3 grs) was statistically similar to all other treatments. SGR and TGC figures were not found to be significantly different between treatments.

Although Otohime performed the best in terms of growth, all treatments performed well, showing none of the diets to be a limiting factor of growth in this experiment.

After radiography analysis, all treatments showed healthy skeletal growth with minor instances of fusion (0.15%), dorsal/ventral shifts (2.48%) and radio opaque objects being present (0.25%) at the end of the trial.

Otohime feed/faecal matter was extremely sticky and adhered to the tank sides and grill bottom more than any of the other feeds. This caused a build-up of what appeared to be bacteria on the grills of the Otohime tanks. The same was seen with AgloNorse but to a slightly lesser extent. ProWean was the cleanest of the four feeds.

> *This project allowed associated academics to be at the forefront of global cleaner fish research.*

3. PEN MANAGEMENT

The ultimate purpose of producing lumpfish is the effective deployment of robust and healthy fish in salmon cages to control sea lice through natural delousing behaviour.

Lessons have been learned from previous ballan wrasse research that cleaner fish need to be appropriately managed to ensure optimal parasite control. Despite much anecdotal evidence from farms, there was a lack of scientific data on the delousing abilities of lumpfish with respect to daily environmental fluctuations, seasonality, stocking ratio, supplementary feeding and salmon husbandry.

Commercial experience suggested considerable individual and seasonal variation in lumpfish delousing performance, but the drivers of this remained unclear.

3.1. Tank delousing and swimming activity and impact of age, gender, size, domestication and water temperature

The tank model developed by University of Stirling (UoS) staff to test delousing activity in ballan wrasse was adapted for lumpfish to test the impact of season, size and lice coloration on delousing ability.

WORK DONE

Trials were performed at the UoS Marine Environmental Research Laboratory (MERL), Machrihanish, in a flow-through indoor tank system (12 × 750-litre circular tanks), supplied with natural seawater and simulated natural photoperiod (17:7 h light:dark).

Atlantic salmon were subjected to a controlled sea louse copepodid infection, aiming for 10-12 lice per fish. For the lice colouration trial, salmon were infected with normal pigmented and cryptic lice.

When the sea lice had developed into motile adult stages, cleaner fish were introduced into treatment and control tanks. Ten salmon from each tank were randomly sampled before the cleaner fish were introduced, and every 24-48h afterwards for up to eight days, and attached adult sea lice (male and female) counted to estimate delousing rates.

For individual trials, each fish was subjected to a novel object behaviour test before being introduced to the experimental tank. This test involved placing single fish into a static 750L tank and recording their behaviour for five minutes using a video camera. A novel object (Lego bricks) was then added to the tank and the fishes' behaviour recorded for a further five minutes. Videos were processed to track each fish's movements and the tracks were analysed for correlations with their posterior delousing rates. Fish were categorised as bold, intermediate, or shy depending on their closest distance to the novel object during the test: less than 5cm for bold, between 5 and 10cm for intermediate, and over 10cm for shy.

Five trials were done in total, in summer and winter, with mean water temperatures of 14.5°C and 9°C respectively.

OUTCOMES

Lumpfish appear to be effective delousers in both Scottish summer and winter water temperatures. This is contrary to anecdotal industry reports suggesting that lumpfish are ineffective delousers in the summer, although temperature may affect delousing rates.

In the summer they were more effective at delousing female than male lice, with numbers of female lice being less than 50% after 96h. While male lice were removed, the delousing rate was lower than for female lice.

Smaller lumpfish are preferred in the pens, as previous studies have shown that there is a negative relationship between lice grazing and lumpfish size. However, in this study large lumpfish were marginally more effective at delousing than small lumpfish, although there was no significant effect of lumpfish size. However, as these trials were completed at different times of year, further testing is required.

Delousing rates in lumpfish were slower for cryptic lice than for pigmented lice: 48% female lice and 79% male remaining after seven days for cryptic lice vs. 11% female lice and 66% male for pigmented.

In the individual lumpfish trial, two fish showed moderate delousing and one other lumpfish showed very little or no delousing. These results may suggest that not all cleaner fish are effective delousers, with a wide variation of delousing ability. While some fish appear to 'switch on' to lice and are very good delousers, others do not appear to identify sea lice as a food source.

Further research to focus on identifying good delousers and improved stocks to increase the proportion of good delousers has the potential to significantly improve the efficacy of cleaner fish for sea lice management.

3.2. Sea pen swimming behaviour using hydroacoustic 3D tracking and impact of daily and seasonal environmental changes, routine husbandry, supplementary feeding

WORK DONE

A hydroacoustic tracking system was set up at a commercial farm site covering three pens of the eight-pen group. At the time of the study, the site contained ~5kg salmon.

In each pen, 24 lumpfish were tagged and allowed to recover in a keep-net for three days. Signals from the tags were recorded by an array of hydrophones allowing 3D positions in the pen to be calculated every 6-9 seconds for three months, between June and August 2018. Acoustic tags were also attached to the artificial kelp curtain hides to track their tidal movements.

During the tracking period, various characteristics were studied such as general behaviour according to daily and seasonal changes and routine husbandry; hide position – switching hide location to the opposite side of pen, hide depth – hides dropped to 8-13m deep, no hides – hides removed for two days; acoustic deterrent device (ADD) off two weeks and on two weeks, passive grading and bath and/or wellboat treatment.

OUTCOMES

The study was conducted during a summer heatwave with surface temperatures exceeding 16°C and not dropping below 10°C throughout the water column. Lumpfish are known to perform best at lower temperatures and the warm weather during the study was not conducive to good welfare or delousing performance.

Even so, some interesting observations were recorded. Lumpfish were at the corners or pen edges ~80% of the time, often aggregated below their feeders during the daytime, also covering the entire pen during the daytime and retreating to the bottom corners at night (daytime mean depth 6m, night-time mean depth 12m). Their distribution was affected by the strong tidal currents at the site, with increased presence at the down-tide edges of the pens during ebb and flood tides.

Several Thermolicer treatments were carried out during the ADD trial, which meant that it was not possible to draw any conclusions on the effect of ADD noise on behaviour.

Few tagged fish remained at the time of the treatment, so it was not possible to draw any conclusions from this data on the effect of the Thermolicer and bath treatment, and further testing would be required in the future.

4. HEALTH MANAGEMENT

There are numerous health challenges associated with successful and best practice in production and deployment of lumpfish, and reports showed that lumpfish are susceptible to a wide range of bacterial diseases.

WORK DONE

Research in this project was prioritised to pathogens that are associated with clinical losses, for example amoebic gill disease, Vibrio-like and Pasteurella-like bacteria, as well as atypical Aeromonas salmonicida.

Although Moritella viscosa, Pasteurella spp. and Atypical A. salmonicida were initially identified as the main pathogen species to focus on, Pasteurella spp. was ultimately decided as the main pathogen of concern for the Scottish sector at the time of this study.

Isolates of Pasteurella spp. were obtained from Norway in collaboration with the Norwegian Veterinary Institute, as well as from Scotland. This material would provide the basis for understanding the homogeneity/heterogeneity between Pasteurella spp. in Norway and Scotland, to determine the feasibility of cross-strain protective vaccine development.

Difficulties were encountered when identifying/characterising isolates based on conventional biochemical tests, probably due to the required supplementation of media with blood/serum. Therefore, proteomic and genomic tools were applied for conducting comparative experiments.

ELISA plates were used to detect lumpfish antibodies post-vaccination (>700 DD) pre-deployment.

OUTCOMES

Results showed that lumpfish do not appear to be producing strong antibody responses post-vaccination, prior to deployment. Only one out of 20 vaccinated fish produced antibodies 740 DD post-vaccination, which suggests that the fish may be beginning to seroconvert and that perhaps fish would benefit from an additional period at the hatchery.

However, further investigations on the lumpfish antibody response to other antigens in the vaccine would be useful to determine if the slow response is specifically to the Pasteurella spp. component in the vaccine, or if antigenic competition has impeded the antibody response.

IMPACT

The project has been very successful, as demonstrated by the new knowledge gained, tools and protocols developed and implemented in the industry, number of students trained, and extensive dissemination activities.

For the academic partners, the value of the project outcomes is mainly associated with the genomic tools with a multiplex microsatellite panel and SNP markers, immune tools with innate and adaptive immune markers and assays, and behavioural tools with the validation and implementation of hydroacoustic technologies.

This project allowed associated academics to be at the forefront of global cleaner fish research. In addition, cleaner fish research featured as one of the three case impact studies submitted by the Institute of Aquaculture to the Research Excellence Framework in 2020. Finally, the project has been a very good platform for training research students. One PhD and seven BSc/MSc students have been involved between 2016 and 2020.

The value for the aquaculture industry has been a vast array of new knowledge to help decision-making and improve protocols to enhance performance, robustness, welfare and ultimately delousing abilities. It has also supported investment into this sector in the form of cleaner fish staff, new facilities, and a vision for the future.

New products have been developed and/or refined because of the project outcomes (and other projects running at the same time in Norway) including specific feeds for the full production cycle of the species, the identification of best milt extenders, and understanding of hydrophones and analysis software capabilities.

Large investments have also been made by the project's industrial partners. Mowi invested in new facilities in North Wales (Ocean Matters Ltd) while recruiting many staff to oversee cleaner fish production, transportation, deployment and management. The same is true for The Scottish Salmon Company and Otter Ferry Seafish Ltd.

Further research is needed to focus on identifying, promoting and selecting good delousers. Improvement of delousing has the potential to significantly enhance the efficacy of cleaner fish for sea lice management while reducing numbers required. Likewise, further work on potential biomarkers for egg quality and ageing would greatly help the success of hatcheries. Finally, health and welfare of lumpfish is a priority for future research.

ADDITIONAL INFORMATION

BSC THESIS DURING THE PROJECT

Tahir Z., (2019). Assessment of the Efficacy and Application of Novel Microsatellite Markers in Lumpfish (*Cyclopterus lumpus*). BSc Thesis, University of Stirling.

Irvine G., (2017). Developing polymorphic molecular markers for lumpsuckers (*Cyclopterus lumpus* l. 1758). BSc thesis, University of Stirling.

MSC. THESES DURING THE PROJECT

Smeaton L., (2019). Examining immune response in lumpsucker, *Cyclopterus lumpus*, to inform on the protective potential of immunostimulants and vaccines for the cleaner fish industry. MSc thesis, University of Stirling.

Kessack L., (2019). Discrimination learning and its application in lumpfish (*Cyclopterus lumpus*) delousing behaviour. MSc Thesis, University of Stirling.

Chéidigh A., (2018). Weaning larval lumpfish (*Cyclopterus lumpus*) on three commercial diets MSc Thesis, University of Stirling.

Keung C., (2018). Behavioural responses of lumpfish (*Cyclopterus lumpus*) to changes in farm husbandry in commercial salmon net pens. MSc Thesis, University of Stirling.

Lambden B., (2016). Growth performance of juvenile lumpfish, *Cyclopterus lumpus*, fed four different commercially available diets. MSc Thesis, University of Stirling.

PHD THESES DURING THE PROJECT

Pountney S., (2021). Research into lumpfish (*Cyclopterus lumpus*) brood stock management and gamete quality. PhD Thesis, University of Stirling.

SCIENTIFIC ARTICLES

Pountney S.M., Lein I., Counter Selly S-L, Migaud H., Davie A. (2022). Comparative proximate analysis of wild and captive lumpfish (*Cyclopterus lumpus*) eggs show deficiencies in captive eggs and possible egg quality determinants. Aquaculture (in press).

Pountney S.M., Migaud H., & Davie A., (2020). Short term cold storage and sperm density assessment of lumpfish (*Cyclopterus lumpus*) milt. Aquaculture 529:735646.

Pountney S.M., Lein I., Migaud H., & Davie A. (2020). The influence of culture temperature on spawning productivity and gamete quality in captive reared Lumpfish (*Cyclopterus lumpus*) Aquaculture. 522:735121.

Pountney S.M., Lein I., Migaud H. & Davie A., (2020). High temperature is detrimental to captive lumpfish (*Cyclopterus lumpus*, L) reproductive performance. Aquaculture 522, 73512.

Brooker A.J., Davie A., Leclercq E., Zerafa B., & 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, 734771.

Brooker A.J., Papadopoulou A., Gutiérrez C., Planellas S.R., Davie A., & Migaud H., (2018). Sustainable production and use of cleaner fish for the biological control of sea lice: recent advances and current challenges. Vet Records 183.

Leclercq E., Zerafa B., Brooker A.J., Davie A., & Migaud H., (2018). Application of passiveacoustic telemetry to explore the behaviour of ballan wrasse (Labrus bergylta) and Lumpfish (*Cyclopterus lumpus*) in commercial Scottish salmon sea-pens Aquaculture 495: 1-12.

NEWS ARTICLES

Lumpfish projects net nearly £3m - FishFarmingExpert.com

Lumpsucker PhD opportunity - FishFarmingExpert.com

Lumpfish research puts student in the spotlight - FishFarmingExpert.com

2.9million Invested into Scottish Cleaner-Fish Projects | The Fish Site

Backing for Research into Projects to Enhance Sustainable Aquaculture | The Fish Site

'Cleaner fish' projects get £2.9m funding boost | The Scotsman

Scotland's sea lice control takes another step forward (seafoodsource.com)

Projects developed to boost cleaner-fish use in Scottish aquaculture (fishbio.com)