# IMPROVING CLEANER FISH VACCINATION METHODOLOGY AND TECHNOLOGY

#### PARTNERS

Aqualife Services | Scottish Sea Farms | The Institute of Aquaculture at the University of Stirling

#### **PROJECT LEADS**

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### BACKGROUND

One of the main obstacles to the expansion of sea-pen based salmon production is sea lice; an ectoparasite that feeds on the mucus, tissue and blood of salmonid hosts causing skin lesions, secondary infections, reduced feed conversion ratios and mortality in severe cases. The economic effects of sea lice are significant, costing the global salmon sector an estimated £300-500m per year in mitigation efforts and direct losses associated with lice infections. Deployment of cleaner fish alongside salmon as a biological control of sea lice is an important part of integrated pest management strategies for the Scottish aquaculture sector.

The two main cleaner fish species used, the ballan wrasse and the lumpfish, are introduced into salmon pens to naturally graze on lice. The majority of cleaner fish deployed by the sector are reared in marine hatchery facilities, and like salmon, can be vulnerable to viral and bacterial diseases. Therefore, cleaner fish are vaccinated against these pathogens to protect them and to reduce the risk of transferring pathogens to salmon. Current vaccination of lumpfish uses autogenous vaccines prepared from bacterial isolates derived directly from the population to be vaccinated. Salmonid vaccination protocols have been adapted for lumpfish, however, are not appropriate due to the unique characteristics of the species, for example, low tolerance to anaesthetics causing the need to vaccinate without anaesthesia. Furthermore, lumpfish have adapted pelvic fins allowing them to adhere to rocks or kelp like a suction cup and, in the absence of anaesthesia, lumpfish stick to flat surfaces of the vaccination table which can lead to injury during the process of vaccination.

Improving vaccination practices and technologies in lumpfish rearing will lead to better health and welfare in hatchery reared lumpfish, increased production of lumpfish for delousing and improved sea lice management in the marine finfish sector.

This project brought together Aqualife Services; global leaders in fish vaccination technology and services, the University of Stirling's Institute of Aquaculture; with research team lead by Professor Jimmy Turnbull, and Scottish Sea Farms; one of Scotland's leading salmon producers, to design and validate an enhanced system for vaccinating lumpfish.

### AIMS

The overarching aim of this project was to assess the current methods of lumpfish vaccination, in terms of efficacy, human safety, and fish welfare. Through this, lumpfish specific vaccination and welfare assessment protocols was investigated, and the effectiveness of the Aqualife Lumpfish Vaccination System (ALVaS) evaluated.

### LUMPFISH BEHAVIOUR AND WELFARE INDICATORS

Maintaining optimal fish welfare, in terms of the health and overall condition, is an important factor for any farmed species. A baseline understanding of the behavioural indicators of welfare in lumpfish is required to assess the effects of current vaccination methodologies and to further optimise these practices.

The particularly unique anatomy and physiology of lumpfish, including their pelvic suction cup and low tolerance to anaesthetic, means that current methods and technology for vaccination may be harmful and need to be optimised to account for these factors.

Improvements in protocols lumpfish vaccination will help maintain the health of deployed fish and so lead to improved sea lice control in Scottish aquaculture. Industry demand for cleaner fish is high; protecting their health improves their welfare, allows them to work effectively and improves sustainability by reducing mortalities.

### Work done

The welfare of lumpfish was assessed on commercial sites and in pilot studies at aquarium facilities at both Stirling and Herriot Watt universities.

A range of fish based operational welfare indicators were examined through observation, analysis of video and clinical examinations including time to return to feeding, physical lesions, blood plasma cortisol assays and mortalities.

A summary of behaviour was constructed from observations of normal fish and feeding response was assessed and used as an operational behavioural test (OBT) for recovery after the stressful tagging procedures.

#### **Outcomes**

Through investigation of lumpfish behavioural qualities and considering a range of welfare indicators it was found that lumpfish are relatively responsive, compared to salmonids, and rapidly learn to anticipate feeding when people approach the tank.

Feeding response was selected as an appropriate indicator of welfare for lumpfish as they recovered from vaccination. This was combined with colour of fish, level of activity and clinical lesions to assess welfare.

These welfare indicators identified were then used as a baseline to assess the impacts of vaccination systems and methods in the trials to follow.

### DESIGN AND OPTIMISATION OF THE AQUALIFE LUMPFISH VACCINATION SYSTEM (ALVAS)

Existing protocols for vaccinating salmon roughly include anesthetising and netting fish onto a moist stainless-steel table, hand administering the vaccine into the abdomen with a calibrated needle gun and recovering fish in a holding tank. This conventional vaccination system is not appropriate for lumpfish as accepted practice does not involve anaesthetising the fish. Therefore, lumpfish often stick to the surface of the table and must be forcibly removed, potentially causing stress or damage to fish.

### Work done

A bespoke vaccination system, ALVaS (Aqualife Lumpfish Vaccination System), was developed and optimised to enhance lumpfish welfare during vaccination procedures.

ALVaS has a flooded table, with constant water flow and the chute to the recovery tank. The surface of the table and the chutes are textured to avoid the lumpfish sticking to the table.

Lumpfish vaccination protocols were developed, including calibration of needle depth for vaccine administration, to enhance fish welfare outcomes.

Previously identified health and welfare indicators were to assess the efficacy of the improved vaccination system to traditional vaccination procedures.

### **Outcomes**

The ALVaS system was developed, key features included a flooded work surface so unanaesthetised lumpfish can continue their normal behaviours and a textured surface that prevented the lumpfish from adhering to the table's surfaces (Figure 1).

Fish vaccination standard operating procedures adapted specifically to lumpfish were created. The protocols included: preparation, fish checks, vaccination procedures, health and safety, fish welfare and guidance for post vaccination audits.



Figure 1. Specially trained personnel carrying out lumpfish vaccinations using the Aqualife Lumpfish Vaccination System (ALVaS).

### EXPERIMENTAL VACCINE TRIAL OF THE NOVEL VACCINATION SYSTEM AND BACTERIAL CHALLENGE

Although anecdotal evidence suggested ALVaS was better for lumpfish than conventional salmonid vaccination methods it was necessary to objectively verify its value in terms of the welfare and health of the lumpfish, including a bacterial challenge to assess response to the vaccine.

### Work done

ALVaS was tested against a conventional vaccine table in a trial representing commercial conditions. Fish were vaccinated with an autogenous vaccine produced against lumpfish bacterial pathogen (\*see section on bacteriology). Thirty fish were vaccinated on the conventional wet table, thirty with ALVaS and six were left unvaccinated as controls.

Due to logistical issues and tank location, ALVaS vaccinated fish were exposed to far more handling, i.e. netting and transport via bucket, than conventional and control fish.

Feeding response post-vaccination was recorded to indicate the rate of recovery from vaccination.

All fish (vaccinated and non-vaccinated groups) were challenged with a bacterial pathogen and monitored daily for behaviour, clinical appearance and mortality. Post-mortem bacteriology and histology samples were taken to confirm cause of death.

#### **Outcomes**

Lumpfish were successfully vaccinated with an autogenous vaccine using the ALVaS and the conventional vaccine system.

The rate of return to feeding was similar in the ALVaS and conventional vaccination methods, all lumpfish had normal feeding behaviour two days after vaccination.

Fish mortality was significantly lower after bacterial challenge in lumpfish vaccinated with the ALVaS (10%) when compared to the conventional vaccination system (43%) and controls, Figure 2.

The improved protection in those fish vaccinated with the ALVaS system was greater than anticipated. Although the study was not able to investigate the low vaccine efficacy in the conventional table group, both groups of fish received the same dose and type of vaccine, it is possible the stress associated with vaccination in the conventional system reduced the response of the fish to the vaccine.



Figure 2. Cumulative percentage mortalities in the three treatment groups following bacterial challenge.

### BACTERIOLOGY

Microbial research in this study aimed to confirm the identity of bacteria associated with disease in lumpfish and to ensure the bacterial challenges were appropriate to the vaccine tested. It was not the aim of this study to evaluate the efficacy of the commercially available vaccines, but instead to determine effectiveness of alternative vaccine administration methods. As such, it was always planned to use a single autogenous vaccine (a vaccine made from bacterial isolates derived from the population to be vaccinated) against one bacterial pathogen for vaccination and challenge studies. This allowed the vaccination method to be investigated in a robust manner. The vaccine used for the challenge study was provided from HIPRA, Spain using one of the Aliivibrio salmonicida strains recovered from the infected Lumpfish at Stirling.

### **Work Done**

Lumpfish transferred from Otter Ferry Seafish originally allocated for the vaccine trial, presented with external lesions, were unresponsive to treatment and ultimately had to be culled shortly after arrival to the Institute of Aquaculture aquarium at the University of Stirling.

Bacteriology samples were taken from moribund and dead lumpfish presenting with ulcerative skin and fin lesions. Large numbers of bacterial colonies were observed in the lesions and these bacteria were found to be invading the underlying musculature.

Bacteriology isolates were identified to select a candidate bacterial species for the vaccine challenge.

Additional work was performed on the antibiotic sensitivity profiles of bacterial strains to assist with disease control strategies. This work was in addition to the original project outputs but considered to be of value given the diversity of bacterial species involved in infectious outbreaks in cleaner fish.

#### **Outcomes**

A total of 14 bacterial isolates were recovered from naturally infected Lumpsucker fish stocks, including *Aliivibrio salmonicida, Vibrio tapetis, Vibrio gallaecicus* and some unidentified Vibrio species. All of these have been shown to be aetiological agents in cleaner fish disease or recovered from moribund cleaner fish species.

*Aliivibrio salmonicida* was selected as the candidate strain for the vaccine challenge as it was present in 7 out of the 14 isolates.

Resistance was found to two antibiotics, ampicillin and amoxycillin. The authors recommended these are not used to treat against disease caused by vibrio species. Following this, antibiotic use to aid recovery after vaccination was re-evaluated at lumpfish hatcheries.

### **IMPACT**

This project successfully achieved its goals of improving lumpfish health, welfare and vaccination protocols while also developing a new and lasting partnership between industry and academic partners. Outcomes developed as a result of this project include:

- Aqualife Lumpfish Vaccination System (ALVaS); a vaccination table system tailored to the specific biological requirements of lumpfish to optimise health and welfare during vaccination.
- A standard operating procedure for vaccination of lumpfish using ALVaS.
- Identification of suitable parameters for assessment of lumpfish health and welfare.
- Novel findings on the bacteria associated with disease outbreaks in lumpfish and their antibiotic sensitivity.

Project lead, Aqualife Services, were very pleased with results and opted not to protect the IP so that learnings from ALVaS could rapidly disseminated to improve vaccination practices and welfare of lumpfish, both in Scotland and internationally. Aqualife now offer vaccination services to all lumpfish producers across the UK, Ireland and Norway.

Speaking on collaboration and industry value of the project, Kathy Taylor at Aqualife said: "The development of the ALVaS system, along with SAIC and other industry partners has been of huge benefit for both Aqualife Services ltd and our customers. The improvements in welfare for the fish and efficiency and quality of the vaccination services has led to increased business opportunities for Aqualife Services. It has helped Aqualife to develop long-term working relationships with all the major lumpfish producers in the UK and provided year-round employment for our specialist lumpfish vaccinators".

Project developments and outputs have contributed to activity across the supply chain with RM MacDonald, a metal working firm based in Inverness, collaborating on the development of the ALVaS system. Lumpfish producers then go on to purchase their own tables for vaccination teams to use when they arrive on-site.

Speaking from the producer's perspective, Daniel Phillips, Cleanerfish Production Manager at Ocean Matters, a subsidiary of Mowi producing approximately 3,000,000 lumpfish per year, said: "The ALVas is a good system for vaccinating lumpfish, it has good welfare at the heart of its operation with fish being maintained in constantly refreshing water throughout the vaccination process. The checker-plate base prevents fish adhering and being damaged whilst the channels allow a swift entry to the receiving tank. Overall, this makes vaccination efficient and safe for the lumpfish."

The findings surrounding bacterial characterisation and antibiotic sensitivity profiles were added value for the project. These were investigated to better understand the bacterial pathogenesis and response to antibiotic treatments. As a result of these findings, recommendations were given for treatment of disease caused by vibrio species and, following this, antibiotic use to aid recovery after vaccination was re-evaluated at lumpfish hatcheries.

Teams at University of Stirling valued the opportunity to conduct research with impact and further develop academic-commercial partnerships. Reflecting on participation, Prof. Jimmy Turnbull said: "Although this was a relatively small project, it produced some useful practical results. We were all quite surprised and delighted to see how much benefit was demonstrable in the fish vaccinated with the new system."

# **ADDITIONAL INFORMATION**

Data on the bacterial recovery, identification and antibiotic sensitivity patterns was presented at the European Association of Fish Pathologists, UK conference "Aquatic animal health in a changing world" (University of Stirling, September 2016).

# **MEDIA**

https://www.scotsman.com/country-and-farming/new-fish-vaccination-research-gets-cash-injection-1498533

The ALVaS system in use: <u>https://www.youtube.com/</u> watch?v=kthzZkWFCI0