Home Office

NON-TECHNICAL SUMMARY

Investigating the genetic basis of salmonid resistance to sea lice

Project duration

1 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (d) Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

Sea lice, Atlantic and coho salmon, Disease resistance, Improved welfare, Sustainable food production

Animal types	Life stages
Salmon (Salmo salar)	Juvenile, Adult
coho salmon (Oncorhynchus kisutch)	Juvenile, Adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to investigate the genetic basis of resistance in salmon to parasitism by sea lice. The project will identify genes and mutations affecting resistance to support the development of sea lice resistance in farmed Atlantic salmon.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Food from water is a critical element to satisfy the protein requirements of a growing global population. As returns from fishing for wild fish in open waters has largely remained constant since 1990 aquatic food production is moving from wild capture to sustainable farming. Aquaculture’s contribution to the supply of fish for human consumption exceeded that of wild-caught fish for the first time in 2014. To meet the continually growing global demand, it is estimated that aquaculture production will need to further double in the period to 2050.

Despite progress aquaculture still faces many problems. Infectious diseases of viral, bacterial, protozoan, and parasitic origins are the most significant constraint, causing lost production, increased costs, wasted resources, major animal welfare problems and concern for transmission to wild stocks. Atlantic salmon aquaculture is worth approximately £1 billion per annum to the UK economy (at first sale) and is a major source of employment in rural communities of the Scottish Highlands. While the Scottish Government and the aquaculture industry has ambitious plans for sustainable growth, infectious diseases constrain this expansion.

Sea lice (particularly *Lepeoptheirus salmonis* in the Northern Hemisphere and *Caligus rogercresseyi* in the Southern Hemisphere) are a significant, perpetual problem for salmon aquaculture globally. These copepod parasites attach to the skin of salmonid fish and feed on mucus, live tissue, and blood. Parasitised fish show impaired growth and increased occurrence of secondary infections. Furthermore, potential transmission of sea lice to endangered wild salmonids is a key issue motivating regulators to adopt more precautionary policies to aquaculture, constraining development. In addition to a significant negative impact on salmonid health and welfare and public perception, lice prevention and treatment cost the global industry about £800 million per year.

A diversity of control strategies including area management plans, reducing the duration of the marine phase of salmon production, feed supplements, cleaner fish, mechanical removal (by water jet, brush, laser), bathing treatments (freshwater, high temperature), and tailored cage design exist or are being developed. These multifaceted strategies are only partially effective and can cause additional welfare issues. Veterinary medicines, which are expensive and potentially environmentally damaging, are still frequently required to control sea lice, however resistance to common delousing drugs and adverse welfare during treatments are constant concerns. Therefore, despite extensive control efforts, sea lice remain a major hindrance to sustainable aquaculture, including negative effects on public opinion of the industry.

A wide diversity in response to infectious disease is a well-known phenomenon both between, and within species. Some host species such as Atlantic salmon are highly susceptible to parasitisation, while others (e.g., coho salmon) are highly resistant. Improving the innate genetic resistance of aquacultured salmon to lice is a promising, environmental and welfare friendly, yet underexploited approach to control.

Traditional controlled breeding programs for key production species have enabled enhancement of key traits, including disease resistance over the long term. In the last two decades advances in genome sequencing allowing cost effective individual and family typing have enabled breeding programs to enhance and speed up this process by using marker assisted selection. The identification of a marker associated with resistance to infectious pancreatic necrosis virus (IPNV) in salmon and its incorporation into breeding programs is a good example of this resulting in major reduction in incidences of IPN disease globally and associated massive welfare improvement. Understanding the mechanisms and genes underpinning the observed variation in resistance or susceptibility to diseases is now key to enabling and implementing more specific and even more rapid future control strategies based on selective breeding.

Future selective breeding programs based on gene associated selection need to be informed by small scale empirical studies. Studies such as those proposed within this licence have the potential to support a sustainable increased production of seafood with immense welfare improvements (due to the large numbers of individual animals farmed) and reduced chemical use and environmental impacts.

What outputs do you think you will see at the end of this project?

The immediate outputs will include fundamental knowledge on the mechanisms of genetic resistance to sea lice parasitism which will be disseminated via high impact publications and international symposia. Important outcomes also include future targets for selective breeding programs and novel strategies to combat sea lice impacting on aquaculture, so industry focused publications, meetings and conferences will also be targets for dissemination.

Who or what will benefit from these outputs, and how?

In the short-term the data will improve understanding of the mechanisms behind increased host resistance to sea lice and academia will benefit from empirical data driven publications. In the short to medium term the aquaculture industry will benefit from knowledge on future relevant targets to include in traditional selective breeding programs enhancing resistance to sea lice in Atlantic salmon.

Beyond the duration of this licence in the medium to long term the outputs benefit rural economies engaged in aquaculture at the national and international levels offering reduced production losses, improved public perception of aquaculture, food security, employment, and associated benefits. Previous work on identifying markers associated with resistance to infectious pancreatic necrosis virus in Atlantic salmon (now used in traditional selective breeding strategies) is estimated to have a value of approximately £26 million per annum to the UK industry alone. As breeding programs are introduced for new targets identified under this licence and become effective there will be further massive welfare improvements for both future farmed fish experiencing reduced impact of disease and added benefit for wild salmonids by reducing parasitism pressure from aquaculture.

How will you look to maximise the outputs of this work?

The work is collaborative across academic and industry partners, as such dissemination will be wide and active via all partners in the project. High impact publications as well as informative workshops and solutions for industry to take forward into breeding programs will be disseminated.

Species and numbers of animals expected to be used

- Salmon (Salmo salar): 1000
- Other fish:
    - coho salmon (Oncorhynchus kisutch): 1000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The fish used are the appropriate species for the investigation, i.e., the relevant farmed species and natural host (Atlantic salmon) and a naturally resistant but non-native, non-farmed species (Pacific, coho salmon).

Coho salmon have been brought into the research facility as eyed eggs under special dispensation from stock enhancement programs in Canada. Juvenile Atlantic salmon of defined family makeup with predicted differing susceptibility will be provided by industry partners. Both species will be held as stock animals and reared until the post-smolt seawater stage when lice exposure will occur. We will also be using juvenile stages of *Lepeoptheirus salmonis* parasitic sea lice obtained from an experimental aquarium facility on the Atlantic coast of Scotland. Copepodid lice are crustacea. Decapod crustacea (class Malacostraca) are now legally recognised as sentient in the UK and elsewhere. Although sea lice are a related class (Copepoda) within the superclass multicrustacea, and may meet some of the criteria around awareness, they are not yet recognised as sentient.

Typically, what will be done to an animal used in your project?

Lice eggs strings will be collected from parasitised salmon, transported to the experimental aquaria held in static aquaria with regular seawater exchange, hatched to infectious copepodid stage, enumerated and added to tanks containing salmon in seawater.

Both species of fish in seawater will be exposed to lice copepodids in controlled disease challenges by immersion followed by sampling at defined timepoints. As it is known that the differential resistance manifests (by rejection of lice) in the first few days after attachment challenges will be held for a short period only (7 days maximum). Fish will be terminated humanely one tank of each species at a time, across up to nine timepoints by a Schedule 1 or an approved non-Schedule 1 method. Fish will experience a single exposure procedure.

What are the expected impacts and/or adverse effects for the animals during your project?

Initial settlement of copepodids can elicit flashing and jumping, indicating irritation, but behaviour usually returns to normal within 1 week. Sea lice challenges will usually be conducted by lowering the water level and adding infective copepodids. Following parasitism with lice, most fish show slight skin damage. This is most often on the dorsal midline, immediately posterior to the dorsal fin and is characterised by a white/grey opacity and thickening of the epidermis with occasional pinpoint haemorrhages. In heavier infestations, lice may induce more extensive and marked damaged to the epidermis, characterised by exposure of the dark pink underlying dermis. This is normally only after the lice have developed to mobile pre-adult and adult stages. In such cases this would be categorised as acute moderate harm and the fish would be killed immediately using a schedule 1 method. This study is short term and will complete well before lice reach the pre-adult stage.

The actual adverse effects are managed by defined humane endpoints implemented by regular appropriate monitoring which involves both direct visual checks and desktop observations of live videos from in-tank underwater cameras. Actual severity experienced will be mitigated by timely removal and humane termination if necessary.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected severity is mild and the maximum severity is moderate.

As this experiment will assess host response to lice attachment in the early stages after attachment, up to a maximum of 7 days, the lice will not develop into mobile pre-adults or adults which are the stages that are most damaging to the fish and when epidermal damage and dermal exposure might be observed. The most likely severity fish will experience is mild, transient irritation.

What will happen to animals used in this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The attachment site of the salmon skin comprises multiple tissues and cell types, and differential resistance may be due to actions of specific cell populations. Susceptibility and response to parasitism thus require complex interrelated metabolic, anatomical and immunological mechanisms that cannot currently be fully adequately modelled except in a protected whole animal.

During the project we will also use a small number of salmon to establish *in vitro* primary cell cultures from skin to investigate and corroborate potential target genes identified in the comparative disease challenge and subsequent host response analysis. This will become a future resource that will be used in future experiments under a different project licence using gene editing to test empirically the impact of these gene targets. However, data derived from *in vitro* studies are not typically entirely accurately reflective of the whole animal response.

The fish used are the appropriate species for the investigation, i.e. the relevant farmed species and natural host (Atlantic salmon) and a naturally resistant but non-native, non-farmed species (Pacific, coho salmon).

Which non-animal alternatives did you consider for use in this project?

Computer based (in silico) models and cell culture based (*in vitro)* models will be used in the project to inform choice of gene targets for future investigation. Analysis of production data, genomic data from earlier experiments, and mining of existing data from online databases as well as the transcriptome data from the current project will be used to inform candidate resistance genes and mutations. High priority targets identified from transcriptome analysis of the comparative host responses will be tested in cell culture models: this will include genome editing of target loci in the cells, enrichment for edited cells and exposure to parasite secreted protein. Future projects under a different licence envisage empirical testing of target resistance genes by genome editing of salmon embryos (*in vivo)*. Measures of cell survival in the cultures developed in this project will inform the likelihood of a gene edit having an effect on resistance in future *in vivo* experiments. These cell cultures, of which there are relatively few compared to terrestrial animal and human models, are necessarily derived from relevant fish species.

Why were they not suitable?

Cell cultures derived from humans or terrestrial animals are not appropriate for fish disease research as in most cases the pathogens of cold blooded fish do not replicate at the temperatures terrestrial animal cell culture models are held. The immune system of invertebrates differs considerably from that of fish, preventing direct comparisons in pathogen challenge with invertebrate models. Fish cell culture models are informative to a certain degree but are not solely sufficient since infection barriers and the immune system have many different elements and span various organs and tissues. Thus, whole animal models remain essential to this research area.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have estimated numbers based on number of experimental units, accounting for replication, husbandry requirements (particularly stocking density) for both species and contingency to ensure sufficient fish at the seawater stage for disease challenge.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We and associated project partners utilise professional statisticians, the statistical software R and, when relevant, packages for power analysis to ensure animal experiments have adequate statistical power to detect at least medium sized effects in the assessment of lice attachment and comparative host parasite expression analysis. We refer to acknowledged tools for best practice such as PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) and fish specific guidelines (NORECOPA) and the Experimental Design Assistant (EDA) for design of experiments, replication will be used, allocation to tanks will be randomised, and sample analysis will be blinded to increase robustness and repeatability ensuring resilience. We use ARRIVE guidelines for eventual reporting. This approach is intended maximise likelihood of a successful challenge experiment providing appropriate results and data and minimise requirement for experiments to be repeated.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

All animal research conducted at the laboratory is undertaken using approaches consistent with defined Good Laboratory Practice regulations. The high-quality standards provide assurance that very few studies need verification or repeat. The number of animals required for each study is calculated in conjunction with our Statistical Services Group. This ensures that the minimum number of fish are used to provide robust data. If sufficient baseline data is available the use of control animals may not be required, to reduce animal usage. Stocking densities and population size remain a necessary consideration to ensure expression of normal feeding and social (e.g. schooling) behaviour and to minimise anti-social (aggressive) behaviour. We continually review and improve infrastructure in the experimental facility to enable further reduction of animal numbers within experiments. Examples include additional sizes of tank and systems to achieve variable depth control in tanks allowing reduced numbers to remain at appropriate stocking densities thus fewer fish required for studies. A detailed formal study plan is prepared for each study undertaken, and contains study specific objectives and justifications for use, severity of expected adverse effects and the number of fish

required. Study plans are reviewed by the local Animal Welfare and Ethical Review Body (AWERB), fish husbandry experts and the Statistical Services Group, ensuring that all the 3Rs have been considered and animal use is reduced to the minimum required to achieve the aims of the study.

The use of effectively specific pathogen free fish by rearing from eggs in the bio-secure experimental facility, means numbers for negative controls can be kept to an effective minimum. Project partners have longstanding expertise in lice challenge models. Tissues from sacrificed fish will be shared across multiple partners in the wider research program, including linked projects not directly funded by the grant awarded for this work. A small pre-study using a small number of fish from each group will be performed to confirm effective transfer of lice challenge skills between project partner establishments and inform lice numbers for the main challenge.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The model to be used is parasite challenge by immersion in water. Salmon are raised according to each species preference across each life stage, the specifications of which are obtained from review of relevant literature and expert opinion and detailed in species specific husbandry cards. Features include (not exhaustive) temperature, salinity, pH, dissolved oxygen, excretory product levels, hygiene, light intensity and photoperiod, stocking density, nutrient requirements, recommended feed types, growth rates, and environmental enrichment (e.g., water movement).

Salmon in seawater will be exposed to sea lice by addition of a defined numbers of copepodids per fish into replicate experimental tanks containing up to 45 post smolts. These will be maintained for up to a maximum of 7 days post exposure. Copepodid challenges will be performed on predicted high resistance Atlantic salmon, predicted low resistance Atlantic salmon, and resistant coho salmon. Demonstrated consistency in fish holding conditions across study groups and appropriate replicates by precise control of environmental parameters will ensure repeatability of the challenges with minimal variation.

Skin and attached live parasites will be sampled from fish in each group per time point, with counts of attached lice and fin clip samples taken. The lice count data will be used to benchmark the resistance phenotype of the Atlantic salmon families and inform on precisely when lice are rejected by resistant groups of fish. Atlantic and coho salmon post-smolts will be kept separate under appropriate husbandry conditions. Each tank will have a predefined sampling timepoint for termination to avoid repeat disturbance of fish during the challenge. Because the study focuses on the early time post attachment and holding is for a short period only, the lice will not mature to pre-adult or adult stages and thus the potential epidermal damage and harm experienced by the fish will be limited.

Why can’t you use animals that are less sentient?

Sea lice naturally exist in marine waters and parasitise the marine grow out stage of the salmon life cycle. Sea lice do not survive in freshwater hence the early life stages of salmon are not appropriate. The study is an in-depth analysis of host parasite interaction in the first days after attachment. It is not pragmatic or sufficiently representative to maintain large numbers of fish in many tanks under anaesthesia for this duration.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Routine monitoring for salmonids is three times daily whilst feed is offered manually. This provides the ability to check the fish as well as assess feeding behaviours ensuring all fish get equal access. Checking will be increased immediately post exposure to lice and throughout the study if signs of epidermal damage are observed. Each individual is checked against agreed criteria and observation recorded on score sheets. Visual checks will be supported by additional video surveillance.

Because behavioural changes associated with irritation such as flashing and jumping are expected transiently after attachment of lice, tanks will be netted and will contain minimal furniture to reduce possibility of physical damage to fish as a result of this behaviour.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We utilise the PREPARE and fish specific guidelines (NORECOPA) and the Experimental Design Assistant for experimental design. We use ASPA codes of practice, Guidelines for the use of Fishes in Research (American Fisheries Society), Guidelines on the care and use of fish in research, teaching and testing (Canadian Council on Animal Care), and any relevant news articles from NORECOPA, LASA, AWRN, and IAT.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The project is short term for one study only; opportunity to implement changes during the life of the project are therefore limited. However, in the lead up to the study the project licence holder is part of the local AWERB which facilitates continual professional development via named information and training officers and regular surveillance and dissemination of new 3Rs literature. The licence holder will also benefit from access to the principal investigator and project licence holder of project partners who have extensive experience of maintaining lice affected salmon populations and running lice challenges.