Home Office

NON-TECHNICAL SUMMARY

Support to provide fish lines

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Sharing, Quarantine, Importation, Cryopreservation, Invitro fertilisation

Animal types	Life stages
Zebra fish (Danio rerio)	Embryo and egg, Neonate, Juvenile, Adult
Medaka (Oryzias latipes)	Embryo and egg, Neonate, Juvenile, Adult
Guppies	Embryo and egg, Neonate, 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?

Providing an importation, cryopreservation and IVF service to support research groups within the UK. This will involve the breeding, maintenance, supply of aquatic species such as zebrafish, guppies and medaka, including genetically modified. This will support studies across a range of biomedical research fields.

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?

Reason 1, importing for others to continue biomedical research and reduce the number of animals imported each year) During 2024, CEFAS rules regarding the importation of some fish species changed due to them being susceptible to 2 viruses: Viral haemorrhagic septicaemia virus (VHSV) and Spring Viremia of Carp Virus. This change to the quarantine process requires all fish to come into the UK with a Health Certificate declaring the country of origin to be free from these 2 viruses, something most country's cannot provide. If this cannot be provided, the receiving facility must have a regulation 15 room for the fish to enter. This room must be clearly separate from other rooms, all waste water must be treated before entering the mains water, 150 fish from the importation must be screened at F1 generation as adults and be free from both pathogens, only then can the F2 generation leave the Regulation 15 room. All animals in the room must be of F1 generation when the screening takes place. This change meant that no biomedical research facilities (that we are aware of) has been able to import Zebrafish into the UK as they simply didn't have this area established, processes in place, staff to support this or resources. Since 2015, we have been running a quarantine facility. Our goal is to make sure the fish are free from harmful diseases. Over time, we've created a detailed system for bringing in, quarantining, and testing these fish, and we've shared our process with other facilities around the world to help them keep their fish healthy. We are also CEFAS compliant, meeting their new regulations for importation with a regulation 15 room, currently the only facility in the UK to achieve this. To ensure others continue to have availability to import animals and continue their science (whilst they establish their own regulation 15 room and quarantine processes where possible), we look to import, maintain, breed, screen for them and send fish to them which have been through this process, this is both Genetically modified and wild type fish.

Along side supporting other UK research facilities with importation, we also look to share the imported lines with multiple facilities in the UK. This will reduce the number of animals imported and maintained overall (as less animals used for breeding and screening in one facility compared to multiple with the same line) and support reproducibility.

Reason 2 cryopreservation and IVF for others to kill specific pathogens and reduce the number of fish held in facilities) We also know how to freeze fish sperm, known as cryopreservation. Some fish diseases can't survive freezing, therefore if we have a positive health screen for specific pathogens,

we can freeze sperm from fish these fish and kill the pathogen, then bring the line back using IVF. This helps prevent future health problems when those fish are used in breeding, improving fish welfare but also ensuring the pathogens do not impact scientific results. We can also send these frozen sperm (and even fish eggs) to other places so they can create their own new fish without having to move the adult fish around. This reduces stress on the fish and helps improve their welfare and assists with reproducibility.

Additionally, we can perform a process called In Vitro Fertilization (IVF) using frozen sperm. This allows us to bring back fish that were frozen earlier for other facilities when they need them. By offering this support, we can help other places avoid having too many fish on hand by cryopreserving, storing and bringing them back only when necessary and ensuring fish with specific genetics can be brought back to life for future research and reproducibility. Part of this will be importing from within and outside of the UK, performing the freezing and storing of the sperm, then providing the sperm to other facilities or storing and performing IVF at a later stage.

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

We anticipate frequently importing fish to our facility from other facilities outside of the UK. These specific fish will be housed, maintained, health screened and bred within our facility before embryos or adults or gametes being sent to the requesting facility (distributed) within the UK. These fish include genetically altered fish also known as GA, which means that the fish's genes (the instructions that make them who they are) have been changed on purpose. These are changed to give the fish special traits, like being stronger or growing faster. We will not be created new GA (F1) modified animals, instead breeding and maintaining and distributing the fish we import.

Long term goal: By doing this we can share these fish with others for their specific research needs, this maybe research in cancers, development or behaviour as examples. As these fish can be siblings, the scientific data collected from experiments is more easily reproduced in multiple labs, as the fish have some of the same genes as they have the same parents.

This project will reduce the number of animals imported, held and bred within facilities. It will also support reproducibility of results by having one central facility, sharing with others. It will also reduce the number of animals held in a facility by having one resource for importation and breeding of imported lines, as well as reducing the animals held unrequired by performing cryopreservation and IVF on those lines for other facilities.

We will continue to share our quarantine methods with others through publications, presentations and articles, aiming to improve welfare across facilities for fish species. We also look to set up a training program to teach others through 1-2-1 training at our facility how to: import, quarantine, cryopreserve, IVF, breed and care for fish species. Eventually we aim to create a portfolio of the fish we have imported, their traits, how to care for them based on these traits as share with others to support their welfare and research.

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

Zebrafish are now the second most popular laboratory animal model, with Killi fish and guppies now also become more frequently used also. This is mostly due to them sharing over 70% of protein-coding

genes with humans. The recent changes to CEFAS importation requiring further quarantine processes to be implemented withing Great Britain means that many GB facilities have not been able to import for over 12 months as they cannot meet these new demands due to funding, space and staffing. This has had a major impact on zebrafish research, including areas such as; eye development, nervous system, heart and gut development as well as cell migration and division. This support is aimed to benefit these facilities which cannot currently import whilst they implement their own quarantine or as a way to meet the new CEFAS requirements whilst also improving animal welfare and reducing the number of animals used.

From this project, fish welfare (for multiple species, not just zebrafish) will improve due to careful importation and clear quarantine processes (ensuring pathogens are not spread between tanks). Also, a clear health checking program which uses as few fish as possible will be used that will ensure accurate results, careful colony management and breeding for other facilities.

We look to reduce the number of fish imported and held within facilities by working as a central importation unit which shares the imported fish with other requesting facilities (reducing the number of imports required), as well as perform cryopreservation and IVF as a support to reduce the number of fish held at facilities.

Our facility and other facilities will benefit from the supply of fish which are specific pathogen free (free from certain harmful germs or diseases), as it will support maintaining the health of the fish already in their facilities and prevent these germs and diseases impacting the research results. As well as a cryopreservation and IVF support ensuring they are not housing fish which are not immediately required.

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

We will ensure that the fish community is aware of this support through publications, articles, forums and presentations.

We will continue to share our processes and procedures for performing a quarantine processes, regulation 15 room design and performing IVF and cryopreservation through publications as well as future 1-2-1 in house training program for other facilities staff members to join us and learn how we care for fish, import, breed and perform cryopreservation and IVF.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 82500
- Medaka (Oryzias latipes): 33000
- Other fish:
    - Guppies: 33000

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.

Fish usage continues to expand in the animal research field, with fish frequently being imported into the UK but not shared as there is no single resource to support this. We already house multiple species at our facilities in the UK, providing a support that will work along side our own research projects and the skills we have in place already can achieve this. The biomedical research projects will include (but not limited to, Cancer, heart disease and neurological disorders). These projects mostly use embryos for research but in some instances, such as the study for Tuberculosis, adults are used to study how the bacteria behaves in an older animal.

We aim to import embryos or sperm rather than juveniles or adult fish where possible, to reduce any potential welfare impacts of importation. We will then raise these fish to adult stage, breed these fish to F1 generation for screening before breeding again to create F2 generation, this is to complete the CEFAS regulations for importing animals into the UK. When exporting we will send embryos or sperm from the F1 generation or greater, instead of older ages (adults) to facilities outside of the UK where possible, again to further reduce the risk of welfare issues during transport.

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

1) Importation of Genetically altered or non-genetically altered fish of any age and housing in quarantine. Where possible we will import embryos before they are capable of independent feeding, where this is not possible, we will import from the closest supplier possible, ensuring the quickest route to our facility is taken by working with the importing courier. We will ensure all animals are held within conditioned water for their species, provided oxygen and insulated containers for the journey. No food will be provided to the animals during transport to ensure water quality is not diminished due to the breaking down of food in their environment. The container will be checked by a Named Veterinary Surgeon (NVS) or Named Animal Care and Welfare Officer (NACWO) upon arrival.

2) Breeding the fish to create offspring. Only fish showing no harmful phenotypes (a fish has something wrong with its body or behaviour because of a change in its genes) will be used for breeding. An example of such harmful phenotypes include: difficulty for the fish to grow, swim, eat, or even stay alive. Any found to have a harmful phenotype will be culled immediately. All animals will be held on a specialist designed fish housing system with enrichment provided (such as live food and planting) and light cycle provided for the species. Welfare checks will be performed twice daily. During breeding, zebrafish will be held off of their housing system for up to 24 hours, within breeding tanks to prevent them eating the eggs produced, food will not be provided during this time to ensure the water quality does not diminish and risk the welfare of the fish. For Guppies and medaka, breeding on their housing system will be used as guppies are live bearers (give birth) and medaka eggs are not released from the female body (so are not eaten by tank mates).

3) Screening of fish for genetic status (genotyping) by fin clipping (tail biopsy) or swabbing (optional). This procedure has minimal clinical signs or pain associated, We check the animals for full recovery post procedure and humanely kill any which do not recover within 30 minutes. During this procedure fish will be housed within a specialist fin clip tray which allows for the fish to see each other, share pheromones and be provided food and fresh water.

4) Screening of imported fish or subsequent generations by culling a percentage of the colony and sending for health screening (optional).

5) Supplying requesting research group with the subsequent generation upon request. Animals maybe moved to external facilities using an approved courier and packing as described (unless cryopreserved). We will ensure all animals are held within conditioned water for their species, provided oxygen and insulted containers for the journey. No food will be provided to the animals during transport to ensure water quality is not diminished. Where possible we will send eggs or fry instead of adults.

6) Cryopreservation / IVF (optional). This maybe conducted if a pathogen is found within the imported colony which cryopreservation is known to kill and to freeze the males down for future projects to share the sperm with other facilities. To achieve this the males from the group are anesthetized, sperm removed and frozen, the male is recovered unless no longer required, in which case he is killed before recovery. IVF can be used to bring a line back from cryopreserved or fresh sperm and using the eggs from an anesthetized female. Anaesthetic will be carefully monitored and discussed with the named veterinary surgeon or named animal care and welfare officer. Any animals which do not recover after 30 minutes will be culled. Fish may also be imported for the purpose of cryopreservation and or IVF as a support to reduce animal numbers held in the UK.

7) Humane killing of animals

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

The GM fish are not expected to show any welfare issues from the import or export process, however it is possible that survival to 30dpf maybe reduced by 10% beyond that of the wild type strains. This is not uncommon when importing eggs and fry and will be reduced where possible by ensuring a quick import and export route is used (within regulations), adequate air and water and heat packs provided where required and phenotype details requested from the supplying facility also. This loss is due to importation and not due to regulated procedures.

Aneasthesia: Fin clipping and cryopreservation is not expected to cause any welfare implications, with fish expected to fully recover from the anaesthetic within 30 minutes. We will check the animals for full recovery and then twice per day following the procedure.

As we are importing fish we have not housed previously, we will ask the supplying facility for any records they have regarding the health status and phenotypes of the colony. We will ensure to not import any fish which have a known phenotype which causes greater than mild clinical signs. Any fish imported which show greater than mild clinical signs will be immediately culled.

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)?

All fish species

Mild 100%

What will happen to animals used in this project?

- Killed
- Used in other projects
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

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 aim of this project is to provide animals for study in other projects, and animals are therefore vital to the success of the work. As the animals we import will be used for multiple studies, for example; in order to understand the effects of modifying genes and the effect of potential drugs in treating disease, the whole "system" must be studied which means live animals are needed to see how these changes affect an organism as a whole.

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

The aim of this project is to provide animals for research projects, and therefore there is no non-animal alternative to this. More widely in the connecting projects, cells grown in the lab or computer models are used where possible. Sometimes, however, this does not answer the research question and studying the whole animal is necessary.

Why were they not suitable?

Alternative methods are not suitable in the context of this project, but they are significantly intertwined with the work which this project will supply animals for. For example, tissues taken from these animals can be analysed in the lab, or even used to grow cells from by other facilities.

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?

Based on the interest we have received so far from the zebrafish community following discussions with the facility managers and users (approx 10 facilities), we have calculated the below. We have also included heath screening for CEFAS, which requires 150 fish per line as well as a 10% fry loss greater than wild types, genotype required, and sex ratios.

Zebrafish:

Importation of 5 lines per month, with 5 tanks per line holding 275 fry per line to allow for up to 10% loss due to importation, genotype results, health screening under CEFAS regulations as well as in house health screening requirements and male to female numbers for breeding = 1375 fry per month

1375 x 12 months of the year = 16500

16500x 5 years of project = 82500

Medaka and guppies:

Importation of 2 lines per month, with 5 tanks per line holding 275 fry per line to allow for up to 10% loss due to importation, genotype results / requirements and health screening for CEFAS and in house and male to female numbers for breeding = 550 fry per month

550 x12 months of the year= 6600

6600 x 5 years of project = 33000

Lines will typically be maintained at 40 fish per line before being frozen down or culled. However we do not anticipate keeping many lines long term, rather importing, screening, breeding and distributing before freezing down and culling.

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

We aim to house as few adults of each sex per line as possible, we therefore calculated the requirements based on the minimum number of pairs required per line for breeding, genotype requirements, typical quality of breeding, rest time following breeding, the number potentially required for screening, loss pre 30dpf of up to 10% above the wild type strain due to importation.

and required numbers of males for sperm freezing and IVF (if this procedure is required).

We will also check if the line is already imported into the UK through the zebrafish locator tool and emails to other labs prior to import. Reducing the duplication of importing lines already in the UK.

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

Efficiency of breeding to fulfil the projects whilst generating as few animals as possible is at the heart of this project. One project licence to cover all breeding for other projects enables significant control over the numbers of animals produced. Each strain will have a dedicated colony manager who will

communicate regularly with the facility manager and NACWO to ensure that breeding levels are kept closely in line with the demands of each project. These teams will discuss animal numbers for the studies to ensure that numbers are kept to a minimum. Where project demand falls in the long-term, a breeding colony will be frozen down as embryos or sperm.

Imported animals will be distributed and shared as wide as possible in the UK.

For lines we import only for cryopreservation or IVF, we will ensure we import the smallest number of animals possible to achieve this.

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.

To support natural behaviours: All imported animals and subsequent generations will be group housed. All fish will be provided brine shrimp (live diet) for chasing behaviour. Paramecia (live protozoa) will also be provided to young zebrafish fry for chasing and enrichment, we have also found this diet increases survival of young zebrafish fry as reduces the need for fry to swim to the surface of the water to collect food and therefore reduce energy expenditure. All tanks are on a light and dark cycle with slow ramp up and down to replicate sunrise and sunset. When genotyping fish, they will be held in a tray which allows for fish interaction, pheromone exchange and fresh water and diet to be provided.

Fin clipping or skin swabbing for genotyping – for fin clipping the smallest sample possible will be removed from the tail only as this recovers / fully regrows within 2 weeks of the procedure, for zebrafish this is approx 10% of the tail removed or less. Where possible we will use skin swabbing as an alternative to fin clipping to reduce the need for anaesthetic. Where required, we will work with the NVS regarding post procedure analgesia. All fish given anaesthetic will be monitored for 30 minutes for recovery, any showing signs of welfare issues will be culled. We will also continue to explore other methods of genotyping such as fin clipping at 3dpf (pre protection) or the use of the ZEG machine which genotypes embryos, we will implement their usage where possible.

We include cryopreservation and IVF as a way to reduce the number of animals kept alive in facilities unrequired, support sharing between facilities and reduce the spread of pathogens, therefore improving welfare. We have performed this procedure a large number of times over several years, therefore we are very skilled in performing gentle massage to the males abdomen to remove the sperm and the same for the female to remove the eggs. For both procedures the fish are anaesthetised. Where required, we will work with the NVS regarding post procedure analgesia.

All tanks which contain only males or more than 75% males or housing less than 2 fish will be provided additional plastic plants to provide a hiding place.

Fish will not be kept over 2 years of age. This allows for adults to be imported and screened and genotyped and bred during this period.

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

The aim of this project is to support fish research projects, no alternatives can be used to meet this project aim.

Fish are already considered less sentient than many other species, we go a step further and where possible we will use eggs for imports and exports and genotyping.

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

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

We will review the ARRIVE and PREPARE guidelines, Code Of Practice for animal housing, FELASA screening and importation guidance.

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

We will attend the 3Rs conferences, online talks, we have joined the NC3Rs website to receive regular updates.

As a NACWO I also review other project licences, attend AWERB, attend and actively participate in conferences, join online meetings, read published articles and receive regular updates during our in house named persons meetings, including those from the Named Information Officer, I am also a member of the zebrafish UK research forum, zebrafish husbandry association and the 3R's enquiry list at my establishment.