Home Office

NON-TECHNICAL SUMMARY

Identifying the molecular mechanisms of appendage and organ regeneration in zebrafish

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Regenerative biology, Regenerative medicine, Appendage regeneration, Heart regeneration

Animal types	Life stages
Zebra fish (Danio rerio)	embryo, juvenile, adult, neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 work is to identify the molecular mechanisms allowing zebrafish to regenerate their caudal fins and hearts following injury.

A retrospective assessment of these aims will be due by 21 June 2029

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

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?

Regenerative medicine is a rapidly evolving field of medicine, which aims to identify and ultimately implement novel therapies that will promote the repair and regrowth of damaged tissues and organs in humans, as popularised by the medical potential of stem cells. Rather than studying stem cells per se, or how they might be used clinically, my lab studies organisms that naturally have high regenerative capacity, such as fish, in order to tease out the mechanisms that bestow them with such high regenerative capacity, such as regrowing fins or regenerating their hearts following injury. The goal of this project is to identify the molecular mechanisms that zebrafish employ, enabling them to regenerate their caudal fins and hearts following injury.

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

The primary output of this project will be a greater understanding of the cellular and molecular mechanisms of appendage and organ regeneration in zebrafish. During the work, we will generate and/or characterise a number of genetic mutant strains in zebrafish, which we will make available to the scientific community. To facilitate the distribution of the genetic mutant strains that we generate as part of this work to the scientific community, we will deposit them in the European Zebrafish Resource Centre. We aim to publish all our findings in high quality, peer reviewed and open access journals.

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

Short-term benefit - the identification some of the key mechanisms that zebrafish employ to promote regeneration of complex tissues, such as appendages and organs.

Mid-term benefit - the development and testing of new drugs that promote regeneration in zebrafish.

Long-term benefit - the development and testing of new drugs and/or therapies that will promote a greater regenerative response in human patients, following injury.

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

This work is part of an International Training Network, primarily funded by the European Union. This network is multidisciplinary and includes internationally leading labs across 10 European countries. As such, this work is part of a large consortium of labs, and we will be meeting and discussing our findings on a regular basis. This collaborative consortium will be a primary avenue for us to disseminate our findings, but also in maintaining abreast of the current progress of the other consortium members. Apart from these mechanisms, we will also attend international conferences on regenerative biology / medicine in order to disseminate our findings to the scientific community and to also learn the latest advances in the field. We will aim to publish our findings, whether negative or positive, in high impact, open access journals, thus ensuring maximum impact of our work in the field.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 26500

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.

Zebrafish have extraordinary regenerative capacities, which allow them to fully regenerate their fins after amputation, and they can regenerate many organs, including the heart, following injury. They retain this high regenerative capacity throughout life. As such, we can investigate some of their regenerative capacity at the larval, pre-feeding and thus pre-protected stages. Indeed, we plan to perform many of our experiments at these early larval stages. However, it is essential that we also carry out some of our appendage and organ regeneration studies in adult animals, because only adults have a mature immune system and greater physiological complexity. Thus, our work aims to investigate the cellular and molecular mechanisms that permit zebrafish to regenerate complex tissues and organs throughout life.

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

The main intervention that will be performed on the animals used in this project will be genetic intervention / alteration / modification. This will involve either the generation or maintenance of

genetically altered (GA) zebrafish lines. Small biopsies or skin swabs may be taken from some of the GA fish in order to isolate genomic DNA for genotyping. In addition, given that the primary aim of the work covered by this licence is to investigate the genetic, molecular and cellular mechanisms responsible for zebrafish's high regenerative capacity in the adult, there are two additional interventions that some of the adult zebrafish will be subjected to. One is caudal fin amputation and the other heart injury. These two injury models are used to activate the regenerative response in the adult zebrafish. However, given their very high healing and regenerative capacities, the adult fish can fully regenerate their caudal fins within 3-4 weeks and to fully regenerate their hearts within 2 months. Finally, some of the adult fish that have had their fins or hearts injured may be subjected to chemical/drug treatments, either via immersion or via injection. The amounts of the chemicals used and their frequency of delivery will be carefully adjusted so to minimise any adverse or toxic effects to the fish, while ensuring maximal efficacy of the chemicals/drugs used.

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

We do not expect that any of the genetic alterations / modifications generated or maintained under this licence will cause lasting harm, therefore this is listed as mild interventions. Indeed, our hypothesis is that mutations in genes we are investigating encoding will be able to heal and regenerate better than wild-type animals. In addition, obtaining eggs and sperm from adult fish will also not cause lasting harm to the fish, and there are also listed as mild interventions. The two injury models induce a full and complete regenerative response, but these take from 3 weeks to 2 months to complete. Therefore, the caudal fin injury model is listed as Moderate given that full recovery of the fins takes up to 3-4 weeks. And, while the adult heart injury model also culminates with full functional regeneration of the heart, this takes up to 2 months, therefore the heart injury model is listed as Severe.

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)?

We expect a significant proportion of the GA animals (maintenance and breeding of GA zebrafish) to experience sub-threshold severity, as the genetic modifications are not expected to cause any harm to the animals. For the subset of the GA animals that need to be anaesthetised in order to obtain samples for genotyping and those anaesthetised for the purpose of obtaining eggs and sperm will be returned as having experienced mild severity. We expect that the majority of adult fish subjected to the injury models (caudal fin amputation and heart cryoinjury) will experience moderate severity, with a minor number of the latter experiencing severe symptoms, such as excessive and persistent bleeding following surgery.

What will happen to animals at the end of this project?

- Killed
- Kept alive

A retrospective assessment of these predicted harms will be due by 21 June 2029

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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 main objective of this project is to uncover the mechanisms that allow adult zebrafish to regenerate complex tissues, such as appendages and organs, following injury. It is currently not possible to fully emulate the complexity of multi-tissue environments present in appendages and organs, including circulation, in vitro over the time required for these complex tissues and organs to regenerate fully in vitro. It is thus necessary to perform much of our work in vivo. While we can and will perform some of our preliminary studies in pre-feeding stage larvae, the bulk of the work aimed at understanding the mechanisms employed by the adult animals to repair and regenerate appendages and organs will necessitate using the adult stages for our analyses. For this project, we have focused our work on adult zebrafish, not only because of its remarkable capacities to heal and regenerate complex tissues and organs, but also, because it is an aquatic vertebrate with lower neurophysiological sentience.

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

While we can perform experiments using cell culture systems, as for example to test whether chemical inhibitors have the expected activity in cell culture assays before using them on zebrafish larvae or adults, these non-animal alternative strategies cannot be relied on to determine fully the genetic, cellular and molecular mechanisms of regeneration of complex tissues, such as appendages and organs, as such complex structures cannot be reproduced using cell culture. Instead, the primary way in which we replace the use of adult animals in some of our work is to use pre-protected larval stages.

Why were they not suitable?

We use cell culture and pre-protected stages for preliminary studies but given that the primary aim of this project is to investigate the mechanisms employed in adult zebrafish to repair and regenerate their appendages (fins) and organs (hearts), the alternative non-animal or pre-protected stage experiments cannot be relied on exclusively to achieve the overall aim of the project.

A retrospective assessment of replacement will be due by 21 June 2029

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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?

The number of animals has been estimated based on previous licences covering our work. We have based our assumption that we will use a similar number of animals as in the previous 5 years.

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

In all our studies we aim at reducing animal numbers to a minimum by using the NC3R's Experimental Design Assistant (https://www.nc3rs.org.uk/experimental-design-assistant-eda) web application and the PREPARE guidelines (https://norecopa.no/PREPARE). We will also consult with our in-house statistical service for advice during this project. More specifically, we have applied to all our experiments very stringent methods to obtain statistically meaningful results with the minimum number of animals possible.

Data analysis will be conducted according to a pre-specified statistical analysis plan drawn up in conjunction with establishment-based statisticians. Important experimental results will be designed with biological replicates and repeated and validated via an alternative follow-up experiment to minimise the likelihood of spurious non-replicable results.

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

Most of the animals estimated to be used in the project will be breeding and maintenance of GA zebrafish. Thus the primary means by which we aim to reduce the numbers will be through improved husbandry and efficient breeding approaches. We will also endeavour to freeze down our GA strains when they are not immediately needed and, in this way, reduce the number of animals needed simply to maintain the strains. We also will aim to reduce the numbers of animals used for the regeneration experiments through refinement of our regeneration assays.

A retrospective assessment of reduction will be due by 21 June 2029

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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.

Zebrafish larvae and adults are particularly well suited to this project because they have remarkable capacities to heal wounds quickly, without leaving scars. In addition, they can fully regenerate complex tissues and organs within weeks following injury. This makes zebrafish particularly useful for investigating the genetic, cellular and molecular mechanisms of tissue repair and regeneration of complex tissues, such as appendages, and organs, such as the heart. While performing injuries in zebrafish will cause discomfort, the fish fully recover and given their remarkable regenerative capacities, they fully recover from these injuries.

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

Zebrafish is a low-complexity vertebrate model with low neurophysiological sentience. While there are some invertebrates that also exhibit remarkable regenerative capacities, such as planarians, hydra and some crustaceans, these invertebrates lack the complex tissue composition and anatomy representative of vertebrate appendages and organs, which are the subject of study in this project.

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

Most of the protocols in this project, including the husbandry and injury models, are well established for zebrafish both in the literature and in our laboratory, and this includes refinements that minimise the welfare costs of the animals. All procedures that may result in transient distress are carried out under anaesthesia, which also helps immobilise the animals during imaging sessions or when gametes are stripped from the animals. Nevertheless, we are constantly looking to further refine all our procedures to minimise any potential harms. As for example, we recently refined our caudal fin regeneration experiments such that we no longer assay regeneration in groups of animals, but rather, in individual animals over time. This has allowed us to obtain much higher quality data on fewer animals, as the animal-to-animal variability is now removed. We will continue to aim to refine our experimental regimes like this so that we can further reduce the number of animals needed to obtain high quality appendage and organ regeneration data. We will also continue to monitor the published literature for further refinements in these injury models in zebrafish, and we will actively investigate the use of analgesics, such as lidocaine, during the injury models, in terms of the efficacy and also in terms of whether their use impacts negatively on the healing and/or regeneration responses that we are investigating.

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

There are many useful resources on the NC3Rs website (e.g. NC3Rs experimental design assistant). We are constantly reviewing and improving zebrafish husbandry techniques following the advise from other labs and from advised from The Zebrafish Information Network (ZFIN).

The new PREPARE Guidelines will also be strictly adhered to (https://norecopa.no/PREPARE.)

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

We have previously submitted several applications for funding from the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs), and we have previously held an NC3Rs grant. We also maintain strong links with local and national advisors. NC3Rs regularly holds online workshops and institutional events aimed to improve experimental procedures with a focus on the 3Rs. We meet regularly, review and refine our protocols following discussions between our researchers, Named Animal Care & Welfare Officers (NACWOs) and NVS.

A retrospective assessment of refinement will be due by 21 June 2029

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?