Home Office

NON-TECHNICAL SUMMARY

Understanding the mechanisms that form the eye and how it consistently occurs with bilateral symmetry

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Retinal development, Zebrafish, Neurogenesis, Tissue Morphogenesis, Developmental Malformations

Animal types	Life stages
Zebra fish (Danio rerio)	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?

This project will look at how the eyes grow in a matching, left–right way and how they build the light-sensitive layer at the back of the eye, the retina. By studying eye development in zebrafish, we aim to understand how the actions of individual cells come together to shape the overall eye, and what can go wrong during this process that might lead to disease.

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?

This work is important because it helps us understand how complex body parts like the eye form correctly during development. The project focuses on how groups of cells move, grow, and organise themselves to build the retina, the light-sensitive tissue at the back of the eye, and how this process happens in a symmetrical and reliable way on both sides of the body. By studying these events in zebrafish, we hope to uncover basic principles that explain how organs take shape and how cells coordinate their actions. These findings are important not only for understanding normal development, but also for gaining insight into how things can go wrong in the context of birth defects and diseases affecting the eye and other parts of the nervous system.

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

This project will generate new knowledge about how the eyes of a vertebrate develop in a controlled and reliable way on both sides of the head, at the same time, and with the right symmetric structure to function properly together. It will show how changes at the cell level can affect the developing tissue, and how the developing organ and organism responds when faced with internal or external challenges.

The results will be published in peer-reviewed journals and presented at international scientific meetings. In addition, the project will produce high-resolution live imaging datasets and detailed visual records of eye formation, as well as analytical tools to study them, all of which will be shared with the wider research community.

While this is a fundamental research project, its outcomes are directly relevant to understanding what happens when eye development goes wrong. By revealing the basic principles that ensure robustness and symmetry in organ formation, this work lays the foundation for better understanding and eventually correcting developmental disorders of the eye including microphthalmia (when one or both eyes are too small), coloboma (when part of the eye does not close properly), or retinal dystrophies that affect how the retina functions .

Downloaded: 1:18pm, 2 Mar 2026

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

In the short term, the community of researchers studying eye development, organ formation, and symmetry will gain new insights into how cells behave and coordinate to build complex tissues. The project will also provide detailed live imaging datasets and tools to support other studies in developmental biology

In the longer term, these discoveries can be used to help explain how developmental eye disorders arise. These include microphthalmia, where the eyes are abnormally small; coloboma, where part of the eye fails to form; and inherited retinal dystrophies, which affect how the retina develops and functions. By revealing how the developing eye usually keeps its shape and structure on track, this work will improve how conditions like microphthalmia, coloboma, and retinal dystrophies are modelled in the lab, and guide future strategies to treat or prevent such defects. While the project is focused on basic research, the knowledge gained may ultimately benefit patients by informing earlier diagnosis or more targeted therapeutic approaches.

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

We will share our findings through peer-reviewed journals open access whenever possible, and preprinting them for the broad community. We will further present findings at international conferenceson developmental and cell biology, neuroscience, and cross-disciplinary gatherings. To further expand the impact of our work, we collaborate with computational biologists and physicists who help us analyse and interpret our complex datasets to get the most information out of as few experiments as possible.

All image datasets and analysis tools will be shared in public repositories, ensuring transparency and allowing other researchers to build on our work. We also routinely share both successful and unsuccessful methods to support collective learning and improve research quality across the field.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 18.500

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 are especially well suited to study how vertebrate organs, including the eye, form. Their embryos develop outside the mother (external fertilisation), are small and transparent, which makes them uniquely suited for microscopy, and grow very quickly. This means we can observe how cells move, change shape, and form tissues like the retina while development is still underway 'where it

happens, when it happens' using high-resolution imaging of living embryos. This gives us a real-time view of how the eye forms and how cells reach their correct locations.

For our experiments, we use only embryos and larvae up to five days after fertilisation. This is before the onset of independent feeding, which is the stage after which zebrafish are considered protected animals under the Animals (Scientific Procedures) Act (ASPA). Importantly, during this five day time window, the visual system is already developed, allowing us to answer all key research questions using these early stages without the need to use older or more sentient animals.

However, to produce these embryos, we need to breed adult zebrafish that carry specific genetic modifications. These genetically altered (GA) fish are necessary to study how different genes affect eye development. Some genetic changes are used to “tag” certain proteins, which allows us to follow where these proteins and the cells that contain them during development. Because generating and maintaining these adult fish falls under the protected stages, they must be covered by this licence.

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

The experimental research in this project will be done using zebrafish embryos and larvae up to five days after fertilization. At this stage, their nervous system is not yet fully developed, so they cannot feel pain or experience distress in the way that older fish or mammals can. This means that we can carry out experiments that help to understand how the eyes form without causing harm or discomfort to the animals. This means we avoid moderate or severe procedures entirely.

Some adult fish will be needed to produce these embryos for experiments. Adults will only be involved in procedures when absolutely necessary. For example: To maintain genetic lines, a small fin clip may be taken for genotyping. This is done under anaesthesia, causes only brief and mild discomfort, and recovery is monitored carefully.

When sperm or eggs are needed, for example for freezing to keep genetically modified lines, they are collected from anaesthetised adults by gently stroking the sides of the fish. If the gametes (eggs or sperm) do not release easily, the procedure is stopped immediately to avoid harm. Some fish may be swabbed or undergo light abrasion for DNA sampling during screening procedures. These are quick, non-invasive methods and cause no lasting harm.

Some of the fish we use carry genetic modifications. These modifications are designed to help us track how cells and tissues behave during eye development in different conditions. We monitor whether fish show any physical or behavioural abnormalities, which is almost never the case. However, in case any genetically modified animals show visible problems, such as deformities or issues with swimming or feeding, we will identify those through routine welfare checks and they will be humanely killed if necessary.

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

For the vast majority of this project, no adverse effects are expected. All experimental procedures are carried out in zebrafish embryos and larvae up to 5 days post-fertilisation, before the onset of independent feeding, when they are not considered protected animals under UK legislation. At these

Downloaded: 1:18pm, 2 Mar 2026

early stages, the animals do not have a fully developed nervous system and are not capable of experiencing pain or distress.

The adult zebrafish used for breeding may undergo genotyping through fin clipping. This is done under anaesthesia. Possible short-term effects include a mild startle response on recovery, temporary uncoordinated swimming (loss of equilibrium), or slightly altered swimming behaviour. These effects usually last no more than 30 minutes and are resolved without intervention. Fish are monitored individually until full recovery. Any signs of prolonged imbalance, refusal to swim or feed, or abnormal posture (e.g., lying on the tank bottom or floating at the surface) would be met with humane killing. Fins regenerate within 4 weeks without causing stress to the animal.

For gamete collection, fish are anaesthetised. The gentle abdominal pressure used to release gametes (eggs or sperm) is not expected to cause harm. However, in rare cases (fewer than 1% of fish), it may lead to minor scale loss or damage to internal organs. These risks are minimised by ensuring that the procedure is only performed by trained staff. Animals are monitored post-procedure and humanely killed if any signs of ongoing discomfort or injury are observed.

The genetic modifications introduced in this project are not expected to cause any adverse phenotypes. If any modified fish display abnormal appearance, movement, or behaviour that suggests suffering, they will be identified through routine checks and humanely killed.

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)?

Sub-threshold: 95–98%

Mild: 2–5%

What will happen to animals used in this project?

- Used in other projects
- Killed
- 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?

To achieve the aim of the project, we require a model that has the potential to image processes in the developing animal while they happen, where they happen using sophisticated microscopy methods. As zebrafish are transparent for the first three days of their development, develop outside the body of

the mother and are small, they are the best system to tackle our goal and understand how the eye is made from cells to tissue.

While some aspects of retinal development can be studied by putting retinal cells in a dish in culture, these approaches do not yet fully recapitulate complexity of eye formation. Particularly, the study of how two eyes of the same size, shape and optical capabilities develop is not possible outside the animal model. Therefore, live-imaging of zebrafish embryos is essential for answering the questions of this application.

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

The use of retinal organoids, which are cells that can, to a certain degree, assemble to form aspects of real organs are considered by us. Such organoids can be made using either human or zebrafish cells.

Why were they not suitable?

Non-animal alternatives like organoids are not yet suitable for the study of eye formation in the depth that we aspire. These models so far lack constraints that are usually given by the organism the organ develops in. They also do not have the same signalling environment as found in the organism so that making them always the same and close to the in vivo situation is not yet possible. Further, it is not possible to understand the formation of two eyes in relation to each other, as it happens in the organism, because organoids develop in isolation.

What we want to achieve, however, is to use knowledge that we gather in zebrafish and to make organoids more reproducible and robust. This can, in the long-term, lead to the development of more accurate and clinically relevant organoid models, including those derived from patient material.

In this way, the project contributes to the long-term goal of Replacement by generating fundamental knowledge that improves alternative models, even though *in vivo* experimentation remains necessary at present.

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 estimate the need for, at maximum, 35 different transgenic and mutant lines of zebrafish. For maintenance purposes and to ensure that we always have egg laying animals, each line will be crossed every 6 months i.e. twice a year.

Downloaded: 1:18pm, 2 Mar 2026

From each cross we will keep 2 tanks per line with a maximum of 25 fish in each tank, for a total of 50 fish per line per cross.

With a 5-year duration of this project, these were the calculations:

35 x 2 x 50 x 5 = 17.500 adult zebrafish; plus 1000 for gamete (eggs or sperm) collection.

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

For our experiments, we will only use zebrafish embryos and/or larvae up to 5 days post-fertilization. All adult animals that entered our calculation here will only be kept and used for crosses in order to obtain these experimental embryos/larvae.

We calculated the necessary number of adult animals considering the variability in the quality and number of the egg-laying and use of the animals until they reach 18-24 months of age. Our experience indicates that at that point the egg quality and number of laid eggs start to become insufficient to assure that experiments can be carried out efficiently and robustly.

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

We will use several measures to reduce the number of animals used, in line with the 3Rs. First, we will perform pilot studies to assess variability and refine protocols before scaling up experiments. This allows us to determine the minimum number of embryos needed to detect meaningful effects. We will maximise data output per embryo using high-resolution live imaging and quantitative analysis, often tracking multiple parameters simultaneously. Whenever possible, we will use embryos from the same clutch for different conditions or analyses to reduce breeding numbers. All experiments will be planned to minimise duplication and to extract as much information as possible from each animal used.

When generating genetically altered lines, we will genotype adults to identify carriers before breeding, ensuring that only relevant animals are used to produce experimental embryos. This avoids the unnecessary use of non-carrier animals and reduces overall numbers.

Furthermore, collecting and freezing zebrafish gametes will allow the preservation of zebrafish lines without having to maintain and breed the fish in case those lines are not being used experimentally at a given point.

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.

This project will use zebrafish embryos and larvae, a well-established model for studying vertebrate development. Zebrafish are particularly suitable because their embryos develop externally, are small and optically transparent, and undergo rapid development. These features allow us to perform high-resolution in vivo imaging and experimental manipulation without invasive surgery or procedures that cause lasting harm.

Experimental work will be carried out on embryos and larvae up to 5 days post-fertilisation, before the onset of independent feeding. These stages are not considered protected under ASPA and are not expected to experience pain, suffering, distress, or lasting harm.

When procedures that are legally considered regulated, meaning they could cause any discomfort or harm, are required (for example, to identify the genetic makeup of individual fish through fin clip genotyping), they will be limited to brief and minimally invasive steps, such as taking a small fin clip, and always carried out under anaesthesia to prevent pain or distress.

Adult zebrafish will be used solely for breeding. No experimental procedures will be carried out on adult fish. All adult animals will be housed and monitored under standard conditions, with appropriate care and oversight by trained animal facility staff and the NACWO.

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

Zebrafish embryos are already among the least sentient vertebrate models available and are used in this project to avoid the use of more complex or sentient species. Under the Animals (Scientific Procedures) Act (ASPA), fish become protected animals once they are capable of independent feeding. Zebrafish typically reach this stage at around 5 days post-fertilisation. In line with UK regulations and the 3Rs, all experimental procedures in this project are carried out before this stage, when zebrafish are not considered protected under ASPA.

Using less sentient species such as invertebrates (e.g. Drosophila) is not appropriate for this research. The aim is to understand vertebrate eye formation, including optic cup morphogenesis, neurogenesis. These processes occur very differently in invertebrate models and are not relevantly comparable to human biology. The zebrafish offers a good balance between biological relevance and low sentience, making it the most appropriate model for this work.

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

We will follow established refinement principles to minimise welfare costs at all stages of the project. For experimental procedures such as genotyping, fish will be anaesthetised using the minimal effective concentration of anaesthetic and for the shortest duration required. Animals will be individually monitored during recovery, and upon showing any signs of distress will be humanely killed immediately.

Downloaded: 1:18pm, 2 Mar 2026

All imaging and manipulation procedures are carried out on zebrafish embryos or larvae up to 5 days post-fertilisation, which are not protected under ASPA and are not expected to experience pain, suffering, distress, or lasting harm. When regulated procedures are necessary, they will be refined through pilot testing to optimise timing, minimise duration, and reduce invasiveness.

Husbandry practices are also constantly refined to reduce background stress. This includes maintaining stable stocking densities to avoid stress, providing environmental enrichment (e.g. shelters), and offering a varied diet including live food to promote natural behaviours and good health. All welfare monitoring is performed in consultation with facility staff and adjusted as needed.

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

We will follow published best practice guidance to ensure that all procedures are carried out in the most refined and responsible way. This includes the PREPARE guidelines for planning animal studies and the ARRIVE guidelines for transparent and ethical reporting. We will also consult relevant documents from FELASA and NC3Rs, particularly those focused on zebrafish welfare and refinement.

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

We will stay informed about advances in the 3Rs through regular contact with the Named Information Officer (NIO), the NACWO, the NVS, and facility staff, who are all valuable sources of up-to-date guidance on animal welfare and refinement strategies.

In addition, we will consult the NC3Rs website for new guidance and zebrafish-specific resources, and will remain alert to relevant publications, internal seminars, and research community discussions. Any potential refinements identified during the course of the project including through published work, training, or feedback will be reviewed with the team and implemented where appropriate. Regular project reviews will include discussion of 3Rs opportunities.

Downloaded: 1:18pm, 2 Mar 2026