Home Office

NON-TECHNICAL SUMMARY

Fish Physiology in the Anthropocene

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (d) Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

temperature, hypoxia (low oxygen), pollution, metabolism, cardiac function

Animal types	Life stages
stickleback (Gasterosteus aculeatus)	Embryo and egg, Neonate, Juvenile, Adult, Aged animal
Zebra fish (Danio rerio)	Embryo and egg, Neonate, Juvenile, Adult, Aged animal
Brown Trout (Salmo Trutta)	Juvenile, Adult, Aged animal
stoneloach (Barbatula barbatula)	Juvenile, Adult
minnow (Phoxinus phoxinus)	Juvenile, Adult
Rainbow Trout (Oncorhynchus mykiss)	Juvenile, Adult
carp (sp)	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 proposed research will investigate how environmental change in isolation and in combination affect key life history traits like swimming, metabolism and behaviour in fish. As cardio-metabolism underlies these key performance traits, we will also study how the environment (temperature, hypoxia, man-made pollution) impacts the fish heart.

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?

Climate change represents a significant challenge for resource managers (e.g. the Environment Agency) who require advice on the resistance, resilience and adaptability of fish populations to environmental change. Fish are ectotherms (i.e. cold-blooded) and can adapt to a range of environmental conditions including acute (minutes to days) and chronic (weeks to months) changes in temperature, dissolved gases (oxygen and CO_2), and even in anthropogenic (human-made) pollution. **However, there is concern that because such changes are occurring more rapidly and in combination, that while these populations may survive, they may not thrive. This is called lack of fitness. This lack of fitness means they may not have the ability to perform activities required for lifetime success like migration, courtship, or withstanding a further environmental challenge. The purpose of this application is to study how fish fitness is impacted living in the challenging environment of today's rivers. Through this work we hope to implement better and more timely resource management for wild and for managed populations**.

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

We expect this project to produce new data that will form the scientific foundation for both academic publication, and public and stakeholder engagement. Advancing knowledge on multiple environmental stressors across life stages in riverine fish (as opposed to model species) will be of interest to river and lake anglers, to canal and rivers trusts, and national parks managers. It could also be used to hold current water management companies (e.g. water companies) accountable. These are realistic benefits as our current work on microplastics in urban rivers has led to water companies having to disclose untreated sewage release events as part of a public inquiry.

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

Fishes are the primary beneficiary. We have chosen the species for this study in collaboration with local angling and rivers trust groups to represent fish that live in different parts of the river (e.g. benthic (bottom feeding) versus open flow). We hope that our findings contribute to river species welfare through initiatives run through these partners like the building of tree-shade or cool refuges. Healthier water ways improve the well-being of greenspaces for humans, animals, and ecosystems. The **short** term benefit will be in defining integrated limits of environmental tolerances for a number of fish species across life stages. In the **medium** term this data can used to create more bespoke management models. Indeed, policy makers, environmental lobby groups, water and environment regulators will benefit from this project as they will be able to use the findings to make decisions for the benefit of the fishes. The **longer** term benefit of the knowledge generated from this license will improve survival and welfare of fish populations both in wild systems and in aquaculture.

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

We will continue to work with local angling groups, friends of the canals/waterways, and Rivers Trusts to improve the condition of the water fish live in. We will publish in open-access journals, presentation at scientific meetings and make our resources available to other researchers including data and tissues. Our findings may include negative results and will publish and disseminate these with the same commitment and strategy we use for positive findings. Our work could impact policy and will be active in this arena and also work together with local policy providers to ensure findings research the public realm. We routinely engage the public through outreach and widening participation activities and will ensure findings from this project are shared here to inspire the next generation of enviormental bioloigsts and fish scientists.

Species and numbers of animals expected to be used

- Brown Trout (Salmo Trutta): 50 (or rainbow trout)* will use either Rainbow or Brown depending on availability, not both
- Rainbow Trout (Oncorhynchus mykiss): 50 (or Brown trout)* will use either Rainbow or Brown depending on availability, not both
- Other fish:
    - stickleback (Gasterosteus aculeatus): 500
    - minnow (Phoxinus phoxinus): 100
    - carp (sp): No answer provided
    - stoneloach (Barbatula barbatula): 100
- Zebra fish (Danio rerio): 2500

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.

<u>Choice of species:</u>

Temperate Riverine fish: Trout (rainbow *Oncorhynchus mykiss* and/or brown *Salmo salar*) will be used as they are a fish that feeds on the surface of the river, are native to UK waters, and are sensitive to environmental stress. They are easily attainable from fish farms and we have more than 25 yrs of experience working with this fish. Common **minnow** (*Phoxinus phoxinus*), **stoneloach** (*Barbatula barbatula*), three-spinned **stickleback** (*Gasterosteus aculeatus*) are all common fish in UK waterways. We have measured their abundance in urban rivers and the presence of microplastics in their bodies and environmental pollutants in their habitats. These 3 fish feed differently in the water column and thus may have variable exposure to pollutants. For example stone loach feed in the sediments whereas the stickleback tend to feed from the surface (as adults). All 3 species are small in size and are readily held in tanks, and are easily attainable from pond shops.

Carp-like fish: Zebrafish (*Danio rerio*) are a model organism whose genetics can be easily manipulated. We will use them in this project to probe questions where altered physiology through CRISPR technology allows deeper understanding of environmental tolerance landscapes. The work with the zebrafish builds directly on the findings from the my previous PPL. Carp species like goldfish (*Carassius auratus*) or common carp (*Cyprinus carpio*) are exceptionally tolerant of environmental stressors **-** sitting at the other end of the tolerance spectrum from trout. These are easily attainable from pond shops.

<u>Choice of life stage:</u>

This project will work across life stages from **embryo/larvae**, to **juvenile**, to **adult** and **aging fish**. It is clear from the literature that embryonic and larval stages are more vulnerable to environmental stressors than adults and thus we must conduct studies at these ages to understand the vulnerability of fish populations. Moreover, as the developmental environment impacts later life fitness though a process known as *developmental programming*, it is vital to follow fish that have been exposed to stress in early life, into adulthood. We have separate protocols for developmental programming work and for life-stage specific exposure work.

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

There are 3 parts of this application that detail what will be done to a fish in this project. Each are described below.

1) The rearing conditions for embryonic fish (developmental programming exposures and holding/maintenance conditions for juveniles/adults/aging adults (environmental exposures).

2) The Environmental and Behavioural Tests that a fish will go through to understand environmental tolerances. For example, assessing min/max metabolic rate; swimming (fish only, not embryos); thermal tolerance test; hypoxia tolerance test; and behavioural tests.

3) The cardiovascular monitoring, blood taking or substances administration at various time points across the life stage to determine physiological mechanism underlying environmental tolerances.

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1) Rearing Water and Holding Water Conditions:

The aquatic habitat is an integrated environment, comprised of multiple changing environmental parameters. Consequently, fish simultaneously experience changes in several environmental factors including water temperature, dissolved oxygen levels and anthropogenic (man-made) pollutants. These environmental conditions can act singly and synergistically on the physiology of fishes. We will rear (during embryonic development) or hold/maintain (in protected life stages) fishes under varying temperature and oxygen levels, with or without pollutants and microplastics.

2) The Challenge Tests

To understand how a rearing/holding environment impacts the ability of a fish to tolerate an acute environmental challenge, fish will experience a set of 5 challenge tests none of which are expected to cause lasting adverse effects.

Maximum and minimum metabolic rate: where oxygen consumption is used as an index for metabolism.

Swimming tests: where fish swim voluntarily in a swim-flume (fish treadmill).

Thermal tolerance tests: where fish will experience an increase in water temperature until they loss equilibrium, from which they recover well.

Hypoxia tolerance tests: where fish will experience a decrease in oxygen levels until they loss equilibrium, from which they recover well.

Behavioural Tests: where fish will have their behavioural monitored whilst making decisions, exploring new arenas, or choosing a thermal preference zone.

3) Tagging, Cardiovascular monitoring, administration or withdrawal of substances

Tagging: Fish may be tagged for individual identification which is not expected to have any lasting adverse effects on the fish.

Monitoring/Instrumentation: Fish will be anaesthetized by immersion in water containing anesthetic and then placed on a surgery table with gills irrigated with a maintenance dose of anesthetic and covered to keep the body damp. Here cardiac parameters can optionally be measured non-invasively using an ultrasound, or more directly with ECG monitoring electrodes inserted just beneath the skin on either side of the heart, or with cannula and flow probes in larger fish.

Administration of substances: In some instances, the effect of the environment on growth and metabolism will be investigated following an intramuscular or intraperitoneal injection of growth regulators or growth assessors in adult fish. These could be substances that increase metabolism like adrenaline, or substances that help us record fuel use during growth like insulin. Such substances can be administered to embryonic fish via the water or directly via injection into the yolk.

Withdrawal of blood: Blood may be taken to assess for hormones, metabolites etc. Usually there will be a single blood sample taken not more often than once a week under anesthesia.

All fish will be humanely euthanized at the end of project.

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

Our environmental exposure groups have been chosen based on our monitoring work in the rivers and water ways around the Establishment. Our aim is to choose experimental group that reflect the conditions fish are currently experiencing in the wild.

We do not expect long term welfare issues with our holding or rearing conditions. In instances where fish are exposed to environmental pollutants alone or in combination with changes in other environmental parameters, we may expect slower growth and reduced activity. However, oxygen, temperature and pollution change routinely in their natural environment across the seasons and with run off into the rivers changing growth and activity as a normal part of the seasonal cycle. We do not expect the rearing conditions will have adverse effects beyond growth and activity levels. But to control for any potential adverse effect, we will screen our combination of environmental conditions during the pre-protected life stages, which are also the most sensitive life stages in fish. We will monitor how the embryos develop and any conditions that cause abnormal development will not be used with protected stage fish.

None of the challenge tests are expected to have lasting impacts on welfare. We also note that some of the 'tests' like the behavioural investigation of a novel object are used as enrichment. However, we recognize that undergoing multiple environmental challenge tests over the course of a lifetime will have cumulative effects and to minimise any adverse effects accruing we will allow ample time (~3 months in most cases) between sets of environmental challenge tests.

All experiments where cardiac function is recorded will be conducted under non-recover anesthesia to avoid any adverse effects.

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 rearing and holding conditions and the behavioural trials are expected to be sub-threshold and 80% of the fish will experience these conditions.

Of the 100% of all fish (80% of which will not be in 'standard conditions') 60% will under go challenge tests that are mild. 40% will undergo the 2 challenge tests that are moderate.

What will happen to animals used in this project?

- Killed
- Used in other projects

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?

This work has to be carried out on fish because, as aquatic ectotherms, their vulnerability in relation to climate change differs from other animal models (i.e. terrestrial vertebrates or invertebrates). Additionally, fish are the prime beneficiaries of the output from this work, understanding tolerance in relation to fish biology is paramount   Although part of the research in this program of work employs non-invasive techniques, such measuring metabolism by recoding oxygen consumption, and tissue levels studies (molecular, biochemical, genetic) following humane killing, to assess mechanisms limiting thermal tolerances, it is necessary to measure the integrated environmental thresholds of the whole animal.

It is important to remember that thermal tolerance varies with level of biological organization: Complex processes requiring peak swimming performance, like migration and reproduction, will have lower thermal tolerance thresholds than single cells or isolated organs. Thus, work must be conducted over a range of levels so that mechanism can be understood at the molecular level and functional consequences for lifetime fitness can be appreciated at the whole animal level. Indeed, advising policy makers on thermal tolerance thresholds arrived at from isolated tissue studies alone may well underestimate the sensitivity of the system.

The genetically altered zebrafish provide the most feasible way to address the underlying mechanism for growth and development limitations under various environments.

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

We have previously provided data for modelling studies of cardiac function in response changes in temperature and to polyaromatic hydrocarbons. Any relevant data from the current project will be used to parameterize these models. Unfortunately, whole animal simulation models do not exist.

Why were they not suitable?

They do not exist - modeling whole organismal responses is currently not possible. But should some be developed we would adopt to reduce animal use.

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?

A statistician helped us with calculations using typical variations from our previous data. We will use different species to ask specific questions relevant to the environment. For example, if we are interested in benthic (from the riverbed) uptake of microplastic from the sediments into a fish we would use stoneloach as they feed in the benthos and this exposure route is more relevant to them than a trout. As we are interested in co-occurrence of different environmental stressors, we will have treatment multiple groups and as we are interested in interactions, which can be harder to detect, we estimate the need for larger sample sizes. For example if we wanted to look at the interactive effects of microplastic exposure with temperature and a pollutant, we will need 6 treatment groups. Sample sizes for our experiments are estimated from past experiments. Calculations typically show that we need group sizes of 10 to achieve the quality of results we need. We've used our annual return of procedures data to estimate the number of animals that we will need to use for breeding.

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

We discussed our design with a statistician such that we used the least number of animals whilst adequately powering our studies.

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 evaluate the effect sizes and samples sizes for each species as we go and revise where applicable. For some species where less is known about their responses to environmental change we have based sample sizes on responses know from more common species. We will check these assumptions routinely.

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.

Our project is trying to understand the implication of climate change on fish and all of the environmental conditions employed in this study are already faced by fish in their natural environment. There are >40 fish species and this project has chosen 6 different species who either occupy different but relevant environmental niches (e.g. benthic stoneloach vs surface feeding stickleback) or have different tolerances to their environment (e.g. hypoxia tolerant carp v hypoxia sensitive salmonids). The project also involves zebrafish as a genetically amenable model fish. This allows us to specifically define certain genes or pathways that are involved in fish environmental tolerance. We may instrument some fish to allow us to understand the role of the cardiovascular system in environmental tolerances and this will always be done under appropriate anesthetic.

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

Our project is for the benefit of fish and designed to help us understand how they respond to the multiple and simultaneous stressors living in the an era of climate and environmental change entails. Cell culture or invertebrate models would not provide the fish-specific, or integrated whole animal performance information that this project aims to gain.

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

We will monitor fish during all protocol steps and if we recognize an occasion where a lower threshold can replace current ones, we will discuss with our animal care facility and implement where appropriate. An example is using agitated swimming responses rather than loss of equilibrium responses to an acute thermal challenge. Ageing animals will be carefully monitored by staff trained to work with aged fish.

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

I keep on top of resources and guidance from the NC3Rs and the Laboratory Animal Science Association and will keep abreast of symposia relating to wild fish welfare.

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

As project license holders and for all personal working under a Home Office license, we will attend lectures and workshops put on by the NC3Rs, our local NC3Rs manager, and our animal care unit.