NON-TECHNICAL SUMMARY

Understanding and mitigating impacts of modified environments on fish

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
- (e) Research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work

Key words

Abiotic stimuli, Environmental change, Sustainable engineering, Telemetry, Restoration

Animal types	Life stages
Brown Trout (Salmo Trutta)	Juvenile, Adult
Salmon (Salmo salar)	Juvenile, Adult
Grayling (Thymallus thymallus)	Juvenile, Adult
European eel (Anguilla anguilla)	Juvenile, Adult
River lamprey (Lampetra fluviatilis)	Juvenile, Adult

Animal types	Life stages
Sea lamprey (Petromyzon marinus)	Juvenile, Adult
Common carp (Cyprinus carpio)	Juvenile, Adult
Roach (Rutilus rutilus)	Juvenile, Adult
Barbel (Barbus barbus)	Juvenile, Adult
Chub (Squalius cephalus)	Juvenile, Adult
Pike (Esox lucius)	Juvenile, Adult
Perch (Perca fluviatilis)	Juvenile, Adult
Bullhead (Cottus gobio)	Adult
Minnow (Phoxinus phoxinus)	Adult
Sea bass (Dicentrarchus labrax)	Juvenile, Adult
Ballan wrasse (Labrus bergylta)	Juvenile, Adult
Sand eel (Ammodytes tobianus)	Juvenile, Adult
Small spotted catshark (Scyliorhinus canicula)	Embryo and egg, 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?

To (1) quantify the impact of modified environments (e.g. from urbanisation or engineering practices) on fish; (2) develop environmental impact mitigation technology to protect fish (such as behavioural deterrents using acoustics); and (3) to monitor the effectiveness of rehabilitation actions for fish (such as river restoration or the reintroduction of the Eurasian beaver).

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 project is important because multiple species of fish in the UK have suffered serious population declines. The information obtained during this project will provide evidence of negative impact of human modifications on fish distribution, movement, and survival to help develop and prioritise conservation actions. This will help regulators ascertain whether current methods to protect fish (e.g. fish ladders and screens) or rehabilitate aquatic environments are effective, and highlight areas where improvements can be made. Measuring secondary impacts associated with modified environments (including during construction and operation of engineering practices), such as the sounds created, will be achieved so that alternative measures to protect fish can be developed.

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

Expected outputs from this project include:

1. Guidance to regulators and other stakeholders involved in development and environmental management / mitigation , e.g. in terms of whether current legal obligations for fish protection are being met and whether current mitigation options (e.g. fishways and screens) are effective.

2. Presentations (e.g. at international conferences), publications (in peer reviewed journals) and attendance at outreach events enabling dissemination of information to a wide audience

3. Validated prototype mitigation technologies (e.g. novel fish passage solutions)

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

Information obtained will benefit the funders by providing them with the information required to assess whether legal obligations are being met (e.g. whether 40% escapement targets for eels are being achieved at selected study sites), and to prioritise actions based on understanding of areas of greatest impact. Novel insights will help regulators ascertain whether current mitigation options (e.g. fishways and screens) are effective and highlight areas where limited resources should be directed to maximise improvements. This is in line with government interests to develop infrastructure (e.g. for renewable energy or for flood defence) in the most environmentally sustainable ways possible. Further data generated will enable the effectiveness of rehabilitation actions, which themselves involve modification to the environment, to be quantified, providing the evidence needed to inform sound future practice. Finally, this project will allow identification and quantification of secondary impacts associated with modified environments and so inform statutory agencies on areas that require greater focus / attention. Such fundamental experimental research on fish response to environmental stimuli will not only help improve understanding on how these secondary impacts effect fish (e.g. induce delay during a spawning migration, resulting in increased energetic costs and predation risk), but also help identify potential methods for alternative mitigation technologies, e.g. behavioural deterrent or attractant devices based on acoustics, light or electric fields.

The research findings will be of interest to DEFRA and 'arms-length bodies' (the Environment Agency and Natural England); engineering consultants and the construction industry involved in the provision of infrastructure and associated mitigation (fish passes and screens); members of the industrial sector who are obliged to develop aquatic environments and urban areas that surround them in a sustainable manner; and fisheries / angling organizations, conservation bodies, and members of the general public concerned with the protection of biodiversity and natural capital.

The probability of achieving these outputs is high, as evidenced by accruing impact of previous work conducted in this area. It is expected that the benefits will include publications, guidance and policy documents, and uptake of validated prototype fish pass, screening, and behavioural deterrent technology by industry and regulatory bodies.

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

Outputs will be maximised through engagement with a range of stakeholders, including developers, engineering companies, environmental NGOs, government agencies (e.g. the Environment Agency), or others tasked with managing aquatic environments. Long standing relationships with many of these stakeholders will help maximise the outputs of this work. A range of approaches to the dissemination of new knowledge will also help to maximise the outputs of this project. For example, policy briefings, conference presentations, open access peer reviewed publications, stakeholder engagement and outreach events ensuring information in disseminated to the scientific community, public and other stakeholders.

Species and numbers of animals expected to be used

- Brown Trout (Salmo Trutta): 3430
- Salmon (Salmo salar): 300
- Other fish:
    - Grayling (Thymallus thymallus): 1500
    - European eel (Anguilla anguilla): 1730
    - River lamprey (Lampetra fluviatilis): 1730
    - Sea lamprey (Petromyzon marinus): 300
    - Common carp (Cyprinus carpio): 1430
    - Roach (Rutilus rutilus): 1100
    - Barbel (Barbus barbus): 800
    - Chub (Squalius cephalus): 800
    - Pike (Esox lucius): 400
    - Perch (Perca fluviatilis): 400
    - Minnow (Phoxinus phoxinus): 300
    - Sea bass (Dicentrarchus labrax): 930
    - Ballan wrasse (Labrus bergylta): 750
    - Sand eel (Ammodytes tobianus): 1200
    - Small spotted catshark (Scyliorhinus canicula): 750

- Bullhead (Cottus gobio): 300

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 target species of the project is driven by the requirements to provide information needed to better conserve fish stocks in the UK. The diadromous Salmonidae, eel and lamprey are selected due to their high conservation significance and protection under environmental legislation (e.g. the Salmon and Freshwater Fisheries Act 1975; Eel Regulation EC 1100/2007; Habitats Directive 92/43/EEC). Although not of the same conservation significance, there is considerable interest also in this project in coarse fish and certain marine species (hence their inclusion) in recognition that previous research to assess the impacts of modified environments and associated mitigation technology has focused on the Salmonidae, and more recently eel. Additionally, rehabilitation actions that will be monitored in this project are often performed with the aim of benefitting the wider fish community (e.g. in efforts to meet ‘good ecological status’ targets under The Water Environment [Water Framework Directive] [England and Wales] Regulations 2017), which is reflected in the number of species (and both juvenile and adult life-stages) included under this licence.

This project will have both field and laboratory components. For the field elements of the project there is a need to monitor wild fish to quantify impact of modified environments, test the efficacy of environmental impact mitigation technology and rehabilitation actions. It is not viable to use captive reared animals in many instances because: (1) they do not exist (it is impossible to obtain species such as eel and lamprey from aquaculture units); and / or (2) for those that do (e.g. salmonids, cyprinids), the behaviour of captive animals differ substantially from those that are wild, and concerns over releasing captive fish in sensitive habitats prevent this option.

For experiments conducted in the laboratory, the use of captive reared fish may be viable under some scenarios and will be considered when appropriate.

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

There are four research components to this project. What will be done to an animal in each component is outlined below:

1. **Field based assessment of modified environments on fish**: For key target species the direct and secondary impacts of urbanisation and engineering practices will be assessed (e.g. water abstraction points, weirs, small-scale hydropower devices, tidal gates, culverts, areas exposed to noise / light pollution). Standard telemetry techniques (PIT, radio, acoustic) will be used to track the distribution and trajectories of fish relative to the modified environment so that impacts can be determined. These relate primarily to the degree of impediment created by physical structure or modifications (e.g. areas of light pollution), such as time taken to negotiate the impediment, any associated mortality (e.g. due to turbine damage) or other costs (e.g. energy expenditure) due to

impeded movement / migration. The regulated procedures will involve electronically tagging the fish to enable quantification of their distribution / movement. Fish will be collected using standard surveying methods (electric fishing, netting, or traps) and tagged at the site of capture. Fish will be anaesthetized and a tag will be inserted through into their body (intraperitoneal) cavity. Typically, the incision would be no longer than 5 mm for PIT tags, and 10 – 15 mm for radio / acoustic tags. The incision will typically be sutured with absorbable suture filament, or left un-sutured for some species if the probability of tag loss is deemed low (e.g. incision is small). Fish will be allowed to recover from the effects of the anaesthetic in well aerated water before release as soon as possible after recovery at the site of capture, typically within 2 hours.

2. **Field and laboratory based assessment of the effectiveness of impact mitigation technology**: For key target species, the efficiency of impact mitigation technology will be quantified both in the laboratory (testing a prototype) and in the field (validation of experimental results).The data required in the case of fish passes will be the attraction efficiency (the total number that approach the fish pass entrance as a proportion of the number of fish released) which indicates how easily the fish find the fish pass; entrance efficiency (the total number of fish that enter the fish pass as a proportion of the number that approach), passage efficiency (the total number of fish that pass the fish pass as a proportion of the number that enter), and time taken to do so (delay). For screens, both mechanical and behavioural systems, the information will enable guidance (total number of fish that were diverted to a fish pass as a proportion of those that approached the screens) and screening efficiency (total number of fish that were prevented from entering a water intake as a proportion of those that approached) to be calculated. The regulated procedures described for (1) above will be used for the field elements that relate to the quantification of distribution and movement of fish as they encounter mitigation technology. In addition, fish response to prototype technology will be tested in large-scale laboratory flumes in which regulated procedures may also be applied (e.g. exposure to abiotic stimuli such as artificial light).

3. **Field based monitoring of rehabilitation actions where positive impact to fish is expected**: The primary focus will be on physical river habitat restoration and reintroduction of the Eurasian beaver (*Castor fiber*). The physical conditions (e.g. water depth, velocity and bathymetry) of study sites before and after rehabilitation actions (including at control sites) will be quantified and the fish community surveyed using standard methods. Once caught, fish will be identified to species level enabling community composition to be determined, measured (fork or total length, mm), weighed (g), and either returned to the river at the location of capture (e.g. if too small to tag) or tagged (PIT, radio or acoustic) prior to release. Tagging will enable standard telemetry techniques to be used to determine habitat use and movements of fish relative to the rehabilitation actions. The growth rates of PIT tagged fish re-captured in subsequent surveys will be calculated. Tissue samples from fin clips will provide insight into food web dynamics relative to the modified riverine environment following stable isotope analysis in the laboratory.

4. **Laboratory investigation of fish response to key abiotic stimuli**: Experiments will be conducted in the laboratory to define fundamental behavioural rules for fish response to abiotic stimuli (e.g. acoustics, light, electric and electromagnetic fields) associated with urbanisation and engineering practices. This information will be used to understand impacts and advance the development of mitigation technology such as behavioural deterrents (e.g. by identifying appropriate stimuli). This will involve standard filming techniques using large scale flume facilities

or tanks. The response of fish to acoustic and light stimuli, and electric / electromagnetic fields will be recorded using video tracking software (avoidance or attraction to the cue, swim speed, tortuosity, distance moved). In some instances, blood samples will also be taken from fish in relation to exposure to the stimuli to determine physiological impacts, such as suppression of melatonin. To determine individual level physiological response over time, blood sampling in combination with PIT tagging will be used.

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

Fish will undergo marking (e.g. implanted with electronic tags) and fin tissue / blood sampling which will require minor surgery, exposure to abiotic stimuli (e.g. electric / electro-magnetic fields, sound and light) and a combination of these. These will include minor discomfort and elevated stress during anaesthesia and insertion of hypodermic needles (e.g. to take a sample of blood) and surgery associated with implanting a tag into the body cavity. Fish are expected to recover quickly with no lasting effects on their behaviour or life span (e.g. taking blood from a fish has similar effects as taking blood from a human). When encountering abiotic stimuli adverse effects are likely to be minor and relate to small changes in behaviour in relation to the stimulus encountered, with a return to normal behaviour when the stimulus is switched off.

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)?

Fish - Mild: 64%; Moderate: 28%; Sub-threshold: 8%

What will happen to animals used in this project?

- Killed
- Set free

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?

We need to use animals as the project aims to understand their natural behavioural and physiological responses to modified environments. Models that effectively predict fish behavioural / physiological responses to modified environments are currently in the early stages of development. Therefore, despite recent advances, such models are based on understanding of response to a limited range of conditions for a small number of species. To improve the behavioural rules that are used in these types of models necessitates research on live animals, both in the laboratory and field as described in this project.

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

There are not currently any suitable non-animal alternatives. To develop models to predict how fish will respond to modified environments it is first necessary to develop the behavioural "rules" based on empirical observation on which these models can be developed. Such a rule base is currently absent for most species responding to most stimuli.

Why were they not suitable?

The use of proxy species or non-animal alternatives is not expected to provide realistic / transferable results at this stage. This is because of a lack of behavioural rules based on empirical data to underpin robust modelling approaches in most instances.

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?

This project is split into two components, one that is conducted in laboratories, the other that is field based. Ascertaining the minimum number of animals to be used for the experimental component is comparatively straight forward, in that a limited and predefined number of fish can be used based on past experience of earlier work (including information on effect sizes) and related published studies.

The number of fish needed for the field component are based on the requirements of the funder who will confirm the number of sites and species of interest on a case-by-case basis. The numbers of fish needed are based on estimates that account for typical sample sizes of past studies, the literature relating to similar field studies, and expert opinions of other researchers in the field and of the regulatory agency in England.

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

Research will follow the principles of rigorous experimental design in which appropriate controls and treatments will be used under experimental settings that enable the test variables of interest to be manipulated while confounding factors are controlled. This will include the use of alternated or randomly allocated treatments / controls, and double-blind replicates where appropriate. For the experimental component of the project we will adopt an iterative process in which assessments of statistical power and levels of variance based on data collected in phase 1 will form part of the decision making process in relation to selection of sample size in future phases. For the field component, we will adopt a process of “real time” statistical analysis, in which as the data is obtained (e.g. for before and after comparisons of the numbers of fish that successfully pass a dam after a fish pass has been installed relative to those released through a tagging study) decisions are based on when to halt the trials because a statistical difference has been identified (or not as the case may be). This approach is a very pragmatic way to minimise the number of fish used. The advice of experts in statistical process control and biological statistics at the Establishment has previously been sought for related experimental designs and has been used to further guide the number of animals used in this project.

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

A key means of optimising numbers of animals used is the control of sources of variability in the data. Key confounding variables are site-specific characteristics, fish characteristics (e.g. size) and time (season). Typically, we ensure that the number of replicates of sites are sufficient to make general conclusions (i.e. avoid basing results on single case study examples), we size grade fish to ensure they are similar for experimental trials and appropriate for tagging, and ensure comparative studies are conducted during the same period of the year (e.g. during the migration of the target species).

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.

Choice of model species: The target species for both experimental and field components of the project are Salmonidae (e.g. Atlantic salmon *Salmo salar*, brown trout *Salmo trutta*, and European grayling *Thymallus thymallus*), coarse fish (primarily from the family Cyprinidae), European eel (*Anguilla anguilla*), lamprey (*Lampetra fluviatilis* and *Petromyzon marinus*), sea bass (*Dicentrarchus labrax*), ballan wrasse (*Labrus bergylta*), sand eel (*Ammodytes tobianus*) and small spotted catshark (*Scyliorhinus canicula*). The choice of model species is based primarily on conservation, economic and ecological significance. Although not of the same conservation significance (as e.g. Atlantic salmon and European eel), there is considerable interest in this project in coarse fish (hence their inclusion) in recognition that previous research has focused on the Salmonidae, and more recently eel, due to their conservation and commercial significance. Thus, a significant bias exists with a lack of current understanding in relation to e.g. those in the Cyprinidae family. Where necessary, proxy model species that exhibit similar behavioural traits and life-history characteristics will be used to replace those of high conservation status (e.g. when the conservation concern of a specific source population is sufficiently high as to preclude its use, e.g. river lamprey as a proxy for sea lamprey). Additionally, rehabilitation actions that will be monitored during this project are often performed with the aim of benefitting the wider fish community (e.g. in efforts to meet ‘good ecological status’ targets under The Water

Environment [Water Framework Directive] [England and Wales] Regulations 2017), which is reflected in the number of species included under this licence.

Why techniques are the most refined: For the purpose of meeting the objectives of this project, we have selected techniques (i.e. experimental flume-based trials and field telemetry) that have been in development and refined for over 30 years. Why each method is the most refined is described below:

(a) Tagging / telemetry (PIT and radio / acoustic)

PIT telemetry is considered to be the “gold standard” telemetry technique due to very low probability of adverse effects and high tag retention rates without requirement for suturing. Radio / acoustic tags will be required in some instances. While PIT tags are advantageous due to their longevity, small size, and ability to equip individuals with a unique identification code, radio / acoustic tags will enable fish to be actively tracked in modified environments and to generate 3D positional data in some instances (e.g. when used in conjunction with an array of acoustic receivers). Commercially available PIT tags typically vary in length from 11 to 32 mm. For this project we will typically use tags that are 12mm in length, but up to 32mm tags in some instances for larger fish. For all tag types, the size will be selected to ensure it is sufficiently small relative to the size of the fish (< 10% tag length relative to fish body length and less than 2% tag weight to fish mass). Based on previous research, after recovery from tagging (immediate to 4 hours) fish are expected to experience a life-expectancy that does not deviate from controls, as illustrated by the ability to monitor Pacific salmon several years after they were tagged.

(b) Tissue sampling via fin clipping.

To ensure passivity during the procedure, especially for larger fish that may be sampled, anaesthetics will be used during fin clipping. Fin clipping involves the use of a pair of surgical scissors to remove a small part of the fin tissue (approximately 1.2 mg wet mass). This is preferentially taken from the pelvic or caudal fin as evidence suggests fast, reliable re-growth and ease of sampling (i.e. handling of fish during procedure). Fin clipping is a very quick and minor procedure thought not to impede the movements or natural behaviours of the fish and is preferred over other methods that would require the fish to be killed to obtain the tissue sample. Tissue samples undergo stable isotope analysis in the laboratory at a later date to determine food-web dynamics.

(c) Quantifying fish response to abiotic stimuli in the laboratory

The experimental approach adopted (use of tanks / open-channel flumes) is the most refined as it enable specific fish species of interest to be exposed to a specific stimulus of interest (sound, light, electric / electromagnetic field), while potential confounding variables are controlled. We can carefully control exposure levels inline with detailed experimental designs and based on the findings of previous research (i.e. expected to illicit a certain level of response while causing the least pain, suffering, distress or lasting harm to the animals). For example, exposure to artificial light at night will be at intensities considerably lower than fish would encounter under natural daylight (e.g. an overcast sky is approx. 5,000 Lux). Experimental light intensities to simulate direct light pollution (e.g. as a result of street lighting, which is typically around 5 - 50 Lux) and skyglow (e.g. from the scattering of artificial light by atmospheric particles and is approximately 0.5 - 1 Lux). Where possible relative to study aims, stimulus gradients will also be generated, enabling the fish to volitionally avoid the stimulus (indeed, areas avoided relative to the stimulus strength is a key metric to be quantified). As such, exposure to abiotic stimuli is likely to be 'sub-threshold' in many instances (i.e. the level of pain, suffering, distress or lasting harm is so low that a licence is not required).

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

In cases where there are specific target species of interest e.g. due to conservation concern, they need to be used directly, rather than a model species used as a proxy, as this will provide the data of greatest value, and limiting the impacts of intraspecific variation in response. Immature life stages cannot be used as they can differ greatly in behaviour in response to stimuli (e.g. in response to light based on photoreceptor sensitivity which can change with life-stage) or will not provide relevant results (e.g. because they do not exhibit the same directed movements and have limited swimming capabilities compared to adult fish).

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

All surgical techniques will use well developed and widely used protocols to minimize handling and associated stress. Effects of the techniques will be monitored to reduce probability of causing pain and suffering during future phases. Behavioural traits will be monitored to indicate humane endpoints, e.g. increased gill ventilation rate measured as the number of gill beats per minute and abnormal swimming behaviour, e.g. swimming in circles or close to the tanks bottom, relative to baseline conditions or naïve fish. These relate to observations of the fish prior to the procedure to develop mean (variance) values. Post-surgery behaviour will be closely monitored over the period of recovery to assess deviation from the pre-surgery condition. The data will be regularly reviewed to assess whether behavioural measures can be refined to enhance the efficiency of identification of humane endpoints based on a relationship between exhibition of aberrant behaviour and resulting deterioration in condition. The humane endpoint will be the early termination of a study or the introduction of changes that avoid, reduce, minimise, or alleviate pain (e.g. administration of appropriate analgesia). In the case of surgery for tagging, the techniques will be conducted under anaesthetic by trained personnel with experience in fish behaviour. In the event that a fish exhibits signs of excessive distress (e.g. gill ventilation rate doubles, swimming become exaggerated and abnormal) for 60-90 minutes, it will be killed by a Schedule 1 method. Previous research involving several species of fish illustrate that success rates of recovery after surgery is high and mortality rates low, when conducted by experienced practitioners.

Analgesics will be used when the potential for fish to experience pain is high, i.e. after surgical procedures associated with telemetry.

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

We follow best practice protocols (e.g. below) where possible, and incorporate these into Standard Operating Procedures. We also follow the prepare guidelines when planning experiments: https://norecopa.no/PREPARE

Beaumont, W.R.C. 2011. Electric Fishing: a complete guide to theory and practice. Game and Wildlife Conservation Trust.

Lawrence, M.J., Raby, G.D. Teffer, A.K. et al. (2020). Best practice for non-lethal blood sampling of fish via the caudal vasculature. Journal of Fish Biology 97, 4-15.

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

We regularly received email updates pertaining to advances in the 3Rs e.g. from the NC3Rs mailing list or the Establishment NTCO. Members of the team regularly attend animal welfare related events, such as the RSPCA 'Focus on Fish' online conference on practical refinements for fishes used in research. Additionally, the team keeps up to date with advances in the field through reading relevant peer reviewed literature and presenting at / attending in-person conferences (including those specifically on animal welfare).