Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 28

Projects with a primary purpose of: Basic Research – Ethology

Project Titles and keywords

1. **Causes and consequences of movement and foraging**
    - Migration, birds, trade-off, carry-over effect

2. **Genetics of behaviour and stress response in fish**
    - Genetics, stress, evolution, fish, behaviour

3. **Causes and consequences of corvid intelligence**
    - Cognition, evolution, corvid

4. **Population effects of individual fish life history**
    - Fish ecology

5. **Mechanisms underpinning cooperation**
    - Cooperation, assortment, neuroendrocrinology, guppies, evolution

6. **Early-life adversity and adult cognition in starlings**
    - Starlings, early-life adversity, welfare, behaviour, resilience

7. **Phenotypic programming across the lifespan**
    - Ageing, hormones, environmental change, stress, development

8. **Environment, phenotypic development and performance**
    - Development, ageing, telomeres, stress, growth

9. **Behaviour of predators in conservation landscapes**
    - Badgers, foxes, predators, management, conservation

10. **Kinship and recognition in a social bird**
    - Kin selection, cooperation, inbreeding, social evolution

11. **Fish developmental biology and behaviour**
    - Maternal, stress, welfare, nutrition, conservation

12. **Behaviour and ecology of wild birds**
    - Birds, behaviour, ecology, mating, evolution

13. **Host-parasite dynamics in wild rodent populations**
    - Zoonoses, wildlife disease, ecology

14. **Linkages between invasive plants and salmonids**

- Riparian invasives salmonids

15. Behaviour of fish in the natural environment

- Fish, telemetry, behaviour, dams, engineering

<table>
<tr><td>Project 1</td><td>Causes and consequences of movement and foraging</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Migration, birds, trade-off, carry-over effect</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td></td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td></td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Long distance migration in birds is among the most dramatic and exciting phenomena in nature. However, despite many years of study there are still huge gaps in our understanding of how this behaviour affects both individuals and populations. For example, we have very little understanding of how migratory animals balance competing needs (trade-offs) –e.g. feeding to improve body condition versus feeding to ensure resources for egg production. Understanding this is important because how individuals approach these trade-offs leads to variation in many important measures of individual fitness such as productivity, survival and timing of breeding. Trade-offs, which occur across the annual cycle, also have important implications for focussing conservation and management of the species concerned.

Our lack of progress in understanding migration is almost inevitable given its complex nature. For most animals, an individual’s state (e.g. reserves,</td></tr>
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	immunological performance, foraging skills, social environment etc) in one season is heavily influenced by previous conditions. Therefore the costs or benefits of behaviours or strategies can be "carried over" into subsequent seasons. However, this is further complicated in migratory species because events may not have occurred in the same location, making it hard to identify the key regulatory processes. For example, selecting a poor wintering site can reduce body condition, leading to a later migratory departure date, and this may carry-over into poorer breeding performance. These carry-over effects are likely to be clearest in species migrating to the Arctic where resources are highly time-constrained. Birds may have to carry the fuel for both flights and reproduction with them, placing huge physiological demands on individuals, yet few studies, if any, have managed to directly link the stress incurred during migration with subsequent fitness in free-living animals. A better understanding of how variation in state drives individual fitness is crucial to virtually every question about the evolution and function of different migratory strategies. It is also key to enhancing conservation initiatives for migrants, enabling us to identify where population regulation occurs. This is crucial for Arctic migrants as the state of the climate will also influence how these trade-offs are balanced throughout the annual cycle. This project would combine examination of the physiological tradeoffs and resource use of individuals with state of the art tracking technologies to follow migratory geese throughout their annual cycles. It will use these techniques to gain an understanding, for the first time, of how interactions among physiological (stress), social (associations and competition), ecological (dietary preferences), epidemiological status and climatic environments affect and individual's state, and in turn fitness, across the annual cycle.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project would change the scale of migration studies, moving from tens of tracked individuals to 100s and covering the entire annual cycle. Given the severe declines seen in many migratory species around the world, generating a fuller understanding of this phenomenon is crucial for ecological and conservation efforts. The novelty and breadth of this research will result in the findings being published in both scientific and popular journals with widespread impact. As a migrant to the high arctic, brent geese will be affected by climate change, and studying their phenology will allow us to understand how changes may be affecting many species (both resident and migratory) in multiple locations. This will feed into the protection of important habitats along the flyway, and into best practice for managing conflict between goose populations and communities. The project involves close liaison between governmental and non-governmental agencies, charities and volunteers in fieldwork, ensuring ideas and results are disseminated widely. In addition, this project will support an outreach program involving schools and the general public to highlight the importance of migratory species – both at a local scale and as sentinels of climate change.
What species and approximate numbers of animals do you expect to use over what period of time?	Light-bellied Brent Geese captured at their wintering grounds over multiple winters (Oct-Mar) 2013 – 2018. Approximately 500 birds will be captured in the first year, with lower numbers in subsequent years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Any adverse effects are likely to be mild. Blood flow will be halted using pressure and cotton wool. In the unlikely event of this failing to stop blood flow, a small amount of super glue or similar will be applied. The tracking devices deployed have been used on this or other goose species with no adverse effects reported. The project requires birds to behave naturally, so devices are designed to have minimal impact on bird behaviour. Following sampling, all individuals will be released

	together into the wild at the capture site.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This work could not be done using computer simulations or modelling since it requires information from animals living in the wild. For example, the physiological responses of individuals to extreme efforts during migration are unknown and so cannot be simulated.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Efforts are made annually to maintain unique coloured leg rings on 10% of the wild population. We will take advantage of catches already occurring to reduce the total number of captured birds.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	East Canadian Light-bellied Brent Geese make probably the longest annual migrations of any goose, flying from Ireland, via Iceland, to breed in the high Canadian Arctic. As highly time constrained migrants they represent an ideal study species for understanding the stresses of migration, as well as acting as sentinels of climate change. Geese will be captured using cannon nets and placed in darkened holding pens until sampling. Blood will be collected via venepuncture of the tarsal vein. A cloacal swab will be taken. The geese will be weighed, measured, aged and sexed. If birds are of sufficient mass, a tracking device weighing less than 3% of body weight may be attached. Total handling time will be around 10 minutes. Upon completion birds will be returned to the holding pens and released together. Total time from catch to release will be no more than three hours (defined in the BTO Cannon Netting Manual). An experienced goose handler will monitor birds within the holding pens. Any birds showing signs of stress (e.g. immobility) or excess bleeding will not be released until the symptoms have ceased. Stressed birds will be placed in a dark bag to recover.

<table>
<tr><td>Project 2</td><td>Genetics of behaviour and stress response in fish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Genetics, stress, evolution, fish, behaviour</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>In biology, stress is often a poorly defined concept, and one that is negatively associated with health in humans and animals. However, stress responses actually play an important positive role in maintaining viability and health. When challenged by a threat in the environment – perhaps a predator, disturbance, or adverse conditions – a set of neuroendocrine pathways trigger physiological and behavioural responses (e.g. fight or flight behaviours) that have evolved under natural selection to counter the threat. Nonetheless, while these acute stress responses are thought to be adaptive, it is also well documented that chronic stress exposure can reduce the health of individuals and sometimes – particularly when mothers are exposed - their offspring. Because chronic stress responses, also called “tertiary stress responses” (TSR) are typically bad for fitness (survival and/or reproduction), natural selection should act over evolutionary time to get rid of them. The fact that the TSR is widespread, being found in vertebrates ranging from fish to humans, therefore poses an important question– what constrains evolution of the stress response towards a state</td></tr>
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	where these harmful, or maladaptive, effects do not occur? The goal of the proposed work is to answer this question by conducting genetic studies. We will use small fishes, and primarily the guppy (*Poecilia reticulata*) as a model system. Practically, the work will involve experimentally manipulating stressors in the environment, determining how different individuals and genotypes respond through behavioural and hormonal processes, and determining the long term consequences of this variation for fitness. In particular we will test two hypotheses about where the evolutionary constraint comes from that maintains the TSR. The first possibility is a trade-off between the effects of acute and chronic stress on fitness. In simple terms, genes that cause the TSR may persist in a population precisely because they are the ones that lead to the most appropriate acute stress responses. A second possibility is that, where mothers experience chronic stress, a trade-off occurs across the generations. Here, some maternal genotypes are better able than others to maintain the mother's own health, but do so at a cost to offspring (e.g. by reducing the amount of care she provides).
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	In the short term the principal outputs of the research will be academic, benefiting the broad set of biologists working from an evolutionary perspective across disciplines and levels of biological organisation. This is because the project will tackle fundamental scientific questions about the nature of evolutionary constraint, a phenomenon that is far wider in scope than the specific context of understanding maladaptive stress responses. However, the work will also provide us with a better understanding of the genetics of chronic stress response. In turn this should yield tangible benefits for improving welfare in captive animals. The presence of genetic variance for the tertiary (chronic) stress response would open up the possibility of using artificial selection as a tool to improve animal welfare in captive populations (e.g. livestock, aquaculture, scientific research). Understanding the genetic architecture of the stress response, its performance consequences under

	chronic stress exposure, and identifying biomarkers for its efficient characterisation is a necessary first step towards this.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years we expect to use approximately: -4000 guppies -1000 swordtails -2000 zebrafish -1000 sticklebacks
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Any adverse effects are likely to be mild. Fish will be subject to tagging for identification (and in some cases fin clipping to obtain samples for DNA analysis). These procedures, which will be conducted under anaesthetic, are routine in fish studies and complications (e.g., unsuccessful recovery from anaesthesia) are rare. Data on behaviour, size/growth, maturation time, hormone levels, metabolic rate and longevity will all be collected using completely non-invasive methods the severity of which is sub-threshold. A subset of animals (up to approximately 2000) will be subjected to a chronic stress treatment that is expected to induce reduced growth (or weight loss) and reduced reproductive output. Since longevity and reproductive performance are traits of interest in this study we expect most animals subject to licensed procedures (e.g. tagging under anaesthetic) to remain “on license” for the duration of their lives. Euthanasia criteria (agreed with the named veterinary surgeon) are in place to deal with any diseased, injured or senescent fish. Where fish are not required for further breeding or data collection they will be euthanized by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no non-sentient alternatives that could be used and in vitro approaches are not applicable to organism level questions. The study aims to test evolutionary theory about the pathways linking animal behaviour, physiology, health and the environment (the source of stress) in vertebrates. It is only possible to do this using a vertebrate model.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Studying the evolutionary genetics of traits in wild-type populations generally requires large sample sizes and our study is no exception. Experiments have been designed using computer simulations to determine the minimum sample sizes that will allow powerful testing of our hypotheses. In doing this we have also ensured that the statistical methods we will use to analyse the data are the most powerful ones available. To reduce animal numbers further we will not address all objectives in all of the study species. Rather guppies will be used to test all our key hypotheses, while other species will be used for smaller parallel studies where they add particular relevance. For example, by verifying our findings in zebrafish we can assess the potential for genetic improvement to improve welfare in captive populations of this widely used scientific model.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The fish models to be used are exceptionally robust, easy to care for, and highly fecund under laboratory conditions. In all these regards they are more suitable than other possible vertebrate models (e.g., mice, rats). All procedures to be applied are mild or sub-threshold. There are no alternative procedures of lower severity. General welfare will be ensured by maintaining housing conditions and husbandry standards (e.g. daily inspections, frequent water changes, a robust program of water quality testing) that meet or exceed all HO requirements.

Project 3	**Causes and consequences of corvid intelligence**	
Key Words (max. 5 words)	Cognition, evolution, corvid	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Recent findings suggest that corvids, members of the crow family, possess sophisticated cognitive abilities comparable to those of primates, but the evolution of cognitive intelligence is not understood. This project aims to further our understanding of the evolution of intelligence by addressing the development and fitness consequences of corvids' cognitive abilities in their natural environment. Most work on corvids to date has been conducted in captivity, limiting our understanding of the factors favouring the evolution of corvid intelligence in nature. This project will use observations and behavioural experiments on wild corvids to: (1) Assess the cognitive processes corvids use to overcome social and ecological challenges. The need to navigate the challenges of social life in complex and dynamic societies is thought to be one of the main driving forces behind the evolution of primate intelligence. The project will use experiments to test whether corvids, like social primates, recognise multiple individuals, keep track of the relationships between them and benefit from their investments in social relationships. It will also examine how corvids recognise and generalise about threats such as	

	predators in their environment. (2) Investigate how learning from others affects corvids' behaviour. Experiments will examine how information about predators and new foraging techniques spreads through groups by learning. (3) Assess the causes and consequences of variation in cognitive ability. I will present corvids with a variety of cognitive tasks to determine (i) why individuals vary in their cognitive abilities and (ii) how this variation influences the ability to survive and reproduce. Hormone analyses (from feathers and blood plasma) will allow assessment of stress (corticosterone) and its relationship to cognitive performance.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By generating insights in into the development and consequences of wild corvids' cognitive abilities, this project will make important contributions to our understanding of the evolution of intelligence. Findings will be of great value to ecologists, biologists and psychologists and, by revealing the striking abilities of British birds, will help to foster a greater respect for wildlife among the general public. Finally, understanding animals' cognitive processes is a critical element in wildlife management and conservation strategies.
What species and approximate numbers of animals do you expect to use over what period of time?	Wild birds (corvids); 2500 individuals over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	With the exception of the withdrawal of feathers (for hormone analysis) and small blood samples (for hormone analysis and molecular sexing analysis, as male and female corvids look alike), the project is entirely non-invasive. Blood and feather sampling will be conducted using aseptic technique, taking only the small volumes necessary for molecular sexing and hormone analysis. These procedures are of mild severity and no adverse consequences are anticipated. Birds will be generally released immediately following sampling to avoid undue stress. To determine whether young birds learn how to respond to predators, I will also conduct experiments in which model predators or unthreatening objects (e.g. plastic bottles, stuffed toys) are presented to birds temporarily held in captivity, in the large trap

	used for capture. These experiments will be of short duration (each presentation will last < 30s and birds will be held in captivity for < 20 mins in total) and will be no more stressful than a brief encounter with a predator under natural conditions. Any stress resulting from seeing a model predator will be mild and transient, and no adverse consequences are anticipated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no alternative to research on non-human animals if we are to gain a broad understanding of the factors favouring the evolution of intelligence. Without conducting research on animals, it is impossible to examine the benefits conferred by elevated cognitive performance and, therefore, to determine how natural selection drives cognitive evolution. Mathematical models and computer be useful in examining the efficacy of cognitive processing, but can tell us little about the ecological pressures driving cognitive evolution under natural conditions. In this project, statistical models will be used to infer the mechanisms underpinning the transmission of information in corvid societies. However, for such models to produce valid results, they must be based on empirical data.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All proposed experimental and observational protocols are based on previous successful research conducted by myself and others. Sample sizes for experiments will be determined using power analysis with significance levels set to 5% and power to 80%. The use of multifactorial statistical models will allow for analysis of normally and non-normally distributed data, controlling for repeated measures where necessary, thus ensuring the greatest possible statistical power for any given sample size. As corvids are sexually monomorphic, it is essential to take small blood samples so as to determine their sex accurately through molecular sexing. Blood samples will also provide accurate point measures of corticosterone in plasma, while feathers provide a long-term, integrated measure of stress throughout development. The total number of individuals sampled will depend on the rate of population growth over the course of the project, but numbers are likely to be 500 individuals or fewer each year.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Corvids have large brains and possess sophisticated cognitive abilities, making them ideally suited for studies of the evolution of intelligence. However, virtually all previous research on corvid cognition has been conducted in captivity. The use of wild corvids represents a significant ethical advance, given that the captive environment may cause substantial stress in these intelligent, curious and highly sociable birds. With the exception of blood and feather sampling, the project is entirely non-invasive and will not require the use of regulated procedures. Blood sampling will be performed rapidly and efficiently so as to avoid unnecessary suffering. Blood sampling is considerably more reliable than feather removal for molecular sexing analysis and provides accurate point measures of stress hormones in plasma. Small blood samples (typically < 100ul) will be taken from superficial blood vessel. The area will be swabbed with ethanol before and after sampling to prevent infection, and any bleeding will be controlled with gentle pressure. One feather will be removed rapidly immediately following blood sampling, to give a measurement of long-term stress levels throughout development. Central tail feathers will be taken as they do not affect flying ability and will re-grow. The birds are small enough to be gently restrained by hand (I have extensive experience of this) and will be released immediately following sampling. The point from which tail feathers are taken will also be swabbed with ethanol before and after the feather is removed, with any bleeding controlled with gentle pressure. Experimental presentations of model predators will be brief (<30seconds per presentation). Models will be presented outside the trap where the focal bird will be temporarily held, at a distance of at least 1m. An opaque screen inside the trap will allow birds to escape and hide from the model.

Project 4	**Population effects of individual fish life history**	
Key Words (max. 5 words)	Fish ecology	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Y	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
	Y	Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	**Summary of project objectives** • To assess the role of individual traits (e.g. growth, migration strategy, etc.) in determining individual life history choices of salmon and trout, and the consequences of those choices for the total population. • To annually quantify the number of Atlantic salmon, trout grayling at various life stages (e.g. parr, smolt, adult etc.). • To identify and/or quantify the pattern of movement of fish species (salmon, trout, grayling and eel) at different life stages and/or at various obstructions or installations in the watercourse • Assess the impact of riparian and in-stream habitat on feeding, growth and migration patterns and life history strategy of juvenile salmon and trout.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	Data on salmonid migration and survival will allow identification of the critical mortality phases of the salmon and trout to be ascertained. Our methodology will allow quantification of variation at the population level and a better understanding of the role of	

project)?	individual traits on freshwater and marine survival. In addition the effect of freshwater habitat type and in-river barriers on migration patterns will allow knowledge-based management of both these impacts. Knowledge of the efficiency of fish passage and any mortality or migration delay around in-river obstructions is vital if negative impacts on fish are to be avoided whilst generating low-carbon energy from low-head hydro-turbines. In-river habitat is being affected by both natural and man-made impacts (climate change, water abstraction and increased nutrient input). This in turn could affect the ability of the river to maintain a good density of salmon and trout. By assessing feeding behaviour in a range of in-river habitat types we will be able to quantify the impact on the growth rate, survival and migration patterns on salmonids. Data from the study will enable promotion of evidence-based management practice leading to improved fishery management. It will enable informed debate about the impact changes to in-river habitat and of fish barriers. Results will also be disseminated by publication in the scientific literature (including publications widely read by fishery managers) and in more popular articles written for the general public.
What species and approximate numbers of animals do you expect to use over what period of time?	Atlantic salmon, 75,000 over the 5-year licence Brown/Sea trout 10,000 over the 5-year licence Grayling, 2,500 over the 5-year licence
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Work will include measuring and weighing fish, marking fish with either visually identifiable marks or transmitting tags, taking scale or tissue samples and stomach flushing the fish. For all of the above sedating or anaesthetising the fish decreases the likelihood of harm to the fish caused by it struggling. With the exception of tagging using acoustic or radio tags all procedures are of mild effect on the fish. Due to the need to make a 2-3 cm incision for the internal radio/acoustic tags this procedure is rated moderate. Fish are released into the wild at the end of the procedures, however, they are still considered to be under the Act if individual identifiable fish could be used in additional procedures. In this instance they remain within the Act until they die (of natural

	causes).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The aim of the project is to examine natural behavioural patterns on free-living wild fish. This cannot be carried out by any other means than using fish either from the wild or released into the wild from hatchery stock.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experimental and behavioural studies undergo a pre-assessment statistical power analysis to determine the numbers of experimental animals required to meet the objectives of the study. Where studies involve estimation of population parameters and comparative mortality rates, we have used a power analysis based on the binomial distribution. The analysis sets a target power and precision, for example we want to be 80% certain of detecting a 40% mortality rate, and then uses a set of scenarios to determine the minimum number of fish required to meet that target. The scenarios make use of known ranges of mortality rates at each life stage (e.g. 3-6% for marine return rates) and for each scenario outputs the number of tagged fish required. . The power analysis has shown that estimating an accurate marine return rate (typically 3-6%), which is the most difficult yet the most valuable rate to estimate for salmonids, requires a minimum of 10,000 fish per year to be tagged or marked. With population sizes that may exceed 150,000, this corresponds to under 7% of the population. Likewise, batch-marking fish through immersion (calcein) needs to be carried out on large numbers of fish in order to increase the probability of recapture later, either at the same site or after dispersal throughout the river.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The purpose of the work is to establish the natural behaviour of fish. As such it is vital that the fish used are acting in a normal manner. At all stages of the marking and tagging procedure therefore the welfare of the fish is paramount and any fish showing abnormal behaviour or not fit for purpose when captured will not be used in the procedure. Fish will only be re-released after Protocols if they are deemed suitably fit by a competent person. If the abnormal behaviour could have been caused by our protocols the fish will be killed by a schedule 1

	method. Most of the personal licence holders in the group have long experience (minimum 10 years) in handling fish and are well qualified by that experience to determine fitness-for-release status of fish. On-going assessment will be made regarding improving and refining methods used.

Project 5	**Mechanisms underpinning cooperation**	
Key Words (max. 5 words)	Cooperation, assortment, neuroendrocrinology, guppies, evolution	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Understanding how a behaviour where an individual pays a cost at the benefit of another, unrelated individual is maintained in a population has remained an evolutionary enigma over the course of the last century. The main objective of this work is to understand the behaviour and the biology that underpins the evolution of cooperation among unrelated animals of the same species.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This work provides a rigorous, empirical test of a decade of theoretical work that has proposed both passive and active ways in which cooperative individuals can cluster in populations and thus support the maintenance of cooperative behaviour. It will also allow us to understand the biological processes underpinning social interactions resulting in and stemming from cooperative behaviour.	
What species and approximate numbers of animals do you expect to use over what period of time?	Guppies *Poecilia reticulata*; 2500 fish over a 5 year period.	
In the context of what you propose to do to the animals, what are the expected adverse	The procedures involved in carrying out the work are mild in severity. Possible adverse effects include transient periods of mild stress, a low risk of puncture	

effects and the likely/expected level of severity? What will happen to the animals at the end?	wounds during marking and an extremely low risk of mortality during application of anaesthesia. At the end of experiments animals will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Key goals of this project are to test predictions resulting from decades of theoretical work and to understand the processes underpinning behaviour. Both of these require using live animals as the field cannot really advance in this case without empirical work. However, in cases where we can avoid using animals we have planned for this. For example, rather than use live animals to investigate how social network structure manifests itself as a result of the behavioural processes we are investigating, we will use simulation models that we have previously developed for the study system. Although something akin to conditional movement strategies appears to be active in humans, more complex mechanisms are likely to be at play to support the success of these strategies. Guppies pose a vastly simpler system where we can run the empirical work that will, for the first time, test decades of theoretical work which suggest that simple behavioural rules can underpin the evolution and maintenance of cooperation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experimental design is supported by pilot work and work published in peer reviewed literature throughout to assist with ensuring we use appropriate sample sizes. However, we will continue to monitor this throughout the project by calculating effect sizes and test power and adjust our sample sizes accordingly.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The Trinidad guppy is ideally suited for the proposed work. Cooperation is well studied in guppies and occurs during predator inspection behaviour whereby individuals leave the relative safety of a shoal to approach and inspect a predator, gaining information on the predator's state and on the probability of attack. This information is transmitted to non-inspecting individuals and provides adaptive benefits to all shoal members. Inspectors pay a personal cost of increased risk of predation, which they can reduce by inspecting in cooperative partnerships. Guppies show consistent individual variation in cooperative behaviour during predator inspection which can be reliably measured. In our previous work we have shown that social networks in wild populations of

	guppies differ significantly from patterns expected with random interactions; individuals form stable social relationships based on active partner choice, which results in stable social communities. Our recent work on a wild population of guppies suggests that behavioural traits associated with cooperation during predator inspection may have an important role to play in structuring social networks. In particular we observed that the population social network was significantly positively assorted by the tendency of individuals to exhibit bold behaviour in the presence of a predator. These attributes of the guppy system make them an ideal model species for fulfilling the objectives of this project. We have very clear protocols in our lab for minimising suffering including: 1) housing experimental animals socially and limiting any periods without at least visual contact to social partners during experimental trials to a maximum of 15 minutes; 2) monitoring fish daily for general health and any signs of stress and 3) always providing fish with a refuge in home and experimental tanks. Laboratory users must go through an induction process before they can begin work in the lab, which includes training in animal handling.

<table>
<tr><td>Project 6</td><td>Early-life adversity and adult cognition in starlings</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Starlings, early-life adversity, welfare, behaviour, resilience</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We know that adversity experienced in early life can have long-term effects on health and behaviour in adulthood, both in humans and in other animals. In this project, we seek to understand more about how animals respond to such adversity. We will use starlings because we know a great deal about the natural biology of these long-lived birds, and it is possible to cross-foster chicks from one nest to another. This allows us to give pairs of siblings different early experiences by cross-fostering them to different nests with different competitors or food supply. This is an experimental design that would not be possible in other species. We will follow the consequences of early experience through into adulthood.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The potential benefits are two-fold. First, we hope to shed light on the effects of adversity on brain and behaviour in other animals, including humans. Greater knowledge in this area will help us understand the biological and psychological consequences of early life adversity, and how these might be mitigated later in life. Second, songbirds</td></tr>
</table>

	are widely kept in captivity, as pets and for scientific purposes, and our project has the potential to help us understand how to optimally raise and keep them in order to maximize their welfare and longevity.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use wild European starlings (*Sturnus vulgaris*) from wild breeding colonies in the local area. Our study will involve up to 800 birds over 5 years. However, for the majority of the birds, their involvement will only be being cross-fostered to a different nest, being weighed, and having small blood samples taken before they fledge. Once they fledge, they will live in the wild as normal and not be caught again. A subset of these birds will be caught and brought into captivity prior to fledging. In addition, we will hand-rear a small number of chicks from soon after hatching.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Starling parents do not learn the identities of their offspring until just prior to their chicks fledge, meaning that chicks can be cross-fostered between nests without parents noticing. Being placed into a nest with more or larger competitors does have adverse effects on early growth rates and biomarkers of future health. However, these effects are subtle and thus the severity is mild. The conditions that we will expose the birds to will be in the natural range found in wild starlings. As many birds will have their conditions improved by the cross-fostering as will have them worsened. The small blood samples we will take are stressful for the bird but there is no evidence that they cause lasting harm; the severity is thus mild. Most birds will remain in the wild after procedures. There is good evidence that disturbance of starling nests post-hatching does not cause abandonment by parents or affect growth rates of chicks. Those birds that come into captivity will live in social flocks in large aviaries. They will periodically perform behavioural experiments. These can involve being handled by humans, social isolation in cages, short periods of food deprivation, eating bitter foods, and exposure to images of predators or other novel stimuli. The severity of these experiments is moderate. An individual bird may live in captivity for several years and will take part in multiple trials for the purpose of measuring different aspects of their behaviour. Individual birds will spend less than 50% of their time being tested and

	will receive breaks of at least a week in the aviary between tests. Birds that have been brought into captivity as nestlings would not be competent to survive in the wild. These birds will either be euthanized or rehomed at the end of the project.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Since we are interested in the impact of adversity on animals, we cannot complete this project without using live animals. Starlings have a lower level of neurophysiological sensitivity than non-human primates, which would be model species of choice. Our questions require a long-lived species with sophisticated cognition. Thus, we cannot use an invertebrate organism or standard rodent model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We design our experiments around pairs of siblings who experience divergent early conditions. This is a highly efficient design as all confounding factors (such as genetics) are controlled, and the individuals only differ on the factor of interest. We constantly strive to minimize numbers of animals by designing experiments efficiently. Typical numbers of animals brought into the lab in a given year will not exceed 40 individuals. Most of our animal numbers (total 800) comprise birds that remain in the wild following study of their development in natural nests.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use starlings because our scientific objectives require a long-lived species with natural genetic variation in which we know the natural range of variation in early-life experience. Starlings are abundant in the local area. We have provided over 100 nesting boxes, and most of the chicks hatched in these fledge and live normally in the wild. Starlings are widely studied and their natural biology is extremely well understood. Unlike mammals, starling parents do not recognize their young and so it is possible to perform cross-fostering experiments without causing abandonment or long-term effects on the chicks from the cross-fostering process *per se*. Wild starling parents tolerate visits to their nests by humans well. Starlings readily take part in complex learning experiments, for which the methods are already developed. We have many years of experience in providing appropriate diets and

	husbandry for starlings in captivity. Mortality in captivity is extremely rare. We keep our birds in large aviaries most of the time. When individually caged starlings are usually in visual contact with other birds. Our testing procedures are designed to minimise the amount of catching and handling possible, for example by training birds to weigh themselves on electronic balances.

Project 7	**Phenotypic programming across the lifespan**	
Key Words (max. 5 words)	Ageing, hormones, environmental change, stress, development	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The main aim of this research is to determine the importance of early life conditions in shaping adult brain, physiology and behaviour. Importantly the work will investigate how early life experiences interact with adult experiences to influence these traits. Another significant focus of this study is trying to determine how early life conditions might affect how fast an animal ages, and we will do this by tracking individuals over their entire life cycle, Finally we will determine if the effects of early life and adulthood conditions are felt in the subsequent generations.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Neuroscientists have become increasingly interested in how the brain changes over the course of the ageing process and into senescence, as this can be highly relevant to several human pathologies. However, there is a paucity of studies that have tracked individuals from known developmental backgrounds through adult life to explore the interactions between environmental conditions at different life cycle stages. The developmental origin of health and disease is an important focus for a range of research areas, including those working in basic biomedical and clinical sciences. This work will provide a novel	

	opportunity to gain an understanding of not only how early life can program adult susceptibility to disease pathologies, but also how the environment experienced during adult life can interact with the developmental phenotype to shape the potential for predisposition for a range of disorders, including stress related disorders and cognitive decline. Data collected on endocrine responses to experimental manipulations could also feed into already well known models of diseases such as depression, cardiovascular disease and diabetes. Animal welfare scientists have increasingly adopted behavioural and physiological measures, such as cognitive performance and stress reactivity, for assessing the wellbeing of animals. This project will add to their understanding of how such traits and several others relate to both adult and developmental environmental conditions. This could facilitate direct changes to animal husbandry protocols that would feed into the 3Rs priorities.
What species and approximate numbers of animals do you expect to use over what period of time?	Avian species. In many cases our experimental groups will consist of between 15-20 animals per group, and we will typically have 2-8 groups within an experiment. We will use a maximum of 1200 embryos and 1500 juveniles/adults over the 5 year life of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We are interested in how individuals respond to natural variations in environmental conditions during their life and therefore all manipulations will be carried out so to induce changes in environmental conditions that mimic those experienced in the natural environment. This will minimise suffering in our study animals and allow us to determine how developmental conditions impact on normal physiology and behaviour. The majority of our procedures are not expected to provide anything other than mild stress or momentary pain during the collection of blood samples or during injection, and indeed it is important that stress is kept to a minimum because this itself could affect the animals normal behaviour. Where blood samples need to be taken amounts obtained will be such that the health and well-being of the animal is not affected. In the few studies where tissue, such as the brain, will be collected animals will be killed humanely prior to this. In addition we will closely monitor the health of all animals and humanely kill any animal that appears to be suffering during the procedures. The fact that we

	will manipulate conditions within the natural range experienced for a given species means that we will minimise any potential adverse effects. Animals that experience negative developmental conditions, for example an increase in stress hormone exposure or reduced food availability, may exhibit reduced growth rates, however, our previous work suggests that the at level of manipulation we intend to use, these effects are slight and cause minimal suffering. We have taken several other measures to minimise suffering and the number of animals that will be used in this research. In many cases we will be able to directly manipulate the pre-natal environment, for example by injecting stress hormones into an egg, without causing any distress or suffering to the mother, reducing the overall numbers required to fulfil our objectives.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to properly meet our experimental objectives experimental adjustment of developmental and adulthood conditions is necessary as is the long-term monitoring of physiological traits into adulthood. Therefore the need to follow living animals through time in this research is essential, since a major aim of the project is to understand how animals respond to environmental conditions and how these can influence a range of important traits in later life.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Sample sizes of animals used in experiments are always determined carefully via statistical power analyses to ensure the maximal statistical power to detect relevant differences between treatment groups. In addition, continued use has been proposed to allow animals to be tracked between developmental and adult conditions, minimising the number of animals used. In addition, we will ensure that maximal use will be made of all tissue/samples collected from animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	We will use birds as a model in this research as we know a great deal about their developmental trajectories, ageing rates and physiology and behaviour. An important feature of avian species is that they overcome a major constraint of mammalian studies that look at the long-term effects of developmental conditions, namely the direct link between the mother and developing animal during both gestation and lactation. This prolonged link, limits the ability to determine the exact conditions

(harms) to the animals.	experienced by each animal and/or the effects of subsequent maternal behaviour on later physiology and behaviour. The applicant has been working with avian species since 1996 and therefore is well placed to ensure the welfare

<table>
<tr><td>Project 8</td><td colspan="2">Environment, phenotypic development and performance</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Development, ageing, telomeres, stress, growth</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2"></td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>x</td><td>Basic research</td></tr>
<tr><td></td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td>x</td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td>x</td><td>Preservation of species</td></tr>
<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td></td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">We still understand relatively little about how the environment can shape the lives of individuals. The overall aim of this project is to understand how environmental conditions influence development and subsequent performance, particularly with respect to behaviour and physiology and the rate of ageing and also at the organismal level (growth, size, reproductive performance, longevity, age related changes in performance, differences between the sexes, and the pattern of senescence).

The objectives are as follows:

1. To understand how variation in environmental conditions (nutrition, stress exposure, social conditions), during different life stages, within the natural range, influences performance at physiological, cellular and organismal levels.
2. To examine the extent to which changes in growth rate influence longevity and ageing markers.
3. To examine how markers of ageing change with body size.
4. To examine the extent to which sex differences occur in terms of responses to early environmental circumstances.
5. To compare the rate of ageing in differentially stressful environments.</td></tr>
</table>

	6. To compare the effects of environmental circumstances in early life in short and long lived bird species.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will advance our understanding of the mechanisms that link conditions in early life to performance later in life. This is beneficial both in terms of advancing the science of ageing and development, and understanding the long term effects of growth conditions and stress exposure. This will be beneficial to both humans and other animals. Humans and many other animals can experience early life adversity due to food shortages, inclement weather, poor physical environments, or being a subordinate. The results of this early life experience can persist long after the conditions have been ameliorated, and in some cases effects may be lifelong and reduce lifespan. This project aims to understand the processes whereby these long term affects can occur, and may assist in the development of mitigating treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will last for five years and involve studies of wild and captive birds at different life stages and living in different environmental circumstances. The expected number studied will be in the region of 3000 zebra finches and 200 quail. This is based on number used in our previous studies. Numbers of wild birds are more difficult to predict, since this very much depends on the number of known aged individuals that can be captured at suitable study sites. Numbers are likely to be less than 1000
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals will live or be kept in variable environments, mimicking or representing the range of circumstances the encounter in nature. Various parameters will be measured from small blood samples. The quality of the diet and hormone levels may be manipulated, always within the range to which the animals have evolved coping strategies. The animals will either be allowed to live out their natural lifespan or be humanely destroyed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project is concerned with individual level outcomes for fitness, and thus replacement is not possible.
2. Reduction	The number of animals will be kept to a minimum by

Explain how you will assure the use of minimum numbers of animals	consideration of the appropriate sample sizes based on the magnitude of the expected effects. We do not plan to study very small effects that can only be measured by very large sample sizes.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	For experiments with captive animals, we have chosen species that breed well in captivity. Some of the work requires samples from wild animals living in variable environments, and these animals will be released at their capture site following small blood samples. All people involved in the work will be very experienced at handling and sampling animals and in undertaking any manipulations required. Experienced animal housing staff will ensure that the animals are kept in enriched environments to the highest possible standards and with veterinary care if required.

Project 9	**Behaviour of predators in conservation landscapes**	
Key Words (max. 5 words)	Badgers, foxes, predators, management, conservation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
	x	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim is to understand how wild mammal predators use landscapes in nature reserves, where they may come into conflict with conservation practice. The objectives are to: 1. Determine the relationships between landscape and conservation management and the movement of predators in pace and time. 2. To use data to see if land management can reduce predation rates to benefit species of conservation concern. 3. Quantify individual differences in resource use within species, to understand whether all individuals are equally likely to predate species of conservation concern. 4. Where possible to track badgers and foxes simultaneously, to understand how these species interact within the landscape.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	Understanding the temporal and spatial movements of predators in conservation landscapes could provide valuable insights into how best to manage predators and their landscapes to limit their impacts on species of conservation concern. Lethal control	

project)?	and predator fencing are resource heavy and limited in terms of the predator species they can influence and the scale at which they can be applied. In contrast, managing landscapes so that predators and prey can coexist is a desirable and sustainable option but can only be derived through a thorough understanding of how landscapes are used by both predator and prey.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 30 foxes and 30 badgers over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse effects can come from trap injuries or long term attachment of collars. The level of severity can be moderate. However, the expected level of severity in lower if trap injuries are minimised by good practice and the most appropriate collar and fitting is applied and not attached to animals for long periods of time.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As our aim is to investigate wild predator spatial ecology in conservation areas, it is imperative that we collect all our data from wild animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Minimum sampling effort will be determined by power analysis. Repeated analysis of data after each trapping period will show if sufficient data has been collected and at which point trapping will not continue. Hence we aim to use the minimum number of animals needed for the analysis.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Badgers and foxes are the two predator species which have been recorded to have impact on breeding success of wetland birds of conservation concern. We will minimise harm in the following ways: We will use cage traps specifically designed for red foxes and badgers. Traps will be checked every 6 hours to reduce time in trap and chance of injuries. Animal stress and disturbance will be minimised during processing. Radio-collars used will be specifically designed or modified for the species. Only non-pregnant, adult animals deemed in good

	condition will be collared. Animals will be carefully observed throughout the recovery process and assessment of collar fitting will undertake prior to release and post release via camera trapping. We will have access to 24 hour ‘on-call’ veterinary support during trapping. Collars will be on animals for a maximum of 6 months. Collars are fitted with a remote trigger for release (drop-off mechanism), this can be programmed to drop off at a specific date, if this fails multiple attempts will be made to remove collars from animals.

Project 10	**Kinship and recognition in a social bird**	
Key Words (max. 5 words)	kin selection; cooperation; inbreeding; social evolution	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Recognition systems are ubiquitous in nature and play a critical role in interactions at every level of biological organization. In social species, the ability to discriminate among other individuals of the same species determines the adaptive nature of behavioural decisions. Long-tailed tits discriminate kin from non-kin in two contexts: (1) helping decisions in their kin-selected cooperative breeding system; and (2) pairing decisions for inbreeding avoidance. Previous work has shown that adults recognize kin using calls that are learned during early development. Objectives: (i) To determine parentage and relatedness of members of an established study population using microsatellite genotyping of DNA samples collected in the wild. (ii) To record calls of birds of known relatedness to determine how vocal similarity varies with social and genetic relatedness. (iii) To determine whether helping and pairing decisions are positive and negative functions, respectively, of call similarity. (iv) To model fitness pay-offs from alternative helping and pairing decision rules, using fitness estimates measured in the field.	
What are the potential benefits likely to derive from this	The ability to discriminate kin is extremely important in social organisms, yet the mechanisms involved are	

project (how science could be advanced or humans or animals could benefit from the project)?	poorly known, especially among vertebrates. This will be the first study to investigate the similarity of recognition cues in the contexts of helping and mate choice and the fitness consequences of alternative discrimination rules. Kin discrimination in cooperative behaviour has been described in this species, although the mechanism for discriminating different degrees of kinship is unknown. Moreover, the recent finding that long-tailed tits actively avoid inbreeding creates the opportunity to study the role of recognition cue similarity in pairing decisions in the field. Current research on estimation of inclusive fitness for a very large sample of birds, also allows evaluation of the fitness pay-offs from alternative kin discrimination rules.
What species and approximate numbers of animals do you expect to use over what period of time?	Long-tailed tit *Aegithalos caudatus* c. 250 individuals per annum for 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The protocols require a mild procedure that entails short-term capture of wild birds and that has no adverse effects on the animals in the long term. All animals subject to regulated procedures will be released to the wild following assessment of their wellbeing.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no non-animal alternatives that could be used to address the objectives of this project. In addition, our current knowledge of the study species makes it ideally suited to achieving the project's aims.
2. Reduction Explain how you will assure the use of minimum numbers of animals	It will be necessary to obtain a DNA sample from all individuals in the study population, but each individual will be sampled on a single occasion.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general	The long-tailed tit is a model system for the study of social evolution. This project builds on the findings of long-term study (since 1994) of the behaviour and ecology of this species, providing a robust rationale for the objectives described above. The regulated procedure required (DNA sampling) is mild and has no long-term effect on the animals, whose welfare is

measures you will take to minimise welfare costs (harms) to the animals.	paramount for the collection of long-term behavioural and life-history data.

Project 11	**Fish developmental biology and behaviour**	
Key Words (max. 5 words)	Maternal, stress, welfare, nutrition, conservation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Objective 1: To understand how factors such as parental condition, diet, social competition can affect physiology and behaviour in fish, and thus the welfare of fish held as pets, in aquaculture, for research and in the wild. Objective 2: To understand how changes in the natural environment, e.g. temperature, oxygen tension, pH, presence of contaminants can affect the physiology and behaviour of fish and therefore their conservation.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Objective 1: Understanding the factors which affect physiology and behaviour in fish is of crucial conservation importance and for determining the best ways to protect their welfare. We now know that if a mother fish is stressed prior to breeding that this will influence the condition of her offspring, and that this can be induced by mild husbandry stressors such as moving fish to clean their home tank. The ornamental fish trade represents a multi-million pound industry where millions of fish are transported and held as pets. Ornamental 'pet' fish (e.g. guppies, zebrafish, neon tetras) lead very different lives to fish such as salmon and trout held in aquaculture, and yet many of the commercial diets available for pet fish are based on nutritional information for large aquaculture fish. Very little research has looked at nutrition, husbandry, stocking and transport of ornamental fish in terms of their welfare. This project looks at ways of improving fish welfare by increasing our understanding of fish behaviour	

	and physiology and how non-invasive methods can be applied to assess fish welfare (e.g. observing behaviour, measuring hormones released into the water and taking mucus samples). Objective 2: Many natural environments of fish are being altered by human actions. Major impacts of environmental change on fish may be easy to detect but many subtle changes to fish behaviour which have previously been ignored, can have significant implications. For example, a small change to a fish's behaviour caused by exposure to a very low level of a contaminant may not seem particularly important within controlled experiments. However, if that change in behaviour prevents a fish from avoiding predators or from reproducing, then that very low level exposure has the potential to have terminal effects for natural fish populations. This project will highlight the fact that small behavioural changes should not be ignored and should be considered by regulators as important factors in understanding toxicant impact. Exposure of early life stages of fish (i.e. before first feeding) to contaminants can also cause subtle changes in behaviour. The use of early life stages to consider the effects of aquatic contaminants has two main benefits; firstly effects can be detected in those life stages most susceptible and secondly the use of sentient animals for toxicity testing can be significantly reduced. With newly emerging aquatic contaminants (e.g. nanoparticles and pharmaceuticals), it is important that we understand whether very low levels of these contaminants have the potential to affect the behaviour and physiology of natural fish populations. Accompanying this, is the need to understand how other environmental changes can affect fish, in a climate that looks set to chanqe significantly in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	The fish species used in this project will be mainly freshwater fish that are either considered to be model species for fish behaviour and physiology (e.g. zebrafish and trout) or are important commercial species (both for aquaculture and the ornamental fish trade). Over a year period, no more than 2000 fish are expected to be used; this will decrease as early life stages are used instead.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What	The overall classification of the project is mild. The majority of work will involve exposing fish to mild stresses that they would naturally encounter in the wild or in an artificial environment (e.g. in aquaculture or as pets) and observing changes in behaviour. The project also looks at the effects of toxicants on fish behaviour; however, very

will happen to the animals at the end?	low levels of contaminants are used to look for subtle changes in behaviour that may occur, therefore it is unlikely that adverse effects will be seen. However, due to the nature of these experiments they are classed as moderate. Effects from this project could include adverse changes in behaviour, lack of feeding, distress (loss of equilibrium/swimming ability, laboured respiration). Any fish showing signs of these adverse changes will be immediately removed from the experiment and killed by a schedule I method if recovery is unlikely. It is anticipated that the majority of fish from this project that have only been exposed to mild stresses will be released from the act either to stock or re-homed as companion animals where possible.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Currently there are no alternatives for studying fish behaviour as this requires a whole organism response. Where possible we use early life stages (>70%) to look at the effects of contaminants, however, to understand effects on fish behaviour later in life this is not possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For all experiments associated with this project, experimental design will be based on statistical planning including power analysis. The degree of individual variation in physiological and behavioural parameters relative to the age of fish will be analysed by ANOVA models as the experiments are carried out and results collected to ensure that animal numbers are minimised and robust data sets are collected.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	For work with embryos, many measurements can be made prior to hatch with only — 25 % becoming licensed animals. The fish used will be mainly freshwater species that are either models for studying fish behaviour and physiology (e.g. zebrafish, trout) or are important commercial species (both for aquaculture and the ornamental fish trade; e.g. guppies, barbs, neon tetras). The majority of work within the project is mild, looking for subtle changes in physiology and behaviour. Fish will be closely observed during experiments, to ensure that there are no adverse effects of treatments. Where effects of environmental change, such as contaminant exposure, are considered, only mild effects are anticipated due to the low concentrations used. Exposure levels will be based on existing literature, and carefully selected to look for subtle changes in fish behaviour and physiology.

Project 12	**Behaviour and ecology of wild birds**	
Key Words (max. 5 words)	Birds, behaviour, ecology, mating, evolution	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
	X	Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives are to understand factors which lead to individual variation in reproductive success in birds. We do not know, for example, how individuals vary in their response to environmental stressors, such as pollution, which may affect their ability to produce offspring, and may have an impact on their costs of reproduction.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We are likely to have a better understanding of the factors which influence reproductive success in individual birds in the wild. Taking a small blood sample can provide a wealth of data, including parentage, sex, indicators of stress and genetic information. Such information can be used directly to inform population models which provide evidence used for conservation practice.	
What species and approximate numbers of animals do you expect to use over what period of time?	Wild birds, mainly blue tits and dippers. Approximately 500 blue tits and 150 dippers each year.	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will	The effects are likely to be minimal. Adverse effects might include temporary bleeding, mild bruising and brief pain felt from a needle. The likelihood of parents deserting their nests due to the procedure is very low (no cases in over 1000 nests studied so far).	

happen to the animals at the end?	At the end of the procedure, animals will be returned to the wild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The research questions focus in the behaviour and ecology of the birds *per se*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This is an epidemiological study of individuals within a population where all individuals are sampled. Generally, the effects we are looking for are likely to be small and require large sample sizes to detect statistically. We will monitor the results as the project progresses, and cease procedures when the statistical analyses are robust (either negative or positive).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	These procedures are carried out by trained personnel with experience from over 30 years in the field. It is also important to the study that the animals return to their normal behaviour quickly and do not suffer long term effects from the procedures. Although some of the traits could be measured from taking a few plucked feathers, blood sampling is the least invasive method which provides us with all the data we need (DNA, blood parasite detection, hormone titre).

Project 13	**Host-parasite dynamics in wild rodent populations**	
Key Words (max. 5 words)	zoonoses, wildlife disease, ecology	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will investigate host-parasite dynamics in natural populations of small mammals. Wild animals can be important reservoirs of disease for humans, domestic animals and endangered species. The emergence of disease from wildlife to humans has been linked to environmental change, including the destruction and fragmentation of natural habitats. It is therefore important to understand host-parasite dynamics in fragmented populations of wildlife. Disease prevalence within fragmented wildlife populations will depend on both parasite characteristics and the movement patterns of wildlife between populations. Differences in factors such as length of infection and a parasite's ability to infect other species mean that different parasites species or genetic variants are predicted to respond differently to fragmentation of the host populations. Similarly parasites that can infect wildlife species that move more frequently and further are predicted to be less influenced by fragmentation than diseases of less mobile wildlife species. Specifically this project will use data from wild rodent	

	populations and a range of parasites to: • determine how the current and previous size and distribution of wildlife populations influence the presence and prevalence of parasites and how this varies between parasite species and genotypes • identify the factors that influence movement rates between fragmented populations of small mammals • show how parasite characteristics and movement patterns of wildlife interact to influence the persistence of different parasite species and genotypes within fragmented populations
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Diseases that are transmitted from animals to humans (zoonoses) pose an increasing public health threat in many parts of the world. Many of these diseases originate in wild animals (e.g. leptospirosis, lyme disease). Diseases can also transmit from wild animals to livestock and from common wild animal species to endangered species. By increasing our understanding of disease dynamics in wild animals inhabiting natural landscapes, this project will provide useful information for management issues ranging from conservation to human and livestock health. Ultimately, this research will hopefully benefit public and livestock health through improved risk predictions for zoonotic diseases. In addition to the benefits related to zoonotic diseases, this research will also have direct benefits for water vole conservation. The research will provide further information for reintroduction and translocation programmes on water vole dispersal patterns and the parasites that can infect water voles.
What species and approximate numbers of animals do you expect to use over what period of time?	Water voles (max 1500), field voles (max 700), bank voles (max 350), wood mice (max 350) 5 year project
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The wild rodents will be live-trapped and a blood sample and small tissue sample collected using an appropriate method. Most animals (>90%) will only have a blood sample collected once. Some animals will have a blood sample taken on more than one occasion, with a maximum sampling frequency of once per month. Each sample is small, ensuring few adverse effects

	and a mild severity level. Consequently, animals can be released back immediately at the site of capture, minimising any suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The only way to investigate disease dynamics in fragmented wildlife populations is to study disease directly in natural populations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Pilot trapping prior to any licensed procedure to ensure that sites with appropriate mixes of host species are selected. Annual analysis of data to enable targeting of population sampling based on project objectives. For example, targeting of populations with specific geographic locations (very connected populations vs very isolated populations). To maximise power and the information that can be obtained from the studies, we will use advanced statistical approaches (e.g. Generalised Linear Mixed Models, Generalised Additive Models, Bayesian approaches). As the same samples are used to test for many diseases, overall this programme of research will use fewer animals than a similar research programme that focussed on one disease.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The water vole is probably the best available system for such a study due to the range of population structures that occur. The other species that will be sampled are the species that are most likely to share parasite species with the water vole. All of the species can be easily handled, facilitating sample collection and minimising any distress. The team has extensive experience of working with and handling wild animals. This experience again minimises any distress. The stresses placed on individual animals are minimised through good training and efficient field technique. This is essential for both ethical reasons and to ensure we have as little impact as possible on the natural processes under study. Appropriate traps are used depending on the study animal Traps are checked at least daily, baited with sufficient food, placed in suitable locations to minimise any possibility of flooding and covered to

	hide them from predators. Methods of identification used will be the most refined for the study and species are used. The blood samples collected are small, allowing animals to be rapidly released back at the precise site of capture. Obtaining a blood sample under extremely wet conditions is difficult and prolongs handling times. To minimise stress blood samples will not be taken under such circumstances.

Project 14	**Linkages between invasive plants and salmonids**	
Key Words (max. 5 words)	Riparian invasives salmonids	
Expected duration of the project (yrs)	3 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
	x	Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Non-native species of plants are commonly found along the banks of rivers and streams in the UK. These zones (termed riparian) are naturally dynamic and critical in maintaining connectivity between aquatic and terrestrial habitats but are also highly prone to invasion as they provide ideal conditions for the establishment and distribution invasive species. Non-native plant species in the riparian zone can change the conditions in the river by effecting the amount of shade and changing the nutirient and silt inputs. Native salmonid fish species (Atlantic salmon, *Salmo salar* and Brown trout, *Salmo trutta*) have specific habitat requirements and are strongly affected by the physico-chemical conditions in rivers. Both theory and expert judgment indicate that seasonal alterations to channel shading, organic material input, channel shape and bank stability resulting from dense stands of the three common invasive riparian plant species, could cause changes to these native fish communities, whether directly or indirectly. Despite various government initiatives, and considerable efforts and resources being invested by river and fishery managers to address the spread and extent of riparian zone coverage by invasive plant	

	species in Scottish catchments it remains unclear (a) how much damage is actually caused to populations of native salmonids by extensive invasive plant cover in the riparian zone, or whether salmonids respond positively to management of riparian invasive plants. This project will explore the type and magnitude of response by salmonid fish species to cover of invasive riparian plants in terms of ecology, population persistence and viability. The specific objectives are to: 1. Assess the impact of varying type and cover of invasive riparian plants on the local habitat suitability for and juvenile salmonid fish species and their prey. 2. Identify the role of invasive riparian plants in determining population abundance, structure and growth parameters in native juvenile salmonids. 3. Identify and quantify the mechanisms underpinning changes in channel morphology, aquatic invertebrates and salmonid populations that are attributable to RIPs. Responses within salmonids will be investigated using quantitative population sampling, growth rate measurement and gut content analysis.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Stocks of native salmonid species (*Salmo salar* and *Salmo trutta*) salmon, are important from a national conservation perspective locally as an economic resource, and require careful management. Both species have local conservation concerns (i.e on local Biodiversity Action Plan lists) and salmon angling is economically important in some areas, bringing in important revenue to many rural areas and supporting local jobs and businesses. A huge amount of work is currently being undertaken in order to improve and restore the quality of UK stream habitats with a view of protecting and maximising the production of native salmonid species. This project will increase our knowledge of the possible effects of invasive plants as a limiting factor on the production of salmonids in streams and identify and focus where possible actions can be taken to assist with species recovery and protection activities. Several major UK river catchments are designated as Special Areas for Conservation with Atlantic salmon a qualifying species in many. The management of riparian zones to conserve and protect habitat quality for the species require resource allocation and prioritisation. In the absence of reliable information to

	prove or disprove a linked effect between RIPs and salmonid fish, the control and eradication of RIP species are often considered by managers to be high priority activities that derive benefit on salmon populations. This project will benefit the future management activities in riverine Special Areas of Conservation areas by assessing the linkage and possible effects on important populations of salmonid fish, enabling managers to prioritise limited resources to riparian management and restoration activities that will lead to maximum benefit.
What species and approximate numbers of animals do you expect to use over what period of time?	Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). A maximum of 2400 of each species will be used for the assessment of diet selection by flushing of stomach contents
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Fish will be carefully anaesthetised and handled during the stomach flushing procedure, and following close monitoring of the recovery from the anaesthetic and release back into the wild , it is expected that individuals will resume feeding quickly and will not suffer any long lasting adverse effects of the procedure. The electric fishing sampling methods and anaesthesia are routinely carried out for fish population study for fisheries management activities and no adverse effects are expected, or have been noted in similar studies. Animals will be returned to the wild alive.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Changes to feeding behaviour and prey selection in the target species are important considerations to include as possible processes that can exert control over juvenile salmonid production. The possible effects of riparian invasive plant species cover cannot be adequately investigated without sampling fish in the wild to estimate their population density, the rate of growth and their feeding behaviour. Examination of the gut contents of a sub-sample of *S. salar* and *S. trutta* at sites with and without riparian invasive plant species will provide important sights into the local availability and selection of prey species, and its possible links to changes in growth and population status. Changes in the diet selection, foraging behaviour and nutritional intake of the target species brought about by the presence of riparian invasive plant species

	may be influencing an individual fishes growth rate, its' risk of mortality and the productivity of an area in terms of the salmonid standing stock (biomass) . This may lead to changes to the number of fish that subsequently successfully enter the adult population and contribute to species long-term viability. Changes in juvenile salmonid production may be mediated directly by changes to the composition and abundance of forage items available in the presence of riparian invasive plant species through alteration of the habitat suitability for forage species. Diet changes in fish may also occur directly in response to changes in the physical habitat resulting from the presence of riparian invasive plant species , and their effect on fish foraging behaviour and prey selection due to changes in shading.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The range of available prey species and their relative abundance are likely to vary considerably, both between sites and within a site over time, and any attempt to replicate or model the feeding behaviour in a controlled laboratory situation without the use of live wild fish would not enable this potentially important mechanism to be adequately tested. Currently there is no local information available for the sites to be sampled that can be used to provide statistical validation for the selection of a suitable sample size of fish to be examined for gut contents analysis. No information is available on the range of prey items naturally available (and seasonal change) at the locations, or the expected occurrence, volume and abundance of prey items in individual fishes stomachs. To this end, it is proposed that this portion of the project will be treated as a pilot study in year 1 (guided by published studies indicating a sample size of 30 individuals of each target species (*S. salar* and *S. trutta*) per site and review sampling in year 2 on the basis of information obtained at this time.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In order to investigate the mechanisms by which Riparian Invasive Plants potentially effect the population status of native salmonid species it is not possible to consider an alternative method that does not evaluate the feeding preferences of individuals in situ, and relate this to the composition of the available prey items under varied levels of RIP cover. The range of available prey species and their relative abundance are likely to vary considerably, both between sites and within a site over time, and any attempt to replicate or model the feeding behaviour

	ex situ without the use of live fish would not enable this potentially important mechanism to be adequately tested. Animal suffering will be minimised by: 1. Restricting the investigation of fish diet to two times of the year. 2. The capture of fish using electric fishing sampling methods will only be carried out by fully trained operators. 3. The safe and controlled administration of anaesthesia following the University of Stirling protocol. 4. The initial checking for clinical signs that fish are injured or in poor health following sampling using electric fishing methods. Fish considered to be suffering due to ill health, severe physical wounding, or in a moribund state will be swiftly euthanased using Schedule 1 methods. 5. Repeated observation of fish during the procedure and recovery period. Fish considered to be suffering or showing adverse effects will be swiftly euthanased using Schedule 1 methods.

Project 15	**Behaviour of fish in the natural environment**	
Key Words (max. 5 words)	Fish, telemetry, behaviour, dams, engineering	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project seeks to investigate the behaviour and movements of fishes in the natural environment Specific objectives are to: 1. Improve our understanding of the migratory behaviour and survival of fish populations 2. Improve our understanding of the use of habitats by fishes and of the influence of environmental factors and/or context of evolutionary process/physiology on their use of space. 3. Measure migration patterns of fishes in the vicinity of structures such as weirs, fishways, water intakes and hydropower turbines, to determine rates of passage and survival by telemetry.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will support conservation and sustainability of the UK's fisheries resources and biodiversity. It will also improve our understanding of fundamental biological processes linking behavioural ecology with physiological and evolutionary processes, using fish as model organisms. By improving our understanding of fish biology and determining what impacts (positive and negative) human interventions have on fishes, a more secure future can be provided for our fish stocks and aquatic	

	ecosystems. More specifically, the project will enhance our understanding of the way in which fish behave in the natural environment and of how human activities, including habitat alteration, dams, turbines and pollution can impact upon them. Improved knowledge of the behaviour of fish populations underpins the advice and actions needed to sustain them. Information from our previous studies in this field has influenced environmental policy in the UK and abroad, and has been acted upon by bodies such as the Environment Agency to improve environmental management practices and biodiversity protection. Similar benefits will accrue from this project.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 8000 fish (Cyclostomata, Chondrichthyes, Osteichthyes) of multiple species covered by the British check list, excluding CITES Annex A species, over the course of the 5-year project, with over 50% of these solely marked with PIT tags or conventional marks such as Visual Implant Elastomer (< 1mm x 4 mm).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Expected adverse effects are infection (low probability) and chronic tissue injury (low probability) in tagged/biopsied fishes. Conventional tagging and biopsy is graded 'low' severity, while attachment of telemetry tags, with or without biopsy is graded 'moderate'. The animals are released to the wild and so 'set free' discharged from the Act.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Study of the behaviour of wild fish in the natural environment requires the use of live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The sample size needed for any one fish tagging/telemetry/biopsy study at a given site and period is governed by the probability of recovering data for each fish and the variability in behaviour (or other study attribute) between individuals. Sample sizes need to be sufficiently large to fulfil the statistical rigour required. These requirements are discussed with statistical ecology specialists and by reference to best-practice literature, other specialist user groups and through use of statistical power software. Typical fish telemetry studies require data

	from upwards of 20 individuals at a single site and tagging period. Since some tags are lost and some fish die naturally, this requires larger numbers. With regard to 'passive' electronic tags (PIT) that are detected automatically very locally but cannot be tracked more widely, data is obtained from a smaller proportion of fish than for active telemetry tags and several hundred tags per study site may be needed. Sample size for some conventional tagging studies (needed for smallest fish or where telemetry equipment is likely to be vandalised) may need to be several thousand per site, if recapture rates are low or the study needs to recapture dispersing individuals, but these represent a small proportion of the population.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The principles of refinement have been developed widely in fish tagging protocols over recent decades and best practice, based upon published information, experience and expert workshops is followed, in order to minimise animal suffering while seeking to achieve the study objectives. The basis of tagging/telemetry is that tagged animals should behave in the same way as untagged ones, otherwise false conclusions may be drawn, so care is taken to ensure that fish collection, handling, tagging and any biopsying is carried out with minimal disturbance. Choice of species and life stage is dictated by the study aims, including species (e.g. salmon, eel), or conservation (e.g. lamprey, charr) concern. Tagging techniques are refined to suit the species, its size and habitat, and the study objectives, taking account of the best available knowledge, to minimise influence on the fish tagged. Biopsies are taken as non-invasively as possible. Care is taken in handling of fish from capture to release, only fish in good physical appearance and condition (e.g. no fish accidentally damaged in capture) are tagged. General anaesthesia is used to minimise stress during tagging/biopsy.