Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 17

Projects with a primary purpose of: Basic Research into Ethology / Animal Behaviour / Animal Biology

Project titles and keywords

1. **Visuomotor Aspects of magnetoreception**
    - magnetoreception, vision, pigeon, navigation, behaviour

2. **The Evolution of Cognition**
    - Pheasants, Cognition, Natural Selection, Evolution

3. **Neural mechanisms of fear memory processing**
    - fear memory, extinction, neural circuits, neuromodulation, anxiety

4. **The effect of genes and environment on the behaviour of zebrafish**
    - Zebrafish, behaviour, genes, anxiety, epigenetics

5. **Study of cognitive behaviour in inbred BXD mice**
    - BXD, recombinant inbred, QTL, cognition, translation

6. **Responses of marine fish to hypoxia**
    - Fish, Behaviour, Hypoxia, Physiology

7. **The Genetics of Local Adaptation**
    - Local Adaptation, Gene-flow, Blue Tit, Phenology

8. **Environmental effects on host-parasite interactions in fish**
    - Fish, parasites, global warming, environmental change, behaviour

9. **Large mammals: nutrition, ecosystems and climate**
    - Climate change, GHG emissions, animal condition, biodiversity

10. **Genomics of communication in songbird models**
    - Brain, learning, genes, zebra finch, chaffinch

11. **Studies of the spatial ecology of wild animals**
    - Wild animals, movement, gps loggers, conservation, habitats

12. **Cardiovascular function and adaption to sepsis**
    - Sepsis, Cardiovascular system, Rodents

<table>
<tr><td>PROJECT 1</td><td colspan="3">Visuomotor Aspects of magnetotreception</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">magnetoreception, vision, pigeon, navigation, behaviour</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</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>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>Yes</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 colspan="3">A variety of animals are known to be able to sense magnetic fields, but how this elusive sense works remains unknown. We will establish and validate a behavioural paradigm for investigating whether vision is a fundamental component of the mechanism.

Many species have been shown to be capable of using features of earth’s magnetic field to aid in navigation, sometimes over long distances. Despite decades of research, the physiological mechanism by which this is achieved is still not established. Several peer reviewed research articles have suggested that light, and by extension vision, are important components of magnetoreception. Our experiment aims include establishing a new behavioural model to allow us to provide a definitive</td></tr>
</table>

	answer as to whether this is in fact the case. This finding will significantly advance the field by narrowing the range of theoretical mechanisms currently proposed. We will also demonstrate the reliability and usefulness of reflexive, minimally invasive techniques, which are likely to be adopted by other researchers. More broadly, an improved knowledge of how magnetoreception actually works is potentially of great importance because it will serve as the basis for better decisions on environmental issues such as planning and impact assessment, by providing a greater understanding of the effects of manmade structures or equipment on magnetically sensitive animals, a significant number of which are currently in decline.
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 behavioural approach will serve as a basis for investigations of the morphological and molecular mechanisms that underlie the ability of birds and other animals to detect and orientate with respect to the earth magnetic field. Most importantly, an improved understanding of magnetoreception and its significance in the ecology of animals will ultimately help inform the protection of animals in their environment. The findings may also provide the basis for industrial applications such as the design of new navigational instruments and/or sensors.
What species and approximate numbers of animals do you expect to use over what period of time?	75 Homing pigeons (*Columba livia*) are projected to be used in the proposed experiments.
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 experiments are largely non-invasive and behavioural in their approach and design, involving in some cases only very brief stressful situations to provoke a response. No significant adverse effects are expected in any of the subjects for these experiments.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This is a study in which the behaviour of the bird is necessary to indicate its ability to perceive and respond to various stimuli, and the receptors and neural pathways involved can only operate normally in an intact living animal. Although several theoretical studies have been carried out to identify various possible physiological and/or neural bases for magnetoreception, a living behaving animal model is ultimately required to confirm or disprove these putative mechanisms.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposed experiments have largely been designed to rely on reflexive behaviours, which can be elicited repeatedly without harm in the same animal, thus reducing the total number of animals required by avoiding the need for a continuous supply of naive subjects and allowing for the much more powerful “within animal” statistical approach for investigating the specific responses to different magnetic and/or visual stimulation.
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.	There is a significant, extensive literature concerning the use of Homing pigeons in various kinds of experiments investigating magnetoreception in the context of their ability to orient and fly to their home lofts over large distances. In addition, there is a substantial literature on the vision and visual psychophysics in this species. For this reason, the same species has been chosen to allow direct comparisons/connections to be made with the findings reported by other researchers in the field. Homing pigeons also have the important advantage that they are common, easy to obtain and to care for, and have excellent navigation skills that are known to include magnetoreception. The experiments have been designed to be minimally invasive, requiring in most cases only a mild restraint with no lasting effects, and the reflexive nature of the behaviours involved removes the need for lengthy conditioning periods, as employed in other lab-based behavioural testing. Most previous studies of magnetoreception have been carried out on migratory songbirds with a

	limited availability and less accustomed to being held in confined cages. This experimentation could conceivably impact on their subsequent migratory success. Whereas homing pigeons are normally kept in artificial lofts and are quite used to being handled by humans.

<table>
<tr><td>PROJECT 2</td><td>The Evolution of Cognition</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pheasants, Cognition, Natural Selection, Evolution</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)[3]</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></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[4]</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>The aim of this project is to determine how cognitive abilities evolve under natural selection. The evolution of cognition is one of the most important, yet poorly understood issues in modern biology. Comparative studies inform us how species or populations differ, and from these, one can infer possible selective pressures. However, studies of how heritable inter-individual cognitive differences relate to reproductive fitness in the face of natural selective forces, and the consequent evolutionary trajectory of cognitive performance, are absent. This study will build on the well-established methods and paradigms developed in comparative psychology, cognitive science and behavioural ecology, applying them to free-living animals, taking into account factors that have previously confounded studies in this area, and so</td></tr>
</table>

	determine how cognition evolves. Pheasants (*Phasianus colchicus*) present an ideal study system with which to tackle this question. Large numbers (several hundreds) of individuals will be reared under controlled conditions, and then exposed to natural selection pressures. Being precocial, chicks will be reared without confounds of differences in parental care. During this rearing period, they will participate in a suite of automated cognitive training and testing, and individual's performance will be recorded. Conditions before and during the rearing period will be experimentally manipulated including maternal investment in eggs and their early-life rearing conditions. Crucially, these captive reared birds will be released into the wild and so exposed to natural selection in terms of their survival and mating success, which will be quantified using established methods. Surviving birds will be recaptured and bred from, producing large clutches of chicks so that heritability can be studied with robust samples of replicates. Empirical work will describe how individuals vary in their performance across a suite of cognitive domains; how such performance links to their natural behaviours; how their performance contributes to their fitness; how variation in performance is inherited; and how variation in performance is influenced by early life maternal or environmental factors, specifically hormonal and dietary effects. These are all significant steps in themselves, but the real strength of this project is that they can be addressed in synchrony in a single, free-living study system. This will provide a robust framework in which to tackle the broad question of how cognitive performance may evolve that can then be applied across a wider suite of conditions and taxa, including humans.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	This research will primarily advance basic knowledge. The study of the evolution of cognition is timely, with recent conferences organized by the Royal Society and the Association for the Study of Animal Behaviour focussing on the question. Consequently, I expect that my work in the area will lead, stimulate and prompt a broader body of

project)?	complementary studies in the field. My empirical work will describe how individuals vary in their performance across a suite of cognitive domains; how such performance links to their more normal behaviours; how their performance contributes to their fitness; how variation in performance is inherited; and how variation in performance is influenced by early life maternal or environmental factors. These are all significant steps in themselves, and I expect each to result in stand-alone publications, but the real strength of this project is that they can be addressed in synchrony in a single, free-living study system. This will permit a more robust framework in which to tackle the broad question of how cognitive performance may evolve that can then be applied to a broader set of taxa and conditions. Because of the subject and the study system, I anticipate much media interest in the research, and I will use my prior experience of interactions with the media to seize on this and disseminate my findings as widely as possible.
What species and approximate numbers of animals do you expect to use over what period of time?	Pheasants (*Phasianus colchicus*) ~1150 birds used over five years. 900 of these will be bred as part of the study. The remaining 250 will be purchased from commercial game breeders.
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. Birds have free choice over how they participate in cognitive tests. Any food deprivation prior to testing will be for a short time period <2hrs. All experimental manipulations of rearing conditions involved enhancing rather than depleting standard rearing conditions. The handling, marking and blood sampling methods are well established and have led to no long term welfare effects when deployed on ~2000 pheasants over previous years. The tracking devices deployed have been used pheasants with no adverse effects reported. The project requires birds to behave naturally, so devices are designed to have minimal impact on bird behaviour. All individuals will be released 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 other modelling since it requires information from animals living in the wild and under natural selection. It also relies on quantifying individual variation in cognitive processes, which would not be possible from artificial study systems. I considered using an invertebrate system, but these presented insurmountable difficulties in following the movement and fates of specific individuals, deployment of batteries of different cognitive tests and the transferral of cognitive paradigms established in vertebrate systems to invertebrates with very different sensory systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	I have calculated expected sample sizes based on anticipated survival and recapture/resighting rates and extrapolated sample sizes from these sufficient to satisfy a power 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.	Pheasants offer an unusual and (in the UK unique) opportunity to rear vertebrates under controlled conditions in large numbers, free from parental care; subject them to a suite of well established and understood cognitive tasks; release them into the wild to track their fitness outcomes; and to recapture known individuals to breed large numbers of offspring from them. No other species permits this degree of both controlled rearing and release into the wild. The rearing and testing environment ensures that all individuals will be subject to regular (daily) inspection, and their care is well understood via commercial rearing operations. All birds have free choice options and can refuse to participate in any test. Birds will be reared at a lower density than that recommended by DEFRA's code of practice, thus reducing likely stress and competition between chicks. All staff handling birds will have prior experience in order to minimise stress. Tags and tracking devices will be fitted by experienced staff, following the manufacturer's instructions.

<table>
<tr><td>PROJECT 3</td><td>Neural mechanisms of fear memory processing</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>fear memory, extinction, neural circuits, neuromodulation, anxiety</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[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>
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<tr><td>Preservation of species</td><td></td><td>No</td></tr>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Anxiety disorders are serious mental illnesses that have high social and economic costs as over 15% of people will suffer with anxiety at some time in their life. Although psychological and medical treatments are available for treating these disorders, many patients still show symptoms even after extensive treatment. Medications can also have unwanted side effects and can take weeks to work. This highlights the need for a better understanding of how these treatments work. Anxiety disorders such as post-traumatic stress, phobias, and panic disorder make up a significant proportion of these diseases. These disorders are associated with unusually strong memories which can persist even with prolonged treatment. Understanding how fear memories influence behaviour may lead to insights on the relationship between these memories and anxiety symptoms. Key to</td></tr>
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	this is understanding how these fear memories are formed, stored, later accessed, and suppressed by the brain. Many patients with these disorders share in common disturbances in cognition (e.g. attention, reasoning, memory) and emotion (e.g. feelings like fear). They also have abnormal brain function in specific areas involved in cognition and emotion. 'Cross-talk' between these brain areas, which is required for cognition and emotion, is also disrupted in anxiety disorders. Importantly, these same brain areas are also involved in fear memory processing. This project will determine how interactions between certain brain regions are involved in fear memory processing. Although previous research has investigated the function of individual brain regions during various fear memory processes, how interactions between these brain regions and how certain chemical messengers are involved in regulating these interactions during fear memory processing is poorly understood. This research will examine brain function during fear memory processing. It will also determine how selected neuromodulators are involved in regulating brain function during certain fear memory processes.
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)?	Investigating how certain brain areas involved in cognition and emotion function during fear memory processing will enhance our basic knowledge of brain function related to learning and memory. It may also lead to a better understanding of how fear memories are related to abnormal cognition, emotion and brain function in anxiety disorders. This, in turn, may lead to the development of new anxiety treatments in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use ~ 2000 rats and ~ 800 mice over five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	Some animals will undergo surgery to implant small devices into the brain which are used to monitor brain cell activity or to inject drugs directly into the brain. Although under general anesthesia during surgery, the animals will feel some pain after surgery which is

level of severity? What will happen to the animals at the end?	reduced by treating them with pain killers. After recovery most animals will be trained to fear a sound by associating it with mild electric shock. This training does cause some pain but it is only temporary and the training is necessary to determine how particular brain areas are involved in certain fear memory processes. The shock parameters used (i.e. duration, intensity) will be the least severe possible while still ensuring that the animals reliably show fear memory after training. The highest standards of animal welfare will be maintained throughout these experiments as minimizing pain and distress is required not only for ethical reasons but also to ensure that the data obtained is consistent and reliable. No substantial severity protocols will be used and at the end of the experiments all of the animals will be humanely culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This research will examine brain function directly at the level of individual brain cells during fear memory processing. It will also determine the effects of inactivating brain cells and the role of certain brain chemicals on brain function during fear memory processing. As this kind of work is not possible to do in humans, it will use animals. It is necessary to use live animals to conduct research on brain cell and chemical function during fear memory processing. Thus it is not possible to use other methods such as cell culture. Using computer models to understand brain cell and chemical function involved in fear memory processing is possible to some extent. Some of the experimental data obtained in this research will also be used to create new computer models of brain function during fear memory processing, which will contribute to the eventual replacement of animal experimentation in the future. However, these computer models cannot fully replace the use of animals in this research until we better understand the biological processes involved.
2. Reduction Explain how you will assure the use of minimum numbers of	The experiments will be designed and analyzed using the appropriate statistical tests to ensure that the number of animals used is kept to a minimum. This research will use newer methods of assessing activity in many

animals	neurons in the brain at the same time in more than one brain area, which will further reduce the number animals needed in these studies.
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.	Rats and mice will be used as many previous studies have shown that similar brain regions in these animals and humans are both involved in fear memory processing. This research will use newer models of determining brain cell activity during fear-related memory processing under general anesthesia. This will reduce the animals' suffering and is a refinement of other methods typically used. Some animals will undergo surgery to implant small devices into the brain to monitor brain cell activity or to inject drugs into the brain. Although under general anesthesia during surgery, the animals will feel some pain after surgery which is reduced by treating them with pain killers. After recovery most animals will be trained to fear a sound by associating it with mild electric shock. This training does cause some pain but it is only temporary and the training is necessary to determine how particular brain areas are involved in certain fear memory processes. The shock parameters used will be the least severe possible while still ensuring the animals reliably show fear memory after training.

<table>
<tr><td>PROJECT 4</td><td>The effect of genes and environment on the behaviour of zebrafish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Zebrafish, behaviour, genes, anxiety, epigenetics</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5C(3)[7])</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></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[8]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Behaviour can be influenced by the genetic make up of an animal and also by the environment. One way of determining the effect of genes on behaviour is to assess how animals behave when they carry faulty genes (mutations) compared with those that do not. This can show us how genes can influence specific types of behaviour. Some of these genes are important in human diseases. Animals can be influenced by various environmental stimuli throughout their life and we are interested in knowing whether these can affect their behaviour. Some of these effects could be long term and changes in behaviour could be passed on to offspring but this needs to be investigated.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td>The proposed work is primarily fundamental research on fish behaviour. However, some of the</td></tr>
</table>

project (how science could be advanced or humans or animals could benefit from the project)?	fish that we will study are those that carry genes that are important in specific human diseases and we aim to determine whether behaviour can be affected by these genes. Furthermore, we propose to determine whether various environmental stimuli can influence behaviour associated with anxiety and if this influences future generations.
What species and approximate numbers of animals do you expect to use over what period of time?	Experiments will be conducted on wild type (normal) and mutant carrier zebrafish. In consultation with the Department of Mathematics and Statistics and through the use of power analysis, it is estimated that 1500 mutant fish will be tested for their behaviour over the course of the project and that 1683 mutant and wild type fish will be exposed to different environmental stimuli over this period and tested for their behaviour. An estimated 15,600 offspring of the exposed fish will be tested for anxiety over the course of the 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?	This project will involve the use of fish that carry various developmental mutations in behavioural tests. Most mutations are fully recessive and so the heterozygous (carrier) fish that we will use will be very similar to the wild type fish. Other experiments will expose fish to physical (e.g. touch), visual (e.g. model of a predator) or chemical (e.g. predator water) stimuli and assess their behaviour as adults after exposure. It is unlikely that the animals will suffer any pain or suffering but if they do, they will be removed from the treatment immediately. The expected level of severity is mild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The nature of the work, which is on animal behaviour, necessitates the use of live animals
2. Reduction Explain how you will assure the use of minimum numbers	Before each experiment is conducted, a detailed protocol will be written covering: (i) a statement of the experimental objectives; (ii) a description of the experiment, covering such matters as the

of animals	experimental treatments, the size of the experiment, and the experimental material; and (iii) an outline of the method of analysis of the results. Factorial designs are preferred, and power analysis is used where appropriate.
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 zebrafish is easy to keep and produces lots of offspring during spawning. It has had its genome sequenced and it shares many of its genes with humans. These factors make these fish a good organism to use when investigating behaviours that are also relevant to human conditions. The behavioural tests, involving single or repeated exposures to the physical, visual or chemical stimuli (whether done singly or in combination) are not expected to cause more than mild severity adverse effects. Genetically altered fish look the same as wild type fish and the genetic alterations do not cause any adverse effects.

<table>
<tr><td>PROJECT 5</td><td colspan="3">Study of cognitive behaviour in inbred BXD mice</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">BXD, recombinant inbred, QTL, cognition, translation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">2 years 4 months</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[9]</td><td colspan="3"><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[10]</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 colspan="3">There is a clear and urgent need to improve the diagnosis and treatment of human brain disorders. The majority of human brain diseases that critically impair cognitive function have some degree of genetic underpinning. Therefore, an improved understanding of the relationships between genes, gene products (proteins) and cognitive phenotypes, both in the healthy and diseased brain, is critical to improve our understanding of brain function and disease, and for the expedient identification of novel drug targets. However, with some exceptions, these relationships remain poorly understood. We will utilise a panel of approximately 20 inbred mouse strains, each of which has a unique combination of genes (and, therefore, characteristics) from the two founding strains, similar to how one might expect children in the same family to share different characteristics in common with their</td></tr>
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	parents. This will enable us to search the mouse genome for DNA regions which differ between the two founder strains that are associated with cognitive phenotypes of relevance to humans and human diseases.
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)?	Potential benefits include: an improved understanding of normal and diseased brain function in mice and humans, including relationships between genes and cognitive traits; identification of novel disease gene candidates; novel disease-related drug targets; impetus for novel mouse models of human diseases; contribution to simulation of human brain function.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 915 mice in 2 years 4 months.
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 (although rare) adverse effects include enhanced aggression, subdued behaviour, abnormal weight gain and body weight reduction to up to 80% of the free-feeding weight in <20% of animals. The severity is likely to be mild or moderate. Animals will be culled using 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	The project is concerned with behaviour, which can only be studied in live animals at the present time. Mice are preferable to humans because their environmental factors (which have a critical impact upon disease risk) can be controlled and manipulated.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The present project was designed taking previous published work and expert advice into consideration. The nature of the BXD recombinant inbred mice means that this project can be extended at a later date, so only a moderate number of mice will be used in this first study.
3. Refinement	Prospects for translating our findings from mice to

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.	humans will be excellent; mice have a high degree of genetic similarity to humans, making them suitable for research relating to human genetics, and highly similar tests of comparable cognitive functions are available for both species. Among recombinant inbred panels, the BXD panel is the largest and most extensively studied. Harm to the animals will be minimised by using only appetitively motivated cognitive/behavioural tests.

<table>
<tr><td>PROJECT 6</td><td>Responses of marine fish to hypoxia</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fish, Behaviour, Hypoxia, Physiology</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[11]</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>
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<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[12]</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>To determine the responses of key European fish species of commercial importance or conservation concern to the low oxygen conditions which are likely to occur in marine environments as a consequence of climate change or other anthropogenic or natural origins, in order to advise on how better to manage their sustainable exploitation and conservation, and protection of their habitats. The objective is that these responses should be expressed as functional biomarkers that can be used in forecast models and biological traits analysis.</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</td><td>By determining the impact of low oxygen conditions on the metabolic scope and the behaviour of UK commercial fish species, we can demonstrate the impact that expansion of low oxygen zones might have on economically valuable fisheries, and so enable effective stock management. For example,</td></tr>
</table>

project)?	if the levels of oxygen are such that metabolic scope is reduced, the fish may have to choose between reproducing, feeding (which can affect growth rates), swimming and predatory response.
What species and approximate numbers of animals do you expect to use over what period of time?	Fish, adults and juveniles. Approximately 1,100 animals will be used in the 5 year programme of work.
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?	Most of the procedures are assessed as Moderate severity, one is severe. Altered temperatures will be within the range predicted to be experienced by each species in the wild. Altered oxygen tension may be below that normally experienced by each species in the wild and in some cases may impact behaviour, metabolic rate, growth and/or development, but oxygen tensions will not be reduced to a point that compromises survival.
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 work is to determine the responses, in terms of functional biomarkers that can be used in forecast models and biological traits analysis, of key European fish species of commercial importance or conservation concern. For this type of investigation there is no appropriate alternative to the use of conscious wild fish.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental methods and numbers of animals used are based on previous experience and research. As part of our Animal Welfare and Ethical Review Process, each programme of study is considered by staff from our in-house statistical team and their sign-off is required before any study is undertaken.
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	The aim of the work is to determine the responses, in terms of functional biomarkers, that can be used in forecast models and biological traits analysis, for key European fish species of commercial importance or conservation concern. Therefore a range of species including cod, eels, sea bass, turbot, plaice, spurdog, etc. need to be studied. The

minimise welfare costs (harms) to the animals.	methods chosen are based on previous experience and research that has been shown to provide useful information on the effects of hypoxia on bio-markers/traits. Such information can be used in forecast models and biological traits analyses that inform advice to Government on factors that may affect fish populations and possible mitigation. Where fish are exposed to reduced oxygen tension, they will be monitored for a suitable period in order to assess any adverse effects and ensure minimum suffering.

PROJECT 7	**The Genetics of Local Adaptation**	
Key Words (max. 5 words)	Local Adaptation, Gene-flow, Blue Tit, Phenology,	
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[13]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The three central questions are a) how different individual are at different locations, and if these differences are genetic or environmental b) do locally born individuals out perform immigrant individuals and is this a consequence of their genes and C) on average how far do genes travel from generation to generation via dispersal of individuals	
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)?	The project should give key insights into the ecology and evolutionary history of a wild animal species, and allows us to measure some key evolutionary parameters for the first time. This information is likely to give us vital information on a species ability to cope with environmental change both in time and space.	
What species and approximate numbers of animals do you expect to use over what period of time?	The species will be Blue fits, and because we - supply a large number of nest boxes we expect to be able to work on 200-300 nests per year. In total we envisage working with up to 7500 birds over 5 years, and a	

	maximum of 2500 individuals in any given 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 happen to the animals at the end?	The severity level is mild - we are only taking blood samples — and there unlikely to be any adverse effects expect some minor transitory discomfort. Once the birds have been bled they will be released back into the wild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project is about the evolutionary ecology of wild animals and so non-animal alternatives do not exist.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical power analyses have been used to ascertain the number of families/individuals required, and an appropriate number nest boxes have been put up given the likely occupancy rates (50-75%)
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.	Blue tits (were chosen because they are a commons species that readily uses man-made nest boxes to breed and is very robust to disturbance at the nest site. In order to minimise harm we work in teams of two or three to minimise the amount of time the chicks are out of the chemical hand-warmers in order to prevent chilling.

<table>
<tr><td>PROJECT 8</td><td>Environmental effects on host-parasite interactions in fish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fish, parasites, global warming, environmental change, behaviour</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[14]</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>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[15]</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>Parasites are natural components of ecosystems and in many cases hosts have co-evolved with parasites to reduce the effects of infections. However, as a result of direct and indirect human influences ecosystems are changing rapidly, and these changes have the potential to alter host-parasite interactions, and to mediate the effects parasites have on hosts. However, most of what we know about the effects parasites have on hosts is derived from studies carried out under pristine environmental conditions. The experiments we propose will allow us to measure how the effects that parasites have on their host fish are being altered by changes to aquatic ecosystems as they become degraded by human influences. In order to</td></tr>
</table>

	do this we need to examine the effects that parasites have on experimentally infected hosts both under ideal laboratory conditions, and also under manipulated environmental conditions. The objective is to quantify how degraded environments are affecting host-parasite interactions, using behavioural, physiological, and reproductive assays that quantify the effects parasites have on their host fish.
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)?	The main benefit of this research is that it will provide high quality experimental data that will tell us whether, and to what extent, different types of environmental stressors alter the effects that parasites have on host fish. This information will allow us, and colleagues working in governmental agencies, to begin to predict how parasites are likely to affect the health, growth, reproductive success and (ultimately) population persistence of host animals in degraded environments.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use no more than 2000 individual fish over the next 5 years. The majority of these fish will be sticklebacks, which are small fish (<5cm, 1g), which are bred in the laboratory especially for this research programme. All other fish will either be bred in the lab or purchased from commercial suppliers. A small number of individuals (typically <50) will taken from wild populations for use as parents, under EA licence. These populations contain many thousands of individual fish, so this removal has a negligible impact on natural populations.
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 expected adverse effects are likely to be mild in severity. The reason for this is that the parasites we will use are all natural parasites of the fish species of interest, and the stressors that we will be exposing fish to will be manipulated within the range normally encountered by these species in natural or degraded environments. In natural populations, fish carry these parasites for all of their lives. At the end of the experiments, all fish will be either killed using HO approved schedule 1

	techniques, or – when necessary to facilitate certain types of analysis – standard non-schedule 1 techniques that we have applied for permission to use.
for Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our research interests focus on the effects that parasites have on the behaviour, reproduction, and sexual development of fish, and how these effects are mediated by changing environments. It is therefore not feasible for us to studies that do not involve animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use our significant research experience and the results of previous studies to inform our experimental design, and we will combine these with statistical power analyses where appropriate to calculate the minimum group sizes required in our treatments. In addition, we can use novel non-invasive molecular sex determination to ensure that we focus only on males or females when this is needed.
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.	Sticklebacks and the other fish species that we intend using are all well-understood lab models for studies of behaviour, parasitology, immunology and physiology. The fish we propose using are small bodied species* that can be kept in the numbers required in existing high-quality facilities. The use of these model species allow us to examine how infection status and changing environments interact to affect host biology at a wide range of levels, including at the molecular, physiological, immune and whole organism. Regarding the parasites, the species we have chosen have effects that are largely confined to the growth, energetics, sexual development and behaviour of host fish. We are interested in these aspects because they are critically important for the ecological and evolutionary processes in which we are most interested; however, these effects are unlikely to have major consequences for fish welfare in our laboratory studies. Furthermore, and

	importantly, none of these parasites can replicate themselves within host fish following infection, so we can carefully manipulate levels of parasitism without risking the possibility of infections spiralling out of control or spreading to other fish within (or outside of) our facility. *NB. Although carp grow to a large size, we will only use 1-year old fish (10-15cm) in our studies.

<table>
<tr><td>PROEJCT 9</td><td>Large mammals: nutrition, ecosystems and climate</td></tr>
<tr><td>Key Words</td><td>Climate change, GHG emissions, animal condition, biodiversity.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</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>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</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>~~No~~</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>~~Yes~~</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>1. Improvement of the GHG emissions inventory, with special interest in wild ungulates and domestic species in extensive grazing systems.

2. Linking nutritional ecology, behaviour parasite/pathogens and animal condition.</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>1. Climate change: improvement of the GHG emissions inventory, with the potential of finding ways to reduce gas emissions.

2. Improve our knowledge on the drivers of animal condition in wild and domestic ungulates. This will help to improve the welfare of domestic species in extensive grazing systems and to understand changes in animal performance in wild ungulates and their impact on the landscape and biodiversity.

3. Help quantify the role that ungulates play in</td></tr>
</table>

	maintaining parasites and pathogen prevalence, essential to parasite and disease control and risk assessment.
What species and approximate numbers of animals do you expect to use over what period of time?	Red deer: 175 Roe deer: 105 Sheep: 400 Goat: 150 Camelid: 150
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 regulated procedures used in this project are all considered mild. The expected adverse effects are mainly due to stress on the first occasion that the animal experience the procedure, or because the animals may be enclosed in individual special pens, although in sight with each other. Animals may have their diets manipulated to look at the effects of different natural or commercial foodstuffs. No adverse effects of the dietary treatments are expected since the dietary plant materials offered to the animals are voluntarily ingested by sheep and deer grazing Scottish hill upland pastures. If animals voluntarily ingest less than 60% their anticipated maintenance requirement for more than 3 consecutive days, the animal will receive a dietary supplement of a standard palatable feed and may be removed from the experiment. Some studies require the use of dosing markers (inert compounds that may occur naturally in dietary plants) impregnated in small pellet-paper devices (<35 mm long x 15 mm diameter) and administrated by mouth using a dosing “gun” like that used to give agricultural animals medicines. This is to measure how fast food is digested or goes through the intestines. Small sensor devices that monitor physiological variables can be used. They are introduced in the animal stomach orally using the same dosing gun described above. In our experience no adverse effects will occur.

	Sometimes we need to collect faeces and/ or fluid from the rumen (stomach) for analysis of how food has been digested. Faecal removal is done by gently removing faeces from the rectum. This is well tolerated by animals and should have no adverse effects, and it may be facilitated by use of an inert, water-based lubricating gel. The process of withdrawal of rumen liquor from sheep by stomach tube by a competent licensee is usually well tolerated by the animals and should have minimal effect on physiological response. However, some stress on the animals is expected. The tube used will be warmed to about 40^{o}C prior to use and will have smooth external surfaces. We test how animals metabolise their food by using special chambers that measure the gases breathed in and out. Animals used in these respiration chambers might suffer mild stress at first housing, but this will be minimised by maintaining animals in sight of each other. We also sometimes use special raised pens that let us collect faeces and urine. Animals will be trained to these over a period of time to minimise their stress. Sometimes we use these types of pens within the respiratory chambers. Animals will be observed by CCTV equipment (so we can watch them without disturbing them) to confirm that they do not suffer stress, and they will be removed from the procedure if they seem distressed or if their food intake drops as detailed above. Natural-like day/night hours cycles will be used in the chambers. Fate of the animals: Animals will be returned to stock or the wild, or sent for commercial slaughter if deemed, by a veterinarian, to be fit and have not received any products not covered by an appropriate withdrawal period under a product licence. Some animals might be euthanased to extract rumen fluid or to recover their GPS collars if they have been marked in the wild.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our project deals with the effect of the environment on the condition and behaviour of large herbivores and herbivores' impact and effect on the environment. No substitutes other than these animal species can be used to assess these effects.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals has been selected based on our existing knowledge of the statistical power of the treatments and of the different techniques used in this project. In general the number of animals used per procedure is small, but because for some treatments is intended to assess their heritability, a greater number of animals is needed to account for natural mortality and for replacement of old animals using standard farming husbandry (i.e. replacement of 5-6 yr/old sheep; selling 80% of lamb crop).
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.	Some of the techniques used are intended to assess differences between species (e.g. methane production between species, sheep, red deer, goats, camelids). If differences between species are not found (controlling for confounding effects), then species, which are logistically and from a welfare perspective easier to use, can be target as model species in future experiments (e.g. using sheep rather than red deer). Some proxies of measuring methane emissions are intended to replace measurements taken in respiration chambers, which will help to reduce the time taking measurements and the number of animals and species used in experimentation. We have a history of developing refined techniques for ruminant nutrition studies and intend to continue this.

PROJECT 10	**Genomics of communication in songbird models**	
Key Words (max. 5 words)	Brain, learning, genes, zebra finch, chaffinch	
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[16]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Songbirds recognize each other and communicate through vocalizations (birdsong), which are partly learned and partly innate (inborn). This research aims to understand the processes in the brain that underlie song learning, development communication.	
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)?	The research may reveal new principles of brain function, which could be useful in therapies for communication disorders and in evaluating animal welfare.	
What species and approximate numbers of animals do you expect to use over what period of time?	Zebra finches: 1300 over five years Chaffinches: 100 over five 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?	Animals may experience mild discomfort from blood collection, and/or from short periods of confinement (up to 2 days for zebra finches or 40 days for chaffinches) in a sound isolation chamber, or longer periods of confinement (up to 120 days) in an isolation chamber modified to allow the bird to control and learn from song playbacks. After all isolation protocols, birds will be euthanized for analysis of brain tissues and extracts.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The goal of these experiments is to understand how animals perceive natural experiences and communicate, and so by definition they necessarily involve animals as study subjects.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We evaluate the statistical power of each experiment when it is designed, and use the minimum number of animals needed to draw meaningful conclusions from the results.
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.	Communication through vocal learning only occurs in humans, songbirds and a few other animal groups (which are more difficult to study, e.g., dolphins). As a hardy, domesticated species, the zebra finch has emerged as the dominant songbird in laboratory. Chaffinches are a complementary example of how different vocal learning styles have evolved in nature. Most of our work involves simple breeding and observation in naturalistic conditions; when regulated procedures are required, the animals are closely monitored for their welfare. Two of our objectives involve developing refinements of common procedures (song playbacks, operant conditioning) that avoid or mitigate effects of experimental isolation.

<table>
<tr><td>PROJECT 11</td><td colspan="3">Studies of the spatial ecology of wild animals</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Wild animals, movement, gps loggers, conservation, habitats</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3"></td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[17]</td><td colspan="3"><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[18]</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 colspan="3">The aim of the project is to increase the understanding of how red squirrels repopulate habitats following the removal of the competing species, the grey squirrel.

To do this we will track individual squirrel movements using novel gps data loggers and radio tracking in areas where the grey squirrel population density is declining.</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 colspan="3">We will gain information on the habitat preferences, spatial movement and home range of the squirrels. Only limited information exists on the spatial ecology of the squirrels as previous studies have used radio tracking of animals which requires the researcher to be in the field to collect the data, providing limited information on position, with</td></tr>
</table>

	limited accuracy. The GPS loggers will allow us for the first time to generate large amounts of detailed information about the movements and behaviour of these iconic species and on multiple individuals in the same area simultaneously. This information will help inform conservation management decisions, particularly about where best to place the effort on removal of the grey squirrels. The information we gain on the social interactions of individuals will also aid in our understanding of the transmission of the squirrel pox virus among squirrels and will be used to update parameters in existing predictive models.
What species and approximate numbers of animals do you expect to use over what period of time?	The initial project will study red (*Sciurus vulgaris*) and grey squirrels (*Sciurus carolinous)*. Up to 100 squirrels will be captured, tagged and tracked over the duration of the 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?	No animals will be killed or held captive by activities described in this licence, which are restricted to sampling and marking squirrels followed by their immediate release at the capture site. Following the period of data collection the squirrels will be retrapped and the collars removed then set free close to the site of capture, no long lasting side affects are anticipated. The protocols described are classed as moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To gain understanding of the animals' behaviour in natural habitats it is necessary to carry out direct observations in the field. There is currently not enough known about the animals to be able to accurately predict the spatial movement of the red squirrel where competition with grey is reduced. As this project takes advantage of the current effort of intensive grey squirrel removal from the areas surrounding a red squirrel reserve this is a unique opportunity to carry out this research that cannot be replicated in a captive environment. Information collected will be used to inform computer models of the animals.

2. Reduction Explain how you will assure the use of minimum numbers of animals	The project will use a maximum of ten data logging collars at anyone time, over time they will be used on around a maximum of 100 individual animals in Northumberland . It is essential to mark the animals to ensure we can account for individual behaviours and allow repeated observations using PIT readers and cameras. No one squirrel will have a collar on continuously.
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 methods proposed to observe the squirrels movement patterns are the least invasive possible. The size and weight of the collars and GPS loggers will be kept to a minimum and will not exceed 5% of the body weight of the animal. No juvenile, pregnant or injured animals captured will be used in the study. Squirrels will be marked using a small data tag beneath the skin that will allow us to individually identify each squirrel using a tag detection reader placed in the woodland. The research described here will also be carried out in compliance with licensing requirements of other relevant regulators.

PROJECT 12	**Cardiovascular function and adaption to sepsis**	
Key Words (max. 5 words)	Sepsis, Cardiovascular system, Rodents	
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
		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[19]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall objective of this project license is to understand the impact of modifying specific biological pathways of interest (e.g. nitric oxide pathway) in the regulation of blood pressure under normal healthy conditions including pregnancy and in the setting of sepsis. Well established models of septic shock will be used. Cardiovascular parameters will be measured and biochemical analysis conducted. These are similar to the measures taken clinically in patients. We therefore wish to develop treatments which restore blood pressure back to normal. We aim to achieve this by modifying certain biological pathways and assess what effect this modification has on blood pressure in healthy animals (naive conditions), pregnancy and in animals where we have induced septic shock. In particular we are interested in modifying a biological mediator called nitric oxide. Nitric oxide is generated in gross excess in response to the infection and this leads to paralytic dilatation of the blood vessels. Blocking the activity of nitric oxide synthesis may	

	therefore be beneficial in the treatment of sepsis.
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 provide key information to develop novel strategies to treat sepsis. Sepsis is the leading cause of death in patients in intensive care units worldwide and leading cause of direct maternal deaths in the UK. Sepsis causes dysfunction of the cardiovascular system with a precipitous fall in blood pressure, which reduces the delivery of oxygen to organ systems and failure of vital organs (e.g. liver and kidneys). Agents are therefore required to restore blood pressure in these patients (vasopressor treatment).
What species and approximate numbers of animals do you expect to use over what period of time?	Initially, we will use cell systems and other in vitro assays where possible but we will be required to conduct experiments on live animals as it is not possible to recreate the complexity of the cardiovascular system in isolated cells in culture. We will be using rodents (naïve and genetically modified) to assess the expression levels or activity of certain biological pathways that control the contraction or relaxation of blood vessels. The majority of techniques will be undertaken on animals under general anaesthesia, thereby minimising any pain and distress caused to the animal. We approximately aim to use a sample size 8 which achieves results with a statistical significance of 5%. Over the 5 year duration of the project we would be using 5300 animals. We have previously demonstrated that mice with certain gene modifications have improved outcomes during sepsis.
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?	Induction of septic shock models in conscious mice may result in a significant departure from health. We are using well characterised models and are therefore aware of the time course of disease progression. However, given the multi-faceted nature of septic shock, some animals may experience an exaggerated response to the insult irrespective of the nature of the gene product modification. This is likely due to variations in immune response that naturally occur from animal to animal. We will use a clinical scoring system and any animals that display undue suffering or distress will be removed from the experiment and

	humanely killed. All animals at the end will be culled by Schedule 1.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of animals will be necessary as the study of sepsis requires a complex functioning of the whole body which is not possible to mimic in the laboratory. However, prior to progressing to *in-vivo* work detailed *in-vitro* experiments such as cell and organ culture will be undertaken. Further studies will be conducted using *in-vivo* non recovery models before progressing to animal work. At each stage refinements are made and if necessary further validation of culture work done prior to progressing with *in-vivo* work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This project uses refined techniques such as radio-telemetry that allow us to make multiple necessary measures whilst minimising any distress to the animal. We will use carefully designed experiments to maximise the quality and quantity of data obtained per animal whilst minimising the total number of animals. We will endeavour, where possible to use the same animals for different protocols.
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 will predominantly utilise murine models of sepsis in these studies. The ability to manipulate specific genes and pathways in the mouse enables us to study complex signalling pathways during sepsis. Experiments in murine models have successfully identified mechanisms that have been shown to play important roles in the progression and outcome of human sepsis (Bernard GR, Vincent JL, Laterre PF et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med. 2001 Mar 8;344(10):699-709) and significant pharmaceutical industry interest is currently focussed on the exploitation of these findings. We will use well characterised models of septic shock and undertake detailed monitoring of all animals to minimise harm.