Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 11

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

Project Titles and keywords

1. **Understanding rodents: communication and welfare**
    - Rodents, communication, welfare, pest control

2. **Ecology of divergence in wild fish populations.**
    - host-parasite interactions, adaptive radiation, three-spined stickleback

3. **Wild mammal population structure**
    - Bats, dormice, conservation,

4. **The evolution of food hoarding**
    - Consumption, stress, neuromodulators, avian, hormone

5. **Ageing, chronic stress and trans-generational fitness in wild birds**
    - Ageing, senescence, global climate change, birds

6. **The neural basis of biological rhythms**
    - Circadian, age, biological rhythms

Project 1	**Understanding rodents: communication and welfare**	
Key Words (max. 5 words)	Rodents, communication, welfare, pest control	
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
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Rodents gain information about family members, potential mates and others within their local environment through communication signals; they can also eavesdrop on signals from other species to gain information about potential dangers such as predators. The main aims of this project are to understand the information in rodent communication signals, how this is encoded, and how rodents use this to respond to opportunities and dangers in their environment in ways that might be manipulated for improved pest control or captive management. To achieve this, our objectives are to establish (1) the role of specific signal components in signalling identity, status, and potential danger or safety; (2) the consequences of such signals for determining social responses and activity patterns; (3) the feasibility of using these signals as tools to improve the effectiveness and humaneness of rodent control; (4) practical husbandry and routine laboratory procedures that minimize anxiety in laboratory rodents.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could	There are a number of likely benefits. Commensal rodents are major pests of humankind. They cause substantial food loss and damage to the built environment, are vectors of zoonotic and livestock infection, and induce allergy and asthma. Because rodents are critically dependent on communication	

benefit from the project)?	signals to coordinate their behaviour, it may be possible to exploit these signals to develop new tools for more effective, efficient and, where possible, more humane approaches to control rodent pests, and to reduce the impact of rodent control on non-target species. Our research will also underpin the design and interpretation of rodent models of behaviour used in biomedical research. These models are extremely valuable for understanding how the mammalian brain controls complex behaviour, but this requires an understanding of the information that rodents gain about their social and non-social environment and how they use this information. In addition, by refining common laboratory husbandry methods, or those involved in routine minor procedures such as blood sampling, our project will also help to reduce background anxiety experienced by laboratory animals more generally, which should increase the reliability of their responses in experimental testing. Understanding the information that animals can gain about each other and how they then use this information is also of fundamental importance in understanding the evolution of sociality and cooperation between animals.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a range of rodent species, including mice, rats and voles regulated. We anticipate using up to 2400 animals in procedures over the five 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?	For the most part, animals in this project will be used in non-regulated procedures that will not have adverse effects on the animals. However, to gain some of the information we require, we will need to use brief regulated procedures that have mild adverse effects, such as taking a very small tissue sample under anaesthetic for genotyping to assess parentage or o assess the genetic information that animals are communicating. Some animals may have a specific genetic deficit in the scent cues that they produce, although this is not expected to have any adverse effects on the animals. Some animals may experience brief exposure to predator odours (in the absence of the predator itself), although in most tests these will be presented so that animals can choose to approach or to avoid such stimuli. We anticipate that most responses will be sub-threshold (no adverse effects), but we may find some cues that produce mild distress

	and attempts to escape from the vicinity of the stimulus or reduced reproduction. After recovery from these mild and brief procedures, animals will rejoin our captive colonies where they will be used in non-regulated behavioural experiments, to collect stimulus cues for tests, and as breeding stock to maintain the colony. To study animals under realistic semi-natural conditions, some animals will need to be housed in mixed-sex groups. However, rodents breed extremely quickly under such conditions. To avoid a substantial over-production of animals that would have to be killed, some young males will be vasectomised, which will involve a surgical procedure. However, they will then be maintained throughout their healthy lifespan and will be allowed to interact freely with females under naturalistic but protected conditions.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As our main focus is on the behavioural responses of rodents and on the communication signals they produce, we cannot achieve this without using animals at all. However, the great majority of the work will involve sub- threshold approaches that will not cause pain, suffering, distress or lasting harm. Regulated procedures will be brief, involve no lasting harm and involve only a very small component of the lifetime of each animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animals used in brief procedures are typically maintained throughout their healthy lifespan and used in a range of sub-threshold experiments and as breeding stock. Genotyped animals typically contribute to several non- regulated experiments, substantially increasing the information gained per animal and avoiding wastage. Numbers per experiment are based initially on effect sizes seen in similar previous studies, based on 80% power to detect departure from the null hypothesis for the weakest main effects to be assessed. Unnecessary variation is minimized by careful matching of animals, avoidance of background stressors that interfere with testing, and comparison of test and control responses within the same individuals where possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	As our questions relate largely to rodents, rodents are the most suitable species for this project (used here as a model for wild rodents and other non-human mammals). We focus particularly on mice and rats, which are the most important rodent pests globally. Other rodent species have been chosen because they

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	are the most important non- target species that are being harmed by current pest control strategies, and because they represent a range of contrasting social systems. We require animals to show natural patterns of behaviour so we take considerable care to avoid any background stress during husbandry. All housing contains enrichments suitable for the species, animals are pre-habituated to testing conditions, ethical rules for prompt intervention are always in place to ensure that tests do not become distressing, responses are typically assessed through remote monitoring, we study animals at appropriate times of their normal activity cycle, and personnel are very well trained in all aspects of husbandry, handling and testing.

Project 2	**Ecology of divergence in wild fish populations.**	
Key Words (max. 5 words)	host-parasite interactions, adaptive radiation, three-spined stickleback	
Expected duration of the project (yrs)	5 yrs	
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)	Objective 1, To quantify the impact of parasites on the fitness of fish in enclosures or mesocosms by using antiparasitic drug treatment and artificial or passive infections. Objective 2, To use artificial and passive infection experiments to determine whether sticklebacks from different populations, or hybrids, differ in resistance to parasites. Objective 3, To use artificial and passive infection experiments to determine whether susceptibility to parasites covaries with phenotype (immune gene expression, morphology, age, life-history or secondary sexual traits) or genotype. Objective 4, To investigate the relationship between ageing, immune function and parasite infection and resistance. Objective 5, To use artificial infection experiments to determine whether parasites from different	

	populations differ in growth rate, virulence or transmission. Objective 6, To examine the immunological basis of resistance to infection in stickleback using appropriate assays. Objective 7, To examine the genetics of resistance to infection and associated traits in sticklebacks using a combination of artificial infections, quantitative and molecular genetics. Objective 8, To use artificial and passive infection experiments to examine the way in which different parasites interact with each other and with immune status in their effects on hosts. Objective 9, To investigate the way in which parasitism interacts with other ecological factors (notably diet) to determine the impact on hosts.
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 has the potential to substantially alter our current understanding of the generation, maintenance and genetic basis of biodiversity in the natural world. Specific benefits that we anticipate as likely are: (i) a novel understanding of whether parasites contribute to divergence and speciation of host populations. (ii) The first thorough understanding of the genetic basis of parasite resistance in natural populations. (iii) An improved understanding of the costs of infection, and the ability to resist infection, in wild organisms. (iv) Increased understanding of the causes of variation in virulence. (v) Improved understanding of the way in which parasite infections and the immune system interact during ageing in natural populations. (vi) Improved knowledge of the effect of diet on wild organisms and their ability to resist infection. Many of these benefits will be realised within the lifetime of this Project Licence, as published primary research. The results will be directly relevant to the large international community of researchers and students studying evolution, adaptive radiation, speciation, host-parasite interactions and the geography of coevolution in general. The results will have relevance to anyone with a practical interest in host-

	parasite interactions, including interactions with diet, and the immunological and genetic basis of resistance, especially aquaculturalists attempting to control parasites in farmed fish. This is a problem of substantial applied relevance, currently being supported by strategic initiatives of the UK Research Councils. In seeking impact for our work, we engage directly with a commercial aquaculture company, to whom we offer training and knowledge exchange. Our work is also likely to be of interest to those studying biological invasions and to conservationists dealing with biological control. Results will be disseminated through presentations at domestic and international conferences and through peer reviewed publications in international journals.
What species and approximate numbers of animals do you expect to use over what period of time?	Three-spined stickleback, Gasterosteus aculeatus, 4930
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 overall expected adverse effects are generally moderate, or less. Fish are generally killed by a Schedule 1 method at the end of procedures, but (rarely) by exsanguination under terminal anaesthesia. Adverse effects are generally associated with the following procedures: Marking: Stress due to restraint and transient discomfort from needle insertion or clipping. 100% likely incidence. DNA sampling: transient discomfort from tissue collection (100% likely incidence). There may also be secondary infection of the wound (<5% likely incidence). Administration of antiparasitic treatment (immersion, food, ip,im): effect of the dose compound. <5% likely incidence. Infection with parasitic organisms: Parasite

	burden causing chronic discomfort, weight loss, morbidity. 80% likely incidence. General and terminal anaesthesia. Potential for inappropriate anaesthetic depth that could result in pain. <1%. Stripping brood fish. Transitory stress due to restraint. 100% likely incidence. Stress resulting from social deprivation, 50% likely incidence. Dietary modification: effect of calorific or protein restriction (100% likely incidence). Long-term monitoring: Overt signs of ageing 10% likely incidence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Replacement does not often apply to studies aimed at understanding the behaviour and ecology of wildlife species, because the animals themselves are the objects of study. Experiments to investigate the evolutionary impact of parasites on fish in natural populations necessarily involves the use of live fish. It is impossible to assay aspects of whole organisms such as resistance to infection and performance (eg survival, reproduction) in (semi-) natural environments on anything other than a living whole organism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have substantial experience in the design and analysis of experiments, upon which we will continue to draw and build. Small pilot studies will be used when necessary to check experimental protocols and to get an indication of likely results, especially in situations where experimental designs are novel in some way. In general, for analysis of statistical data, we use powerful maximum likelihood methods. These include generalised linear models (GLMs) when all data points can be considered independent, and generalised linear mixed models (GLMMs) when we need to incorporate hierarchically structured data (e.g. use of blocking to control for ‘enclosure’ or ‘loch’ effects). Factorial experimental designs will be used,

	where appropriate (e.g. when more than one treatment factor are included in experiments), to maximise the information obtained (power) from the minimum resource. For most quantitative experiments, sample sizes may be determined using power analysis (generally 5% significance level, 80% power, 25% least practicable difference between groups). Otherwise, we will use the least number of animals to provide an adequate description, generally on the basis of previous experience (ours, or from the literature). We expect that 8-10 animals per treatment group should often be sufficient to obtain required results. However, because some parasites exhibit very variable infection profiles, and some experiments will use genetically heterogeneous fish, we may use larger numbers of animals per group to obtain satisfactory 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.	Fish are the vertebrates with the lowest neurophysiological sensitivity. The evolutionary response of vertebrates to parasite imposed selection is likely to be qualitatively different from invertebrates because vertebrates possess more sophisticated adaptive immune systems. Three-spined sticklebacks (*Gasterosteus aculeatus*) are the most appropriate species because: (i) they are tractable for fieldwork and common. (ii) They are straightforward to keep and rear in the lab. (iii) Their parasite fauna is well known. (iv) Their population structure facilitates the evolution of local adaptations (iv) There have a fully sequenced and annotated, small (463 Mb) genome. This is highly unusual for a ‘non-model’ organism, but central to much of our research. Our (now substantial) experience of three-spined sticklebacks and the methods we use allow us to identify reliably the types of behaviour or signs of pathology which we use to define endpoints (and see general guidelines for fish, below). Specific signs of pathology or distress that will be used at all times include abnormal orientation (i.e. ‘positional

	disorders': substantial departures from horizontal) or failure to control buoyancy (always at the surface or bottom: if also overturned, this is likely to indicate a severe effect warranting immediate Schedule 1 killing), gasping/flaring opercula ('hyperventilation') or breathing at the surface, significant changes in pigmentation ('dark or blanched body') and signs of opportunistic infection (e.g with *Saprolegnia* or *Ichthyophthirius multifiliis*). In most cases of seemingly abnormal behaviour, we are aware that 'symptoms' can be trivial and transient. Thus it is usually appropriate to ascertain whether the individual is still showing such signs after a period of an hour or so before taking action (which would normally be to Schedule 1 the individual), although this would not be the case for the most severe symptoms (see below), when fish should be killed immediately.

Project 3	**Wild mammal population structure**	
Key Words (max. 5 words)	Bats, dormice, conservation,	
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
	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 objective of the project is to provide better estimates of wild mammal population sizes for species of conservation concern in the UK. It will also provide information on the threats posed to populations by habitat fragmentation caused by factors such as road building and changes in agricultural practice.	
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 work will provide a better evidence base for the conservation management of the species. It will also help stakeholders discharge their statutory requirement to monitor and report on the size of British populations, their change over time, and the primary threats facing the target species.	
What species and approximate numbers of animals do you expect to use over what period of time?	This project will focus on dormice and bats. It will involve approximately 100 dormice which will be radiocollared and also microchipped (using the same technology used for identifying pet animals). Samples will be taken from approximately 500 bats for analysis of their genetic relatedness and place of	

	origin.
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 of the procedures is low. The marking procedures (PIT tagging and ringing) and fur clipping on their own would fall outside the remit of the Scientific Procedures Act. The main risk is that fixed radiocollars are carried by the animal for life (<5%) and present a small risk of entanglement (<2%). Therefore a range of different approaches are being deployed to try to ensure that the collars are removed at the end of the monitoring period. There are small risks of abrasion (<2%) from the collar which are being addressed through the use of appropriate materials and careful monitoring of recaptured animals. There is a small risk of bleeding or infection from the wing puncture site and site of insertion of the PIT tag (<1%). These risks will be minimised by the use of new sterile equipment and careful technique. All the animals will be released back into the wild at the point of capture.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project specifically investigates wild animals in their natural environment so non-animal alternatives are not possible. The data will help to improve non-invasive approaches such as the use of faecal samples for genetic monitoring.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The principal investigator has in-depth knowledge of statistical design, and will keep the proposed sample sizes under constant review during the project. The sample sizes will also be agreed by Natural England which has statutory responsibility for safeguarding the conservation status of the species concerned.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	The study aims to gather species-specific information in order to inform dormouse and bat conservation. However they are also good models with which to assess the effect of habitat fragmentation. Radiocollars have previously been

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	used with a range of small mammals to provide detailed information which cannot be gathered in any other way. Steps are being taken to minimise the likelihood of any adverse effect including ensuring that collars never exceed 10% of body weight, making exhaustive attempts to retrieve all animals in order to remove collars; and use of appropriate materials and fitting techniques to minimise the chance of abrasion. Wing punch samples are generally considered the best means of obtaining high quality DNA samples and are rarely associated with adverse effects. The welfare costs will be minimised through the use of sterile equipment and appropriate technique.

Project 4	**The evolution of food hoarding**	
Key Words (max. 5 words)	Consumption, stress, neuromodulators, avian, hormone	
Expected duration of the project (yrs)	2 - 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)	Evolutionary selection on behaviour has changed brains. However, we know very little about which changes in brains can lead to changes in behaviour. Here we study which changes in brain structure and/or function have led to the evolution of food-hoarding behaviour from ancestral animals that did not hoard food.	
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)?	A better understanding of brain evolution, and what kind of changes in the brain lead to evolutionary changes in behaviour. This has a larger relevance for understanding ourselves and our own evolution.	
What species and approximate numbers of animals do you expect to use over what period of time?	Coal tits (Periparus ater) and great tits (Parus major); 100 in the first protocol, although this could be increased if more funding is obtained. Maximum 300 birds 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?	We will house coal tits (hoarding birds) and great tits (non-hoarding close relatives) in conditions which we expect to increase hoarding motivation (half the birds) or conditions which should minimize motivation to hoard (the other half of the birds). These conditions mainly consist of an unpredictable food supply (high motivation) vs. predictable ad libitum food (low motivation). We will also look at the effect of social rank (being dominant or subordinate) on the motivation to hoard. We will then verify the success of our environmental manipulation by measuring both consumption and hoarding behaviour in the animals. Because we believe that the mechanisms that control hoarding motivation work through the stress hormone corticosterone, we will also monitor corticosterone levels in the blood stream of the animals. The adverse effects are minimal: - Stress of captivity: mild; minimized by habituating them to captivity in a large aviary with many hiding places and by housing them in pairs in relatively large cages. - Effects of temporary food restriction: mild; minimized by never food restricting for more than 90 minutes at a time, and providing enough food through the day; body mass monitoring - Effects of blood sampling: mild: small possibility of too much blood loss. Minimized by taking very small samples and stopping the bleeding with cotton wool, We always check bleeding has stopped before the birds are returned to their cages. At the end of the study, the birds will be humanely killed to collect brain and other tissues for further examination and comparison between the two species.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	We are interested in the physiological basis of animal behaviour. Only animals can behave and we therefore need to use live animals.

use non-animal alternatives	
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental design is well balanced and multifactorial in order to increase statistical power. We are using the minimum number of animals required to pick up expected effect 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.	Coal tits are the most common food-hoarding birds in England. The only alternative that is relatively abundant as well are rooks and magpies, and these would be much harder to work with; and would probably be affected more by the studies. Great tits are the most common close relative of the coal tits and therefore provide the best non- hoarding comparison species for the physiological responses of the coal tits to the environmental manipulations we will perform. The refinement measures are how we habituate the birds to captivity (large aviary, places to hide); how we house them (in pairs); and how we avoid handling the birds as much as possible. For example, to shuttle birds back and forth to the behaviour testing aviary, we let them fly from the home cage to the room, and train them to fly back by turning off the light in the aviary and on in the home cage.

Project 5	**Ageing, chronic stress and trans-generational fitness in wild birds**	
Key Words (max. 5 words)	Ageing, senescence, global climate change, birds	
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)	Our study aims (1) to understand how the age trajectories can explain variation in life expectancy and reproduction of the next generation, and (2) to understand how chronic stress links with age, and how chronic stress affects fitness of the next generation(s). Three key features of our wild house sparrow and blue tit populations make the objectives feasible: complete sampling and monitoring of all individuals, virtually no immigration to and emigration from the population, and house sparrows and blue tits being model species in ecology and evolution. Notably, none of the birds of our populations will be experimentally exposed to stress that they do not experience anyway. This way, we can make opportunistic use of natural experiments without affecting wild animals in any way they are not affected naturally. House sparrows will be subjected to blood sampling from all birds and feather sampling from selected	

	adults. Blood sampling is essential for detecting the presence of malarial parasites in the blood, and to extract DNA, which will be genotyped. To quantify genetic reproductive success, we must obtain comprehensive DNA profiles of all individuals, since fitness is a property relative to the combined fitness of the population. Hormonal profiles are traditionally obtained by measuring blood plasma. However, due to the diurnal and seasonal variation in hormones this field would benefit from alternative tissue sources. It is possible to quantify a range of hormones by sampling feathers, and we will fine-tune this method. We catch adult birds annually in Dec-Feb after annual moult to take biometrics of adult birds, blood and feather samples. During a breeding season, blood samples of minimal volume will be taken from chicks, and we will also capture adults to monitor breeding progress, and to sample DNA. Only a “natural laboratory” of the type we have will allow our objectives to be pursued. We expect to answer several major, longstanding questions in behavioural ecology and evolutionary biology that are of wide interest to the research community and the public. The results will be of immediate significance to conservation biologists and will add significantly to the body of knowledge on our study species in particular, which has been declining rapidly without explanation in recent years in the UK and other European countries. Our results should also contribute significantly to our understanding of the evolution and maintenance of the differences between the sexes and individuals. Furthermore, if sUccessful, hormonal measurements from feathers will provide a novel, minimally invasive method of determining the hormonal status in birds.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could	NA

benefit from the project)?	
What species and approximate numbers of animals do you expect to use over what period of time?	House sparrows, Passer domesticus. We expect to annually, catch a maximum of 300 adults, and a maximum of 500 chicks. Blue tits, Cyanistes caeruleus. Annually about adults and 700 chicks (they have larger clutch 200 sizes).
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 procedure we conduct is taking a small blood sample from a vein in the wing. The blood sample is so small, that we do not insert a needle in the vein but just prick it with a very thin needle, and suck up the blood from the droplet that forms on the spot with a capillary tube. Then we treat the wound with cotton wool that is applied to it until the bleeding stops. The birds are expected to experience some possible hand ling stress, which we will minimize by expert and quiet handling. We expect the birds to experience some possible discomfort caused by the needle prick and minimum bleeding. There may be potentially bruising/haemorrhage/haematoma at the collection site. We will minimize this by applying adequate pressure for a sufficient period, at least 1 minute, to ensure the wound stops bleeding. Studies have repeatedly, and robustly, shown that this procedure does not affect the bird's later life in the wild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We study wild animal behaviour. Natural systems are extremely complex, and while population modelling is a very useful tool, one needs data from the wild to parameterize these models. We have no data available for these types of trans-generational effects.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use state-of-the-art, Bayesian statistics to conduct power analyses. Our study sites are chosen such that the populations are large enough that we can cover all individuals (as we need complete coverage for the genetic pedigree), and for statistical power, but not too large such that we would use too many animals.
3. Refinement Explain the choice of species	We are interested in the behaviour of wild birds. Our study species are model species in animal behaviour.

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 are interested in the natural behaviour of birds, as such, the research calls for us, as observers, to have a minimal impact on the birds. We work according to the The Bird Bander's Code of Ethics (in NABC 2001): "Banders should not consider that some mortality is inevitable or acceptable in banding. Every injury or mortality should result in a reassessment of your operation. Action is then needed to minimize the chance of repetition."

Project 6	The neural basis of biological rhythms
Key Words	Circadian, age, biological rhythms
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

This project investigates the internal body clock located in the brain which controls many aspects of cyclic behaviour in animals. We are particularly interested in how social signals act on the biological clock to help keep animals synchronised to the external light dark cycle and the consequences of not synchronising. In addition, we are interested in how the biological clock changes with age as there is a clear breakdown in synchronisation over time. These are important questions because there are significant implications to being out of synch with the environment and improvement may be seen in groups including older animals by increasing the effectiveness of certain stimuli such as social signals.

The duration, timing, and value of sleep is controlled by an internal body clock located in the brain. This clock is known as the circadian clock, and controls a wide variety of behavioural and physiological cycles that repeat themselves in a pattern of approximately 24 hours. This pacemaker has been well studied in a variety of organisms and is well conserved through evolution.

Many things in life can disrupt clock function- shift work schedules, jet lag, medications, and ageing to name a few. Currently we do not have effective strategies- behavioural or pharmacological to help improve synchronization of the biological clock. Our work aims to establish how various brain chemicals are involved in normal synchronization in young and old animals with a view to making recommendations as to interventions to improve function.

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)?

Most people have first-hand experience that sleeping poorly can leave them worn-out and ill-tempered. However, many remain unaware of some of the more serious side effects of not sleeping properly (a consequence of not synchronising to the environment). Lack of sleep can impact on conditions such as diabetes and high blood pressure, and also impair the activity of the immune system. Drowsiness can affect how we think and make decisions. Sleep disruptions are particularly common in patients with dementia. In these cases, sleep, and more importantly activity at inappropriate times of the day, can have significant implications for carers. Our work may be particularly important for individuals who regularly disrupt the synchrony of their internal clock with the environment by working shifts or via the use of smart phones and tablets late into the night. This work is also important for the elderly, as older people report irregular patterns of daily rest and activity. As many as half of all older people show disruption in their regular pattern of sleep, and they report that this significantly diminishes quality of life. This is because lack of sleep, or not sleeping at the right times, can impact on mood, performance and behaviour.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use small rodents for our work – mostly mice, but at times rats or hamsters if that model better suits the aim of the experiment. We would expect to use less than 100 mice each year and less than10 rats or hamsters each year 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 levels of severity? What will happen to the animals at the end?

Much of the work is exclusively behavioural. A great deal can be deduced from observation of behavioural patterns, which allows the use of invasive techniques to be kept to a minimum, with as many as 3/4 requiring no invasive tests at all, participating only behavioural observations under different light conditions. When we do conduct either surgery or administration of test substances we do so in consultation with the University vets and take on board their strategies for procedures to support the animal welfare. As we are interested in old age, many of our rodents live a long life and either die of natural causes or receive an overdose of aesthetic when the University vet detects a problem. Mice not used in age experiments will be humanely killed (typically overdose with aesthetic via inhalation).

Application of the 3Rs

Replacement

There are currently no non-sentient alternatives for the observation of neurochemical actions within a functioning nervous system. Cell culture experiments would be ineffective for duplication of the various pathways through which neurochemicals act upon each other and there would be no possibility of observing the behavioural results of the experiments. A great deal can be deduced from observation of behavioural patterns, which allows the use of invasive techniques to be kept to a minimum. From the literature, it is clear that rodents are the most widely used subjects in circadian rhythm experiments. They have a clear circadian rhythm in wheel-running which means that often no invasive methods for measuring circadian rhythms need to be used. It has been shown that the circadian clock is well conserved among mammals

Reduction

The estimates of the number of animals required for each experiment has been prepared by considering past experience with similar experiments and in consultation with our unit's statistician. In all cases, we will initially conduct a power calculation- this statistical test uses results from previous experiments to mathematically insure our studies will have the minimum number of animals to produce reliable statistical results. Also our additional electrophysiological work with neuronal clock cells is important as this serves to inform the experiments we conduct in the whole animal and limits the number of animals needed at this stage.

Refinement

These studies will test if the experiments we conduct in neuronal clock cells translates to the whole animal. Since we are interested in the effects of behavioural signals on the circadian clock and how altered signals change synchronisation with age, we must use awake and behaving animals for many of our studies. Much of the work is exclusively behavioural. A great deal can be deduced from observation of behavioural patterns, which allows the use of invasive techniques to be kept to a minimum, with as many as 3/4 requiring no invasive tests at all, participating only behavioural observations under different light conditions. When we do conduct surgery animals are given analgesia in consultation with the university animal care staff and vets for as long as required.