Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 30

Projects with a primary purpose of: Protection of the natural environment in the interests of the health or welfare of human beings or animals

Project Titles and keywords

1. **Bala sluices salmon smolt tagging and tracking**
    - Salmon, Acoustic, Tags, Salmonids, Tagging

2. **Ecotoxicology of micro pollutants for fish protection**
    - Fish, ecotoxicology, micro pollutants, environment

3. **Ecology, genetics and conservation of ruminant populations**
    - Sheep, deer, ecology, evolution, ageing, parasites

4. **Exposure of rainbow trout to crude oil**
    - Fish, crude oil, taint

5. **Exposure to and effects of contaminants on birds**
    - Birds, environmental contaminants, behaviour, ecology

6. **Assessing freshwater fisheries**
    - Wildlife, population, Salmo, Anguilla

7. **Biological Control of Invasive Signal Crayfish by European Eel and Associated Effects on Sediment Dynamics**
    - Eel, crayfish, sediment release

8. **Investigation of ecotoxicology and pathophysiology of fish**
    - Fish Pathology, Fish Physiology, Fish Toxicology

9. **Ecophysiological studies of fish**
    - Fish, Growth, Behaviour, Life history

Project 1	**Bala sluices salmon smolt tagging and tracking**	
Key Words (max. 5 words)	Salmon, Acoustic, Tags, Salmonids, Tagging	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
	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 purpose of the project is to understand salmon smolt behaviour around an artificial structure, and investigate if the operation of the structure is having a negative impact on this species population.	
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 potential benefits include: - Improvement in scientific understanding about fish behaviour around structures - Better operation the structure for the benefit of salmon to maximise passage and therefore survival Better understanding of fish behaviour to ensure future designs and operation of such structures causes the minimal amount of disruption to salmon, within the UK and wider as applicable.	
What species and approximate numbers of animals do you expect to use over what period of time?	This work will be carried out on approximately 60 - 100 Atlantic salmon (Saimo salar) smolts per year, total of approximately 500 salmon smolts 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	Expected level of severity = moderate The fish will be involved in tagging studies and animals will be discharged from the Act and returned to the wild at the end of the procedure.	

to the animals at the end?	Fish will be caught using a bespoke netting method and individually anaesthetised. If each fish is over a certain size threshold it will be tagged with a small acoustic tag. At the end of the regulated procedure the fish will be released back into the wild to complete its lifecycle with no expectation of long term negative effects upon the fish.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project must use the animals described to ensure natural behaviour is assessed. Computer generated particle tracking can be used to estimate behaviour where currents and 'normal' fish behaviour are fed into a program to estimate fish behaviour, however this does not allow for the actual behavioural characteristics displayed by fish when complex flows are exhibited.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A trial of this project was first carried out in 2015 using 94 smolts. During this project the majority of the fish were tagged on a limited number of nights. Reviewing the results from 2015, experimental design has been modified to tag fewer fish each night, but over a wider range of flow conditions. Therefore we will be tagging in the region of 60-100 fish each year. Sea trout may also be affected in the same way as salmon smolts by the sluice structure, however, by focusing on salmon smolts, we can use these results as a proxy for sea trout to afford them the same protection. Once the results for 2016 tagging are analysed, a review of numbers will again be carried out to assess if reduced numbers of fish can be tagged in subsequent years.
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.	Any impacts of the salmon used in this experiment will be reduced by: • Covering tanks that fish are held in to minimise stress • Maintaining low light levels in the working area to minimise stress • Reducing temperature shock between water and air by carrying out the procedure at night, aerating gills of the fish during surgery if required • Use of correct suture material • Use of correctly sized tag

	• Release of tagged fish with other untagged fish to ensure natural shoaling behaviour is maintained • Constant monitoring of the fish to ensure normal behaviour is observed

Project 2	**Ecotoxicology of micro pollutants for fish protection**	
Key Words (max. 5 words)	Fish, ecotoxicology, micro pollutants, environment	
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
	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)	We are aiming to better understand the effects of micropollutants including for real world mixtures on physiological function and behaviour of individual fish and their effects on wild fish populations. The overall purpose of this research is to create knowledge for the better protection of wild fish populations**.** None of this work will involve testing substances for regulatory purposes. Specifically the objectives of this programme are: Objective 1: Determine the integrative physiological and behavioural effects in fish for exposure to environmentally relevant micropollutants. Objective 2: Determine the impact in fish caused by micropollutant exposure including their genetics and adaptive genetic mechanisms in wild populations Objective 3: Characterise the molecular mechanisms mediating physiological and behavioural responses to micropollutants in objective 1 using genetically modified 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	We will provide deeper understanding on how micropollutants and their mixtures affect health in fish and the mechanisms through which these effects occur for supporting the development of more effective and informative environmental protection and reducing animal usage.	

project)?	Transgenic fish we will develop and employ under this project licence provide more integrative and systems-wide effects analysis for the micropollutants we will study. The combined work on physiology and behaviour will allow for a greater appreciation on how micropollutants impact on fish populations in the wild, as well for identifying potentially more sensitive and non-invasive biomarkers. Collectively this understanding will help develop better management strategies for the conservation of natural environments in the interest of fish and fisheries.
What species and approximate numbers of animals do you expect to use over what period of time?	For the evaluating the effects of micropollutants and their mixtures on fish physiology and behaviour we will use a range of fish species including roach, carp, stickleback, zebrafish, fathead minnow, medaka, rainbow trout, salmon and brown trout, and the live bearing fish, *Xenotoca eseini*. The total number of animals used each year will be approximately 500 adults, 1900 juveniles and 3000 embryos. The maximum severity for this work will be moderate, but for the vast majority (>80%) mild (the animals will be exposed to environmentally relevant exposures). Developing and applying transgenic fish lines for advancing our mechanistic understanding on how chemicals mediate their effects in exposure fish, will use approximately 2000 zebrafish per year with a maximum severity of moderate. Approximately 2000 fish (predominantly (>90%) zebrafish with the possibility of medaka we may obtain from other facilities) will be used for the maintenance and breeding of transgenic lines. The maximum severity classification for this is mild.
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 our studies to assess the biological exposure effects in fish to individual contaminants and their mixture for environmentally relevant exposure regimes it is extremely unlikely that at the concentrations we will use will induce any toxic side-effects in our routine experiments. The micropollutants and effluent treatment regimens should thus induce minimum suffering in the exposed animals. In some of our studies however where the toxicological and pharmacological properties of the test substances are unknown in fish we may see effects that inhibit fish growth rate, but we do not expect to see lethality at an incidence of more than 5% in any of our studies. Possible adverse effects may occur associated with

	the procedure of tagging via implantation of PIT tags, removing a small section of fin (for genotyping) or blood sampling, but it is very rare (<0.05%) for the wound not to heal after tag implantation or removal of a small section of fin, or for continued bleeding to occur after withdrawal of a peripheral blood sample. In our genetic manipulations of zebrafish embryos to create transgenic fish lines and for our work with morpholino and CISRPR-cas to manipulate specific genes to study the mechanisms of contaminant effect(s) pathways), the physical trauma of the DNA injection process and/or the subsequent disruption in the expression of DNA may lead to death or altered development that is harmful to the fish. Almost all of these effects will manifest early in development and before the stage of first feeding (i.e. in non-regulated animals). Fish at the end of an experiment will be terminated by a Schedule 1 method unless they are maintained for the purpose of breeding for maintaining genetic lines of fish, and/or supplying these lines to other laboratories designated to receive them, or for native fish sampled from wild for studies on population genetics, where they may be returned to the wild at the site of their capture. Fish showing any adverse effects as a result of treatment or their maintenance in the laboratory will be terminated in a timely manner by a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where ever possible relevant alternative methods will be used to undertake this research. However, use of whole animals is essential to quantify the relative sensitivities/susceptibilities of different organ systems to micropollutant effects, to establish how these effects are integrated in the whole animal and to understand the complexities of developmental processes more generally. Transgenic zebrafish we have developed, and others we will adopt and/or develop, will allow us to understand the potential health effects of the micro pollutants we will study in a more integrative manner, thus reducing future numbers of different whole animal tests required for hazard identification and environmental risk assessment. Studies into the effects of contaminant exposure on the genetics (including genetic adaptations) of wild fish populations, as we propose to do, can only be done through the sampling of tissue collected (in a non-lethal manner) from fish in

	the wild.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In all cases the minimal number of fish will be employed. For every endpoint investigated, the numbers used will be minimised by employing careful experimental design (e.g. utilising shared control groups and/or intra-individual controls where possible, power calculations, and previously generated datasets) to ensure our methods obtain useable data with the minimum number of fish. For studies on the effects of micropollutants on breeding in our fish models (zebrafish, fathead minnow) we adopt an experimental design where each pair/colony of fish acts as its own control, again minimising animal usage, whilst maintaining good experimental rigor. Consultation with well qualified statisticians will take place prior to all *in vivo* experiments with animals to ensure optimal experimental designs are employed throughout allowing for maximum power of analysis with minimal animal usage. For most of the micropollutants we will study there are good data for their toxicity effects. The possible exceptions to this might be for a new emerging pharmaceuticals or nanoparticle substances of possible concern. In this instance, we will use all available information for known effects in other animals and if there is good reason to expect the compound might induce adverse in the fish, we would first apply a battery of *in vitro* tests prior to any fish exposure studies. This will be followed by embryo-larval tests (up to 96hpf). We will also gather all available information on known and predicted environmental concentrations. For any juvenile or adult fish exposures a pilot (sighting) study will then be undertaken using a reduced number of animals (n=6 per treatment, 3 treatments) to establish an appropriate exposure regimen, prior to commencing the main study. The overall aim is to minimise any compound-exposure related adverse effects in the definitive assessments. We expect the transgenic zebrafish models we develop and apply - that allow for assessments on the interaction sites and biological effects of specific classes of micropollutants across many organ systems in the same fish - will allow for a significant reduction in the use of animals in the future.
3. Refinement	The most relevant fish species will be adopted according to the particular environment at risk and

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.	considering experimental convenience. Fish species include those native to UK Rivers that are known to be affected by the micro pollutants we will study or are laboratory models for which specific test guidelines have been established for chemical testing. The latter group have significant genomic resources facilitating studies on the mechanistic basis of substance disruption at the molecular level, and optimising our ability to interpret any effects seen. *Xenotoca esieni* is adopted as a live bearing fish to study the hypothesis that micro pollutants (most notably nanomaterials) can be passed to developing embryos from exposed female fish during gestation. The purpose of this work is to understand what long term health outcomes can result from chronic exposures under environmentally realistic conditions and only rarely might studies with micro pollutants or their mixtures (effluents) induce toxic responses. Our exposures will almost always be at or below regimes that occur naturally in the environment. Given these factors, the micro pollutant and mixture treatment regimens should induce minimum suffering in the exposed animals. All fish will be carefully monitored on a very regular basis (at least twice daily) via persons with considerable experience in good fish husbandry and fish that show any symptoms of harm will be dealt with in a timely and humane manner. Most of the proposed techniques are non-invasive observations of physiological function in free swimming or anaesthetised embryo larval animals. The only invasive procedures under this project licence in intact animals include the removal of peripheral blood samples, tagging of fish, or the removal of small sections of a fishes fin or small number of scales (for genotyping and sequencing of fish). All of these procedures are undertaken after immersion in anaesthesia in the water and adverse effects resulting from any of these procedures are rare events. Fish will be held in tanks appropriate for their size and where they can move freely and in a manner to reduce stressful social hierarchies**.** In addition to the use of generic indicators of adverse effects as humane endpoints, information will be gathered to provide more specific humane endpoints for use on both existing and future models.

Project 3	**Ecology, genetics and conservation of ruminant populations**	
Key Words (max. 5 words)	Sheep, deer, ecology, evolution, ageing, parasites	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	Basic research
		Translational and applied research
		Regulatory use and routine production
	√	Protection of the natural environment in the interests of the health or welfare of humans or animals
	√	Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	In this project we will study a population of conservation concern (Soay sheep) and a population of red deer representative of Scottish hill red deer across a range of topics that have relevant to conservation, wildlife management and fundamental science. (i) Response to climate change. Human-induced climate change is having dramatic effects on natural systems across the globe. However, remarkably few studies of free-living mammals have been able to link changes in climate to changes in population size. Our two study populations offer the detailed environmental and genetic information combined with data on the lives of many individuals that allows us to bridge this gap in our knowledge. Interestingly, the two populations appear to be responding to climate change, but in different ways. We aim to understand how climate change is impacting on our study animals by dissecting the roles of different underlying processes that might be involved. (ii) Ageing Individuals vary dramatically in the onset and rate of the ageing process, and the causes of this variation and its evolutionary origins remain poorly understood. We want to understand how and why individuals age differently in wild populations experiencing natural conditions. The fact that	

	we are able to monitor animals very closely all the way from birth to death means we can ask how early life experience and environment go on to impact the ageing process many years later. (iii) Genetics and evolution We plan to measure inbreeding depression, whether it is worse under poor environmental conditions such as bad winter weather and measure whether there really is selection to avoid inbreeding in the study populations. We will also apply an approach to estimating an individual's genetic merit for a trait, which combines genetic information from throughout the chromosomes, in order to study whether natural selection targets this variation and whether a genetic response to selection is therefore likely. (iv) <u>Parasites, pathogens and host responses.</u> Parasites and pathogens represent a major threat to free-living populations and a major financial and welfare issue for farmers, since parasites are becoming resistant to most available drugs against them. Investigating alternative approaches to treatment and management that do not involve drugs could be important in future, but we have only a very limited understanding of how these parasites interact with their hosts' immune systems and health in the absence of drugs. Our aim, using detailed parasitological and immunological data from both study populations, is to tests how and why individuals differ in their worm burdens and their immune responses to these worms under natural, untreated conditions.
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)?	Understanding the mechanisms by which populations respond to climate change will allow us to forecast into the future what is likely to happen to populations and the likely need for management actions to mitigate the effects of climate change. Improving our understanding of inbreeding depression and how evolution occurs in real time will add to our understanding of evolutionary history and improve our knowledge base for the future conservation and management of natural resources. Improved understanding of the causes of the ageing process, due to studying it in the environment in which it evolved, is of potential value in the future management of ageing. For example, our study may suggest early interventions with potential beneficial effects in late life. Our work on worms in sheep and deer will help address fundamental questions about the origins of individual differences in parasite resistance, as well as offering

	important potential insights how animals cope with parasites when not treated with drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	Soay sheep, 1,800 over 5 years. Red deer, 800 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 catch and handle animals for as short a period as possible in order to release them back into the wild. The expected level of severity for the vast majority of animals is therefore mild. Occasionally animals may sustain minor bruising or react badly to immobilisation drugs, and we may disrupt the mother-offspring relationships, for all of which we have mitigation procedures. Released animals live out their natural lives.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In situ ecological, evolutionary and *in situ* conservation studies such as ours cannot be replaced by studies of cells *in vitro* or simulations *in silico*, because we need to observe individuals over lifetimes as they face the variation thrown at them by the (unpredictable) natural environment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We study all the individuals living in specific study areas on each island, because we wish to understand how events at the level of individuals build into the higher properties of the populations (e.g. population dynamics). The size of these study areas is set so as to be practically manageable for field data collection and to provide sufficient sample sizes for statistical analysis. Although it does not result in any reduction *per se*, we are extremely keen on sharing our data with other researchers for analyses of new questions, to maximise the use of the data collected across research questions and scientific disciplines.
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 Soay sheep on St Kilda is an island isolate of conservation concern in its own right and our research on this population is primarily directed at ensuring its safety. Abundant, diurnal ruminants living on tree-less islands allow very efficient collection of individual-based life history information by non-invasive observation. It is hard to think of other kinds of populations that could be studied in detail so easily. The relatively simple ecosystems are also an advantage in terms of studying population processes: at each site our study species has no predators to speak of and no competing herbivores. Finally, by now the backlog of information for each study population is also of huge value: to switch to alternative study organisms would mean

	jettisoning 30 years and 43 years of previously-collected data. Our research relies on observation of what happens to individuals due to natural processes. It would be counter to our objectives to cause such suffering that the lives of animals were affected by capture. Therefore our protocol is designed to cause minimal suffering before the animal is released back into the wild. We are always looking for ways that might improve our capture and processing for the animals. Whenever more refined approaches become available during the course of this study either through personal communication, publications or veterinary advice, we will investigate their use.

Project 4	**Exposure of rainbow trout to crude oil**	
Key Words (max. 5 words)	Fish, crude oil, taint	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
		Regulatory use and routine production
	x	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
	x	Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The main objective of the project is to produce a reference material for training and performance monitoring of an expert sensory taste panel trained in the detection of taint in fish and shellfish. Taint is a sensory experience, defined as 'an odour or flavour foreign to the product', that can only be measured by a sensory procedure (smelling and tasting) using human assessors. The trained panel assess samples every 2 months allowing their performance to be monitored. The fish are filleted and a portion of each fish reserved for chemical analysis. The remainder of the fillet is cooked and presented to the panel. Each assessor is provided with a score sheet to record their individual assessment of the sample. Samples are first smelled, then tasted. The intensity scale ranges from 0 (absence) to 5 (extremely strong). A sample, or individual fish, is considered tainted if 50% of the assessors record a positive response (intensity I or above).	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could	Taint assessment provides a rapid and sensitive method for detecting taint following pollution incidents. This allows the Government to take action, closing Fisheries where necessary, preventing potentially contaminated food reaching the commercial market.	

benefit from the project)?	Human health is protected arid the reputation of the seafood and aquaculture industries safeguarded reducing the potential to damage the economy following a pollution incident.
What species and approximate numbers of animals do you expect to use over what period of time?	Rainbow trout (Oncorhynchus mykiss) are used for the project. 64 trout are required annually for the duration of the licence period. Of these 48 are exposed to crude oil and the remaining 16 unexposed and used as controls.
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?	Rainbow trout are kept in normal aquarium conditions to acclimatise before moving to a smaller tank for exposure to oil. The fish show no signs of distress when moved to a smaller tank. No visible signs of stress have been recorded during the exposure period. The severity of the procedure is classified as mild. All fish are killed humanely at the end of the procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Replacement is not possible as it is the response of the living animal to the uptake of oU that is required. Taint which appears in the fish muscle, is a result of the oil entering the fish through the gills and skin from the water. The intensity of taint resulting from fish exposed to crude oil will vary according to the length of time the fish is exposed to the oil. Spiking a sample (fish fillet) with crude oil will not give an accurate representation of taint which would naturally accumulate in a live animal exposed to the same oil as the whole oil would be present in the sample. Chemical analysis cannot replace the use of live animals for taint analysis as it is a sensory procedure (determined by smelling and tasting). Chemical analysis is a lengthy and costly procedure. In the event of an oil spill an expert sensory panel can rapidly assess many samples within a couple of days, giving a rapid response to the potential contamination of a fishery and its subsequent closure to protect human health.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of fish used is based on providing sufficient material (fish muscle) required to run a taint panel every 2 months. A panel will typically consist of between 7-12 assessors, each assessor is required to taste each sample. Within each training session a range of samples are presented ranging from absence of taint tà extremely high taint. This is achieved by

	exposing live fish to crude oil over a period of time. A suitable range of intensities can be achieved by exposing fish for periods of time extending from 20 minutes to 3 hours. Each year the project requires 64 fish, of these 48 are exposed to crude oil and the remaining 16 unexposed and used as controls.
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.	Rainbow trout (Oncorhynchus mykiss) are used they are a farmed species available in the size and numbers available for the project. Farmed trout offer a consistency in raw material over time as they can be obtained from the same farm where conditions are controlled with respect to diet, water quality, disease and size. Rainbow trout can tolerate a high stocking density and show no signs of distress when moved to a smaller tank. No visible signs of stress have been recorded during the exposure period.

Project 5	**Exposure to and effects of contaminants on birds**	
Key Words (max. 5 words)	Birds, environmental contaminants, behaviour, ecology	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
	x	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To investigate the exposure risk to and the effects of pharmaceuticals in the environment on birds.	
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 will help us to understand the complex links between environmental contaminants and physiological responses, including stress hormones, behaviour and life history. This will have broad implications for both our understanding of avian physiology and how animals are likely to cope with environmental change. This project will investigate sublethal effects of environmental relevant concentrations of antidepressants. We are not conducting toxicity studies.	
What species and approximate numbers of animals do you expect to use over what period of time?	The work will use 27 starlings over a four month period.	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	The adverse effects are minimal and the severity rating for this work is MILD. Apart from taking occasional blood samples, our work mainly involves observing behaviour. At the end of the experiment,	

level of severity? What will happen to the animals at the end?	the birds will be humanely euthanized and the tissues analysed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are investigating the behavioural and physiological responses of birds to environmental contaminants. Thus, we cannot use non-animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use statistical methods to determine suitable sample sizes based on previous work that indicates likely effects sizes.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	For the work in captivity, we will be using starlings which regularly feed on sewage treatment plants so are exposed to pharmaceuticals in the environment, thus are an ecologically relevant model for this work. Also starlings are relatively common in the wild and tolerant of being held in captivity. We have prior experience of working with this species so our methods have been refined and the personnel have become highly experienced.

Project 6	**Assessing freshwater fisheries**	
Key Words (max. 5 words)	Wildlife, population, Salmo, Anguilla	
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 aquatic environment is undergoing rapid change from a wide range of factors, such as climate effects, and more localised variations associated with industry and farming. Salmon and trout are emblematic of good habitat quality, support valuable fisheries, and are of noted conservation importance. The European eel is now endangered and requires particular close study to develop suitable conservation measures. Knowledge of the ways that individual fish and their populations respond to environmental changes is required to develop appropriate mitigation and conservation strategies for these species. The objectives of the project are to provide information on the numbers of salmon, trout and eels in Scottish waters and the responses of populations of these fish to changes in their habitat including temperature, forestry and disease.	
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 information is needed to conserve fish populations and allow for effective management of the fisheries that exploit them, and mitigate against other impacts resulting from human activities such as aquaculture and climate change.	

What species and approximate numbers of animals do you expect to use over what period of time?	The species used are Atlantic salmon (*Salmo salar*), Brown/sea trout (*Salmo trutta*) and the European eel (*Anguilla anguilla*). It is estimated that 162,500 fish will be used in the studies over the 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The studies to be conducted involve measuring and counting fish, applying a range of types of tag, removing scales to assess age and genetic constitution, using anti-parasite treatments, and observing growth and behavioural responses to changes in temperatures. All fish will be handled under mild anaesthesia and the level of severity is considered moderate. The animals will be released back into the wild. Occasionally individual fish may be damaged, or killed during capture. Fish identified with severe skin or fin damage, or displaying impaired swimming behaviours will be killed by a schedule 1 method as a humane endpoint. Since the work is to monitor populations and biology of fish in good condition, every effort will be made to use the least adverse methods available to obtaining data.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Since most of the work involves counting fish in natural systems or observing their behaviour, there are no alternatives to using live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Numbers of animals studied are minimised by looking only at sufficient specific study sites to provide a suitable index of population health. Similarly, behavioural studies will use only the number of fish required to identify the scale of any effect of habitat change, however, certain monitoring work requires an entire population to be sampled. In the case of experimental work we will adhere to the principal where existing pilot work will be used to estimate an appropriate sample size to identify effects, typically at the 95% level of confidence. Advice will be sought, as required, from professional statisticians with regard to numbers of animals to be used.
3. Refinement Explain the choice of species and why the animal model(s)	The species used are those whose populations need to be assessed. The proposed work is primarily focused on Atlantic salmon and brown (sea) trout,

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.	which are the species of most importance in supporting fisheries. Fish will be handled under mild anaesthesia to minimise stress. Gentle handling and aseptic technique will be used to minimise the possibility of secondary infection. All efforts will be made to use the smallest tags possible while allowing the required information to be obtained. Fish will be monitored to determine their recovery and assess their well-being post capture, and prior to release. All staff involved in the procedures are skilled in fish capture and handling techniques using defined protocols. Accurate records, including comments/ observations are made allowing for review of procedures. Procedures and their possible refinements will be discussed with the NACWO and NVS where necessary.

Project 7	**Biological Control of Invasive Signal Crayfish by European Eel and Associated Effects on Sediment Dynamics**	
Key Words (max. 5 words)	Eel, crayfish, sediment release,	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	y	Translational and applied research
		Regulatory use and routine production
	y	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)	Whether European eel can be used to naturally control invasive non-native signal crayfish.	
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)?	Control of signal crayfish will reduce their impact on rivers, banks, sedimentation and affects upon small fish and invertebrate species	
What species and approximate numbers of animals do you expect to use over what period of time?	925 eels over a 5 year period	
In the context of what you propose to do to the animals, what are the expected adverse	Introducing eel to effected rivers where signal crayfish are present. Monitoring their rate of crayfish consumption. Monitoring reduction in crayfish	

effects and the likely/expected level of severity? What will happen to the animals at the end?	interactions, reduced small fish and invertebrate predation and reduced bank and bed damage and sediment generation
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	For biological control to work it is essential to use animals. Animal predation using a native species is considered to be more effective (biologically), and more cost effective (financially).
2. Reduction Explain how you will assure the use of minimum numbers of animals	Since the project will run for 5 years this leaves opportunity for continual assessment. When the objectives have been met there will be less reliance upon further data to show the same effect.
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 peer reviewed literature (papers and journals) show that eel are an effective predator of signal crayfish in trials. We wish to explore this to discover if they can be used to control crayfish in the wild. This has the benefit of providing escapement for landlocked populations as well as additional feeding opportunities. Comparing other animals, eels appear to be most favoured for such a study.

Project 8	Investigation of ecotoxicology and pathophysiology of fish
Key Words	Fish Pathology, Fish Physiology, Fish Toxicology
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	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;

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

The overall aim of this project is to enhance understanding of the effects of environmental toxicants and other stressors in fishes. This project addresses an urgent need to provide information to guide environmental protection (e.g., assess environmental risks posed by substances), improve fish health (e.g., within aquaculture industry), and for basic scientific research (fish pathophysiology).

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)?

Thousands of new substances are produced each year and toxicology of these substances must be established before mass production and incorporation into consumer products. Testing toxicity of substances in standardized ecotoxicity tests is required by regulatory authorities (e.g., OECD, U.S. EPA), and this project is directly

involved in developing novel standardized tests with zebrafish. A prominent route of exposure for toxicants is via ingestion, and effects on digestive system physiology can have substantial consequences on overall organism health and survival. Among the substances that are likely to be ingested are engineered nanomaterials (ENMs), which are extremely small (<100 nm in size) and have unique properties and toxicity based on their size. In this project, substances including ENMs, will be incorporated in formulated fish feed pellets and fed to fish for extended periods (up to 8 weeks). These exposures are expected to have minimal grossly observable effects on fish (cause minimal distress to fish), but have consequences on overall health including immune system function and resistance to pathogens. A sub-set of exposed fish will be used in pathogen challenge tests to determine if consequences of toxicant ingestion cause increased susceptibility to disease.

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

Over the five years of this project the number of fish anticipated to be used is less than 5000. Zebrafish are the primary species, but species including rainbow trout Oncorhynchus, carp Cyprinids, and lambari Astyanax are also to be used.

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?

The majority of the fish used in this project will experience only minimal distress (i.e., no changes in behaviour, feeding, or morphology). Endpoints of the experiments will be changes in gene expression of which minimal levels of severity are expected. Acute toxicity (morbidity as an endpoint) will be investigated in fish from age 120-168 hpf and the number of fish that experience acute toxicity will be <1000. Exposure to pathogens will occur in a minimum number of juvenile/adult fish and the endpoint will be severe (morbidity) for some fish (<100 fish). Some (<100) zebrafish will have moderate cardiac deformity that may lead to decreased cardiac output and moderate distress at heightened activity levels (e.g., swimming). Respiration of adult zebrafish will be assessed in a respirometer chamber, and this procedure is non-invasive and is expected to only cause minimal distress to the fish. All fish will be euthanized upon completion of experimentation either with an overdose of fish anesthetic or snap freezing (zebrafish age <168 h postfertilization).

Application of the 3Rs

Replacement

Use of live fish rather than cell cultures or perfused organ preparations is necessary for this project because the integrity of the intact fish is essential for the research questions under investigation.

Standardized ecotoxicity tests are based on early life history stages of fishes, and all aspects of toxicology from absorption, tissue distribution/accumulation, metabolism/toxicity, and excretion are under investigation. This cannot be replicated in vitro.

Dietary toxicology experiments to investigate effects on gut physiology and overall fish health require intact and integrated organ systems.

Reduction

The experimental designs are based on the minimum numbers of fish needed for robust statistical analyses of the effects under investigation. For toxicology experiments, there will be three independent replicates of each treatment and control, which is standard within environmental toxicology. For zebrafish larvae used for gene expression, up to 25 fish will be used in each treatment (test concentration) and control, based on our experience for obtaining sufficient amounts of total RNA.

Refinement

Zebrafish have emerged as a prominent model organism for standardized testing and research across numerous disciplines. The zebrafish model is supported by an extensive scientific community (e.g., U.S. NIH supported repository ZFIN.org), scientific literature (>>10,000 peer-reviewed scientific journal articles), a sequenced genome and tools for molecular biology, extensive information on developmental biology, and availability of mutant strains for hypothesis testing. While zebrafish are the primary species, other species including *Oncorhynchus, Cyprinus and Astyanax* are also to be used because these species are environmentally relevant, relevant within aquaculture, and to enable results to be compared with existing published results. *Astyanax* is a freshwater fish species native to Brazil that we are developing as a model for ecotoxicology testing. This species is found in most surface waters, it is cultivated in aquaculture facilities, and is an important commercial species. The majority of fish used in this project will experience only minimal distress (i.e., no changes in behaviour, feeding, or morphology), and the majority of fish that experience more than minimal distress (e.g., morbidity and mortality) will be aged <168 h postfertilization. Reduction of welfare costs (harms) is a critical element of investigations within this project and this is accomplished by reducing numbers of older fish exposed to harms to the lowest that enables robust statistical analyses, and responding swiftly (e.g., ending experimentation) when harm (e.g., morbidity and changes in behaviour) is observed.

Project 9	Ecophysiological studies of fish
Key Words	Fish, Growth, Behaviour, Life history
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	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;

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

To determine the influence of environmental conditions on behavioural and physiological performance, and the fitness consequences of intraspecific variation in behaviour and physiology.

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)?

An understanding of how animals respond to environmental change An understanding of the effect of metabolism on potential growth and reproductive rate, and on the rate of ageing An understanding of the links between gut microbiota, energetics and animal performance

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

Freshwater fish, predominantly salmonids (Atlantic salmon and brown trout) and three spined sticklebacks – up to 9000 over 5 years (up to 3000 in the wild and 6000 in the laboratory).

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?

The severity experienced by the great majority of fish used in this project will be mild since they will only be subjected to benign procedures such as anaesthesia (for purposes of measuring and/or photographing), manipulation of environmental conditions (e.g. diet, temperature) or measurement of metabolic rate or swimming performance. None of the procedures have an expected severity greater than mild. At the end the fish will be humanely killed; as an option and if stringent conditions are met the fish may be released from control of the Act or released into the wild.

Application of the 3Rs

Replacement

The research programme described here addresses questions about the behavioural and physiological responses of whole fish to their environment and so the objectives cannot be met without conducting field and controlled laboratory experiments using fish.

Reduction

Both field and laboratory experiments will be designed to minimise the number of fish used. These experiments will combine measurements across a broad range of traits to reduce the total number of fish used while maintaining high statistical power.

Refinement

Freshwater fish exhibit pronounced behavioural and physiological variation, thus making them ideal for this kind of study. Moreover they live in simple environments, the essential features of which can be replicated in the laboratory; this has the combined benefit of reducing stress on the fish while generating results that are applicable to the real world.