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NON-TECHNICAL SUMMARY

Phenotypic plasticity and adaptation of cichlid fish in response to novel environments

Project duration

3 years 0 months

Project purpose

- (a) Basic research

Key words

Adaptive evolution, Phenotypic plasticity, Epigenetics, East African cichlid fish, Environmental perturbation

Animal types	Life stages
Haplochromine cichlid fish of the East African Great Lakes	adult, neonate, embryo, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To characterise the impact of dietary change in East African cichlids.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In order to protect global biodiversity it is essential to characterise the impact of environmental changes on a range of animals. Research in the past few decades has established that changes in diet and temperature can lead to changes in parts of the body without requiring changes in DNA. These changes are referred to as phenotypic plasticity and can result in rapid adaptation of parts of the body and behaviour. Understanding the molecular basis for these changes is crucial to predict how animals will respond to a shifting climate.

Fluctuations in environmental conditions often lead to changes in available food sources. This can result from a range of factors including altered prey populations, vegetation dispersal, and migration patterns. In aquatic systems, changes in the quality of the water are leading to the rapid decline of microorganisms crucial in maintaining the marine food chains.

For instance, measuring the abundance of phytoplankton - a type of small aquatic plant - can indicate harmful effects of environmental change in aquatic environments. The upper water column, or shallower level, of the ocean has warmed ~0.1C per decade throughout the past 40 years. Linked to this warming, studies have measured a global decline in phytoplankton levels over the past several decades, as well as changes in their dispersal and yearly life cycles. Because phytoplankton make up much of the basis of the aquatic food chain, changes in the abundance and locations of these small organisms can impact every level of the aquatic environment, placing resource strain upon a range of animals. Currently, the effects of such a widespread change remain uncertain.

For this reason, we are simulating altered dietary conditions in a fish model to study the short-term adaptive process. In the proposed research, we will use East African cichlid fish—a fascinating system known for their rapid adaptation to novel environments—to investigate the responses to changing environments. Our research has two main aims. First, we will characterise the changes that occur in response to altered environmental inputs (e.g. diet) across multiple generations. Second, we will determine whether these changes persist in future generations after returning to the ancestral environment.

To address these two aims, a population of juvenile *Astatotilapia calliptera* bred from stock tanks will be randomly divided among three diet treatments: a marine diet, an algae diet, and an omnivorous commercial food that is normally fed to this species (referred to as the ‘control’ diet). Fish from generation 1 (G1) will be bred within their tanks to produce generation 2 (G2). The G2 offspring under treatment will be randomly divided and will continue on either the marine or the algae diets. Throughout subsequent generations, all fish will return to the omnivorous ‘control’ diet. Relating to objectives 1 and 2, objective 1 is fulfilled by G1 and G2, objective 2 is fulfilled by all subsequent generations.

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

The outcomes of the proposed work have the potential to result in several publications reviewed by other scientists. These publications will report findings related to the following main topics:

1) Identification of genes involved in diet adaptation across several parts of the body, including muscle and liver tissue.

2) Characterising how diet change alters body parts in the absence of genetic change and determining the molecular processes behind those changes.

3) Determination of whether non-genetic changes as a result of altered environment are inherited after returning to the original environment.

We expect the data generated in our work to be useful to other researchers and lead to collaborative work. We will deposit our data in publicly available, free repositories, so that other researchers can download it and use it to answer their own biological questions. This is also a reduction measure, as it should avoid repetition of the same work by other researchers.

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

Our work is of a fundamental nature and will build important knowledge of the impact of altered environments on the control and usage of genes. This will increase our understanding of short-term adaptation in the absence of changes in DNA. As our project touches upon so many fundamental biological aspects, we expect our results to impact several fields of the biological sciences, e.g. evolution, molecular biology, and genetics.

Given the fundamental nature of the proposed work, the research output is not likely to produce directly any product or method with commercial potential. However, the impact of altered diet on fish growth and reproductive success may be used to enhance productivity in commercial fish farming by informing the composition of fish food designed for optimal growth. For instance, as this project involves feeding a population of fish multiple different diets, one of these diets may result in a significant increase in health and growth rate. This finding would suggest that a similar nutrient composition may lead to increased commercial output in fish farming.

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

We intend to present our results in local and international scientific conferences or other scientific meetings. We will attend at least one major scientific conference per year on topics related to our proposed research. These conferences will provide opportunities to broadly communicate our results. Collaborators and other persons directly involved in this work will also participate in dissemination efforts.

Our investigations have already led to several new collaborations with other researchers. We will disseminate all findings, including negative results. Our work will be published in free, public repositories online and in international scientific journals.

Species and numbers of animals expected to be used

- : 1500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using East African cichlids of the African Great Lakes due to their unique and fascinating diversity of body sizes, shapes, diets, and behaviours. These characteristics influence each other and together indicate how well prepared the fish is to deal with and explore its surrounding environment. In fact, the diet of these fishes strongly influences the shape of their skulls and jaws. The combination of these features make East African cichlid fishes, as a whole, an ideal system to identify the genes underlying the bodily changes relating to diet.

In order to understand the effects of a specific diet, these fishes will be fed the same diet throughout their entire life, from the time they initiate independent feeding.

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

The environmental variable perturbed in this experiment is diet. The diets of the animals in this project will be altered to represent the food regimes of different fish in the lake. Specially formulated fish feeds have been designed to represent the diet of algae eaters (algae-eating diet) and of fish eaters (fish-eating diet) while preventing any malnutrition in our experimental group.

Two groups of fish will be fed two distinct carefully determined diets. One group of fish will eat an algae diet, while another group of fish will eat a marine (fish-eating) diet, both in the form of pellets.

A control group of fish will eat the conventional diet of our aquaria stocks, in the form of flakes.

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

We carefully defined each diet so that all the nutritional requirements are met. Thus, we expect our proposed procedures will impact the animals' health and welfare minimally and will not lead to long-lasting pain, suffering, or distress.

In the cases where the diet change produces effects at the level of the skull and jaw, the animals will be killed if their feeding, reproduction, or behaviour are impaired to a point inconsistent with a healthy and normal life. Animals whose diet strongly affects their feeding, reproduction, or behaviour, will be humanely killed immediately to prevent any pain, suffering or distress.

The conditions of the animals will be monitored daily. Weight and length will be recorded once every two weeks to ensure the animals remain at healthy levels. Tanks will be cleaned at least once a week

and water parameters will be measured regularly. In the case that tanks need to be cleaned more often to maintain optimal water parameters, for example when fed with one of the altered diets, we will incorporate this change for all tanks involved in this experiment. No adverse effects are seen from additional cleaning.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Fish: Mild severity: (less than 10%).

What will happen to animals at the end of this project?

- Killed
- Kept alive
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

It is important to understand how animals react to a changing environment, and in particular to dietary changes. To research this topic, we need to feed animals with particular controlled diets.

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

We have considered computer simulations and cultures of cells.

Why were they not suitable?

At the moment there are no alternative resources capable of recreating the natural development of fish. In order to fully understand how animals react to a changing environment, we need to observe how animals develop and behave in a natural context. Other alternative systems, such as cell culture, or computer simulations, are not able to recreate the complex interactions that occur at the level of the organism and during development, between neighbouring cells and tissues. These interactions are most important for proper animal development. Thus, our aim cannot be accomplished without using animals.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We plan to produce four generations of fish throughout this experiment (maximum of 1500 animals):

1. In order to produce juveniles for the first generation (G1) of the experiment, we will breed our stock fish of that species. We expect to use a maximum of 30 stock animals to produce a founding population of 360 fish. We will divide the founding population across three treatments.

2. For the second generation (G2), we aim to produce 370 fish through controlled breeding of G1. We will include at least 120 animals per treatment.

3. For the third generation (G3), we aim to produce 370 fish through controlled breeding of G2. We will include at least 120 animals per treatment.

4. For the fourth generation (G4), we aim to produce 370 fish through controlled breeding of G3. We will include at least 120 animals per treatment.

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

A pilot experiment was performed using a smaller subset of fish and commercial diets of similar types to the ones created for this experiment. The results from this pilot verified that significant gene regulatory changes can be observed within one generation of diet perturbation in this fish.

Meetings with collaborators who performed similar experiments in other fish models assisted us in determining the minimum number of fish required for significant sampling power. The diets will be meticulously defined in order to meet all the nutritional requirements of the fish. These measures ensure that the experiment will not have to be repeated in a large scale.

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

Throughout the duration of the project, we will use the minimum number of animals required to acquire satisfactory data. Experiments will be designed, conducted, and reported according to published guidelines (namely the PREPARE and ARRIVE guidelines).

The pilot experiment, mentioned above, allowed us to define an adequate experimental design in terms of diet and number of animals used. The fishes from the founding population will be randomly assigned to experimental and control groups. A range of tissues will be dissected from killed animals, and those tissues not immediately used for the purpose of this project will be available to colleagues and collaborators.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use East African cichlid fish for the proposed work plan. As these fish species are large and robust, they can be effectively bred and grown in an aquaria environment. Moreover, these animals can tolerate novel diets with no major biological consequences. Thus, these dietary experiments are expected to cause little to no pain, suffering, and distress to the animals.

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

The work proposed here takes advantage of the extreme diversity of shapes, sizes, colours, diets, and behaviours of East African cichlids. The diversity of these cichlid fishes is unique amongst vertebrates and even amongst the entire animal kingdom. Therefore, other less sentient, or invertebrate, animals will not allow us to answer the same biological questions.

Tissue dissection and extraction will be exclusively performed in dead animals.

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

We will incorporate new recommendations on animal welfare, as soon as possible after their publication.

Fish will be checked daily for any adverse effects of the diet treatment. We will alter the level of food given to the fish on a regular basis to ensure the fish are consuming optimal levels for their life stage. Additionally, the algae and marine diets, both in pelleted form, will be ground up to smaller sizes for younger fish to ensure they are as easy to consume as possible. It is important to note that food pellets soften upon contact with the water so, at any life stage, remain easy for the fish to consume.

Lengths and weights of the fish will be measured every two weeks to ensure they remain at healthy levels. These measurements will be taken with minimal stress to the fish as they remain in water throughout the procedure. No anaesthesia will be used when weighing or measuring. The weights and lengths of fish under the algae and marine diets will be charted over time to determine growth rate. These rates will be compared to those of fish consuming the omnivorous parental diet (referred to as the ‘control’ diet) and comparisons will be used for monitoring purposes, including surveys of overall condition via body mass and comparing growth rate to that expected for this species (i.e. growth rate of the omnivorous population). Body mass of the fish will be assessed relative to breeding frequency to determine optimal endpoints for each generation.

Cichlid housing will be continuously improved to faithfully mimic the sandy areas rich in vegetation and hiding places characteristic of their natural environment. This will be achieved by introducing sand, artificial plants, clay pots, and plastic tunnels in the aquaria. Fish will be housed in groups, in order to decrease stress and aggression levels. Manifestations of aggression, including chasing, fighting, and injuries will be monitored daily by the scientific and animal care staff. These aggressive behaviours are normal to these species and not a product of captivity. If required, fish will be temporarily separated and groups redesigned to avoid further injury and aggression. Continuous monitoring of water parameters, food regimen, and breeding will ensure the wellbeing of the animals.

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

We will follow the best practice guidelines available at www.nc3rs.org.uk. Experiments will be planned, documented, and reported according to published guidelines (namely the PREPARE and ARRIVE guidelines). In accordance with suggested practice for fish models, enrichment will be added to each tank and individuals will be housed in social groups to minimise stress and increase quality of life.

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

We will follow the website of the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs, available at www.nc3rs.org.uk), and stay up-to-date with new information and new resources that become available. To achieve this, I have signed-up to the NC3Rs e-newsletter.In addition, named persons at my establishment (i.e. the Named Animal Care and Welfare Officer, Named Veterinary Surgeon, and Named Information Officer) will inform us of relevant new information and resources, and will advise, together with the animal care staff, on the best ways of implementation.