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GPO Box 1536 Canberra City ACT 2601 Australia
telephone 02 6247 8333 • freecall 1800777 231
info@churchilltrust.com.au • churchilltrust.com.au

THE WINSTON CHURCHILL MEMORIAL TRUST OF AUSTRALIA

Report by:

Dr Avnika Ruparelia
2017 Churchill Fellow

The Dr Dorothea Sandars and Irene Lee Churchill Fellowship to investigate the molecular cues that drive age-related muscle wasting using Killifish

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Avnika Ruparelia

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TABLE OF CONTENTS

TABLE OF CONTENTS.....	2
KEYWORDS.....	3
INTRODUCTION	4
EXECUTIVE SUMMARY	6
Fellow details.....	6
Project Description	6
Highlights and lessons	6
Information dissemination strategies	7
PROGRAMME	8
FELLOWSHIP FINDINGS	9
Killifish maintenance and husbandry	9
Sex determination	9
Water parameters and quality control.....	10
Killifish pair mating and embryo collection	10
Embryo hatching	11
Housing densities	12
Feeding regime	12
Health monitoring	12
Killifish injection protocol.....	14
Preliminary experiments	16
Validation of antibodies	16
Collection of muscle samples	17
CONCLUSIONS AND RECOMMENDATIONS.....	19
REFERENCES	20

KEYWORDS

Ageing
Sarcopenia
Muscle
Muscle wasting
Killifish
Fish models
Atrophy

INTRODUCTION

Ageing is an inevitable, multisystemic disorder characterized by a progressive decline in the ability of organs to perform their physiological functions. Sarcopenia is the age-related decrease in muscle mass and strength, both of which occur at striking rates of 1% and 3% each year respectively (1). The age-related decline in muscle mass and function places a great burden on the health care system. Despite this, very little is known about this process and this is the focus of my research theme at the Australian Regenerative Medicine Institute.

A key factor that has limited our understanding of ageing and sarcopenia, is the availability of suitable model systems. A model that has the potential to fill this gap is the African killifish *Nothobranchius furzeri* (*N. furzeri*), an annual fish species that lives in temporary fresh water bodies of East Africa. As an adaptation to the ephemeral nature of their environment, *N. furzeri* have evolved to have the shortest known maximum lifespan (average of 15 weeks) of a vertebrate species that can be bred in captivity (2), making them an optimal model for the study of ageing and sarcopenia.

As a result of the ephemeral nature of their environment, killifish have evolved to have a very specialized life cycle. As such, for successful breeding and maintenance of killifish colonies, specific protocols need to be established and followed. Unfortunately, there are no killifish research facilities in Australia, and there is a lack of researchers with the appropriate expertise to work and handle killifish for research. I therefore wished to travel to the laboratory of Prof Christoph Englert (Leibniz Institute of Aging, Jena, Germany), who have not only established these protocols but also currently run one of the largest killifish research facilities in Europe – and learn first-hand on how to successfully use killifish for research. Here, I learnt specific protocols such as killifish breeding, egg collection and incubation, embryo hatching, killifish dietary requirements and other husbandry protocols. These protocols are detailed herein.

Additionally, given my interest in muscle and my hope to use killifish as a model to study the mechanism of sarcopenia, I needed to demonstrate that they muscle age-dependent muscle weakness like humans. Therefore, as part of my Churchill fellowship, I also collected muscle samples from killifish of different ages, using which I will investigate the prevalence of sarcopenia in aged killifish.

The knowledge gained during my Churchill fellowship has been very important to the research community, and as evidence to this, we have now established the first Australian killifish research facility at Monash University, Melbourne. Killifish have triggered an immense amount of excitement

in the research community and using this model, we hope to make significant discoveries in the field of ageing and age-related diseases.

For the very unique and enriching experience I have had in Germany, I would like to appreciate some very important people:

I would like to thank The Winston Churchill Memorial Trust for giving me an opportunity to pursue my aspirations and having faith in my research ideas. A special thank you to Mr Adam Davey, Ms Meg Gilmartin and Ms Sally Campbell, from the Trust, for your endless support. Additionally, I would like to thank the late Dr Dorothea Sanders and late Ms Irene Lee whose support has provided a platform to Australians like myself, to make a difference and contribute to the betterment of the Australian community.

To Professor Christoph Englert and his research group at the Leibniz Institute in Jena, Germany – you are all incredible and I thank you for hosting me and giving me an experience of a life time. I would like to thank Beate Hoppe and her team at the Nothobranchius fish facility, for very generously sharing your knowledge with me – I am very grateful.

Additionally, I would like to thank Professor Peter Currie and the Australian Regenerative Medicine Institute for continually supporting my research endeavours and supporting the establishment of the first Australian Killifish facility.

Finally, I would like to thank my parents, family and friends, for having confidence in me and encouraging me in every step of the way. I would not have been where I am without you.

EXECUTIVE SUMMARY

Fellow details

Name: Dr Avnika Ruparelia

Address: Australian Regenerative Medicine Institute, 15 Innovation Walk, Monash university, Clayton, Victoria, 3800

Position: Post-doctoral researcher

Email: Avnika.ruparelia@monash.edu

Telephone: +61 3 9902 9638

Project Description

The aim of the project was to gain knowledge on how to handle, breed and maintain killifish (*N. furzeri*; Figure 1) for research. Additionally, I wished to collect muscle samples from killifish of different ages, using which I will determine if aged killifish undergo sarcopenia similar to humans. While in Germany, I generated some preliminary data revealing muscle aging in killifish. Finally, as part of the Churchill Fellowship, I attended the killifish symposium in Cologne (Germany) where I learnt about the latest research in the field and built collaborations, which will be beneficial for future research endeavours.



Figure 1: Image of a 22 week male killifish, captured during my Churchill Fellowship

Highlights and lessons

The majority of my fellowship was spent at the Leibniz Institute of ageing in Jena (Germany) under the guidance of Professor Christoph Englert, a pioneer of killifish research. Here, I collected muscle samples from killifish of different ages, which will be used for histological, molecular and metabolomics studies. Additionally, from Beate Hoppe and her team at the killifish facility I obtained knowledge on how to effectively run a successful killifish facility.

Based on the biology of killifish in their natural environment, standard operating protocols for using killifish for research have been generated. These include protocols for killifish breeding, egg collection and incubation, hatching, feeding regimes and maintenance of water quality. These protocols need to be followed to successfully run a killifish research facility. Additionally, cleanliness in the facility is imperative. Killifish are very susceptible to disease and therefore it is important to use clean tanks and ensure the facility is kept neat and tidy. It is advisable to control the number of people who have access to the facility and ensure all personnel wear laboratory coats and shoe covers – thus preventing transmission of pathogens into the system.

With regards to use of killifish for research, many of the tools, and techniques used for zebrafish research are also suitable for killifish research. There is a gender bias in the growth trajectory of killifish in that males are significantly bigger than females. As such gender of the fish must be taken into consideration when using killifish for ageing research. Finally, whilst the appearance of typical ageing biomarkers have been reported in various tissues, including the skin, liver, kidney and brain, of aged killifish (3) the effects of ageing on muscle have not been investigated. Therefore, before killifish can be used for understanding the mechanistic basis of sarcopenia, the muscle of aged killifish needs to be characterized.

Information dissemination strategies

Since being awarded the Churchill Fellowship, I have established, at Monash University, the first Killifish research facility in Australia. The lessons learnt during my fellowship have been pivotal in the successful start-up and running of the facility. As Australian researchers begin utilizing the Killifish model to answer their respective research questions, I will not only give them access to this innovative model organism but I will also provide guidance and training – therefore directly disseminating knowledge gained during the fellowship. Additionally, I will supervise and mentor PhD students teaching them killifish breeding and maintenance techniques therefore sharing the knowledge to young Australian scientists.

Once I characterize the muscle wasting feature of aged killifish, I will publish the findings in a prestigious, readily accessible scientific journal. In the years to come I will also present my findings at various national and international conferences.