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Grant: PhD student mobility

Home Institute: Department of Botany and Zoology, Stellenbosch University

Host Institute: Department of Biosciences, Durham University, United Kingdom

Time period: 7 November 2019 to 7 February 2020

Report on my experience

I was recently lucky enough to be provided a Filamo PhD student mobility grant which allowed me to be able to travel to the United Kingdom and undertake laboratory analysis of bottlenose dolphin tissue samples collected in Namibia between 2008 and 2019 by the Namibian Dolphin Project. The 12 weeks in the UK provided me with an opportunity for personal development, capacity building and to develop laboratory skills which will aid me during my PhD and in future. As I am only just starting the second year of my PhD this grant has provided me with an invaluable opportunity that will certainly help me during my PhD and beyond.

My Project

My PhD project combines photo-identification and genetic data to determine the social relationships between individuals and if there are underlying genetic relationships that help shape these associations. Additionally, the genetic data will help determine how this small localised population of bottlenose dolphins in Namibia is genetically related to other populations of common bottlenose dolphins. This population of common bottlenose dolphins, in Namibia, appears to be stable at only approximately 100 animals (Elwen, Leeney and Gridley, 2019); however, this abundance estimate was prior to the extensive habitat modifications that have occurred in Walvis Bay since 2015. In order to aid conservation of this population as much information on the genetic health of the population as a whole needs to be gained including links between social structure, behavioural specialisations, the genetic relationships between individuals and the habitat use within Walvis Bay of the various social groups of bottlenose dolphins. This information will aid conservation of this population through better marine spatial planning and evaluation of threats to this population due to anthropogenic factors. As few genetic samples from this population have previously been analysed, this study will provide valuable genetic data that can be used in future evaluations for the designation of protection to this small localised population by the IWC, as has been done for the Doubtful Sound bottlenose dolphin population (Currey *et al.*, 2009). Without comprehensive baseline data, including relatedness of animals, inbreeding coefficients, levels

of heterogeneity and the sex ratio of the population, it is impossible to determine if a population is being affected (positively or negatively) due to habitat modifications (natural: climate change; anthropogenic: pollution, shipping, habitat destruction).

My time in Durham

This was the first time I have undertaking laboratory analyses since my undergraduate studies and will helped me refresh those skills which was a good opportunity in and of itself, however being able to do so in the laboratory of a world-renowned Professor such as Rus Hoelzel who specialises in whale and dolphin genetics was a once in a lifetime opportunity. Currently there are very few people in South Africa that have undertaken analysis of cetacean genetic material, let alone cutting-edge ddRAD and next generation sequencing, with the majority of cetacean genetic samples sent to overseas laboratories in the UK and USA for analysis. As an African researcher having the opportunity to analyse my samples in such a high-tech lab with such an expert in this field was incredible. Being based in the Department of Biosciences at the University of Durham allowed me to cross-foster links between Stellenbosch University in South Africa and Durham University in the UK. During my time there I integrated into the lab through regular attendance of the lab meetings and academic engagements with other researchers in the lab.

Being based in Europe during this period also allowed me to more easily attend the World Marine Mammal Conference (WMMC) and associated workshops held in Barcelona between the 7th and 12th of December which facilitate networking with my peers as well as experts in the marine biology field and allowed me the opportunity to present work from my PhD (oral presentation on 12th December 2019). The workshops held before the conference were particularly valuable for my PhD project. I attended one full day workshop on the use of environmental DNA and its application to marine mammals (7th December 2019) and one full day workshop on the genomics of marine mammals (on the 8th of December 2019).

Skills I learned in the lab

- I learned how to extract DNA from tissue samples using standard phenol-chloroform protocols and using commercially available kits.
- I learned how to prepare DNA libraries using the ddRAD method (Peterson *et al*., 2012) which requires the digestion of extracted samples with two different enzymes, in this case HindIII and MSpI to prepared samples for genetic analyses using next generation sequencing.
- I was able to determine the sex of many of my animals using primers that amplify specific chromosomes (ZFX and SRY) for sex determination (Gilson *et al* 1998).
- I also learned that laboratory work can be frustrating and challenging and that much patience and attention to detail is required during all steps.

During my time in Durham I was only able to complete the processing of my samples to the point before next generation sequencing as samples are “sent away” to a specialist centre at the University that does the actual sequencing. As the sequencer requires a certain number of samples and lanes to be filled the actual sequencing will only occur in March 2020 (hopefully). As such no bioinformatics has yet been conducted but it will include: demultiplexing raw sequences (process rad-tags), loci detection and assembly to the *Tursiops* reference genome using program STACKS (Catchen *et al*., 2013). Discriminate analysis of principal components (DAPC) for clustering of individual samples into populations. ARLEQUIN 3.5 (Excoffier and Lischer 2010) will be used to determine genetic diversity through pairwise divergence between areas (Ho and He) using genome wide heterozygosity, mean nucleotide diversity (π) and percentage of polymorphic loci while Bayesass will be used to determine gene flow between areas. Stairway plots – demography or general population trend back through time to provide the effective population size (Ne). For relatedness (chapter 2), Genome-wide Complex Trait Analysis (GCTA) software (Yang *et al*., 2011) which is designed for use with genome-wide SNPs, such as those generated with ddRAD sequencing, will be used for GRM analysis of genetic relationships among individuals, PCA analysis and estimation of F_{st} between individuals.

The genetic data provided from these analyses will be used to form one chapter on the population genetics of this population of bottlenose dolphins and how they are related to other coastal populations elsewhere and provide the relatedness and sex of bottlenose dolphins within Walvis Bay. This information will provide key evidence to aid in the conservation of this genetically isolated population of dolphins. This combined with published information on the population size (less than 100 adult animals, Elwen *et al*., 2019) and population trend may lead to the classification of this population as endangered under IUCN criteria as has been achieved by other bottlenose dolphin populations under similar threats (Fiordland, Currey *et al*., 2009).