B. Title of Research Project

An epigenetic methylation clock for age estimation of common dolphins (*Delphinus delphis*) in New Zealand

C. Details of Proposed Activity

☐ Take ☐ Hold ☐ Import ☑ Export

NB please tick all applicable activities

D. Applicants/Key Researchers

List the names and institutional affiliations of all the key individuals involved with the research. List any convictions or offences, of any of the applicants or key researchers, against the MMPA 1978 or any other Act involving the mistreatment of animals.

- Prof. Karen Stockin, Massey University, School of Natural Sciences, k.a.stockin@massey.ac.nz
- Eva-Maria Hanninger, Massey University, School of Natural Sciences, e.hanninger@massey.ac.nz
- Dr. Katharina Peters, University of Wollongong, School of Earth, Atmospheric and Life Sciences, katharinap@uow.edu.au
- Dr. Livia Gerber, CSIRO, Canberra, livia.gerber@csiro.au

E. Description of Proposed Research

- Abstract

Provide an abstract of the proposed research project, emphasising the research objectives and the manner in which such activity involves the taking, import or export of marine mammals.

In wildlife management, age determination is important to acquire knowledge about population structure, the reproductive state of an individual and its lifespan (Barratclough et al., 2019). Knowledge on demographic age can provide crucial insights into population viability and population resilience to anthropogenic and environmental pressures (Betty et al., 2019; Heydenrych et al., 2021; Manlik et al., 2022; Palmer et al., 2022). Indeed, the study of life-history features is crucial for effective conservation and management of protected species, such as cetaceans (Moore & Read, 2008).

In cetaceans, it is challenging to determine an accurate age as these animals do not show any external signs of aging (Barratclough et al., 2019). While total body length can assist with age class assessment (neonate/ juvenile/ adult), body length is highly variably between individuals of the same age and accordingly, does not allow accurate age estimation (Betty et al., 2019; Peters et al., 2023). Instead, age estimates require either long-term photoidentification surveys (Connor & Krützen, 2015; Peters et al., 2023; Würsig & Jefferson, 1990), physical examinations or invasive tissue extraction (Barratclough et al., 2021; Peters et al., 2023). Invasive methods, such as the quantification of growth layer groups in teeth (Barratclough et al., 2019; Evans et al., 2002; Lockyer, 1993, 1995; Maas, 2009; Murphy et al., 2014; Westgate & Read, 2007), are restricted to post-mortem investigations (Barratclough et al., 2021; Peters et al., 2023).

Recent advances in the assessment of DNA methylation provide a minimal-invasive alternative that can be applied to free-ranging cetaceans by biopsy sampling of skin (Peters et al., 2023; Robeck, Fei, Haghani, et al., 2021; Robeck, Fei, Lu, et al., 2021). DNA methylation describes the occurrence of methyl groups at cytosine–phosphate–guanine sites (CpGs) in the DNA sequence (Bocklandt et al., 2011), which serve as regulator for gene expression (De Paoli-Iseppi et al., 2017; Sen et al., 2016) in multicellular organisms (Hayano et al., 2019), and prominently in mammals (Sen et al., 2016).

Due to a phenomenon called the 'epigenetic drift', individuals accumulate epigenetic changes with age (Issa, 2014; Poulsen et al., 2007; Sen et al., 2016). The correlation between age and DNA methylation of CpGs allows the calibration of epigenetic clocks (De Paoli-Iseppi et al., 2017), which are considered the most reliable age predictor that is currently available (De Paoli-Iseppi et al., 2017; Guevara & Lawler, 2018; Jylhävä et al., 2017; Peters et al., 2023).

Epigenetic clocks have been developed for multiple odontocetes, such as belugas (*Delphinapterus leucas*; Bors et al., 2021), bottlenose dolphins (*Tursiops truncatus*; Barratclough et al., 2021; Beal et al., 2019; Robeck, Fei, Haghani, et al., 2021), Māui dolphins (*Cephalorhynchus hectori maui*; Hernandez et al., 2023) and Indo-pacific bottlenose dolphins (*Tursiops aduncus*; Peters et al., 2023). To date no species-specific epigenetic clock exists for common dolphins (*Delphinus delphis*).

The initial calibration of a methylated clock requires tissue samples from known aged animals. The tissue archive of the Cetacean Ecology Research Group (CERG) at Massey university holds 30-years of tissue samples – including skin and teeth samples – from stranded and bycaught cetaceans, which underwent routine post-mortem examinations under DOC permit 39239-MAR and iwi approval. The age of the animals has been previously assessed post-mortem by quantification of growth layer groups in their teeth (Palmer et al., 2022, 2023). The availability of skin samples of known aged cetaceans provides the great opportunity to calibrate a species-specific methylation clock for New Zealand's common dolphins (*Delphinus delphis*).

Objective: Based on known dental age, we aim to develop an epigenetic clock for New Zealand's common dolphins (Delphinus delphis). Specifically, we will extract DNA from skin samples of 129 individuals.
Globally, there is currently only one lab in the world with the precise patented method to evaluate DNA methylation using a Infinium methylation array (HorvathMammalMethylChip40, Arneson et al., 2022; Barratclough et al., 2021; Peters et al., 2023). Accordingly, we seek support to export a tiny quantity of DNA (note, no tissue and only enough DNA for this mahi) to the Clock Foundation (California, USA).

- Duration of Proposed Research

Provide a detailed description of the overall duration of the proposed research.

We will extract DNA from skin samples during October and November 2023. On receipt of appropriate export and CITES permits, we aim to ship the DNA to the Clock Foundation (California, USA) for further processing, which will take around 6 weeks.

- Species Name and Status

Provide a list of all the species (common and scientific names) involved in the research activities. Describe the status and factors that affect the species i.e., incidental bycatch, pollution etc.

The project focuses on common dolphins (*Delphinus delphis*). New Zealand's common dolphins are affected by a range of anthropogenic threats, including incidental bycatch in trawl fisheries or coastal set nets (Stockin and Orams, 2009) and tourism (Stockin, 2008), as well as environmental contaminants, such as polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides (Stockin et al., 2007).

- Sample Size

Provide sample size for each species, method of sampling and location.

Skin samples were routinely collected during post-mortem examination of bycaught and stranded common dolphins (*Delphinus delphis*) from New Zealand's coastline. The post-mortem examinations took place between 2000 and 2023 (currently held under DOC permit 39239-MAR). We will extract DNA from 129 individuals. Stranding locations are provided as appendix and as map under the following link:

https://www.google.com/maps/d/edit?mid=1rYbXTTwebdvweNN1WyQ0Jf1TopTnifl&usp=sharing

- Justification of Proposed Research

Describe why this work is necessary, clarify if it has been done before and if so why it needs to be repeated. It is especially important to identify and justify all procedures, which have the potential to cause pain or distress to the animal(s), and details of the steps to be taken to avoid or minimise the pain or distress.

The study of life-history parameters is a central aspect for determining the vulnerability and recovery potential of a population (Betty et al., 2019; Heydenrych et al., 2021; Manlik et al., 2022; Palmer et al., 2022). New Zealand common dolphins are subject to a variety anthropogenic impacts (Au & Perryman, 1982; Stockin, 2008; Stockin and Orams, 2009). Knowledge on the age structure of a population, the reproductive state of an individual and its lifespan (Barratclough et al., 2019) are crucial for an effective conservation (Betty et al., 2019; Heydenrych et al., 2021; Manlik et al., 2022; Moore & Read, 2008; Palmer et al., 2022). Current aging methods are largely restricted to post-mortem investigations. An epigenetic clock is less invasive and would be applicable to free-ranging common dolphins.

- Risk Mitigation

Outline what steps you will take to limit or mitigate any potential adverse impacts the proposed research may have. Impacts include any aspect that may affect the health and safety to the animal, or to members of the public; adverse effects on public relations, or any loss or destruction of cultural or historic resources.

Our research goals can be achieved without involving live animals, as all required tissue samples have already been collected during routine post-mortem examinations under Iwi approval and DOC permit 39239-MAR.