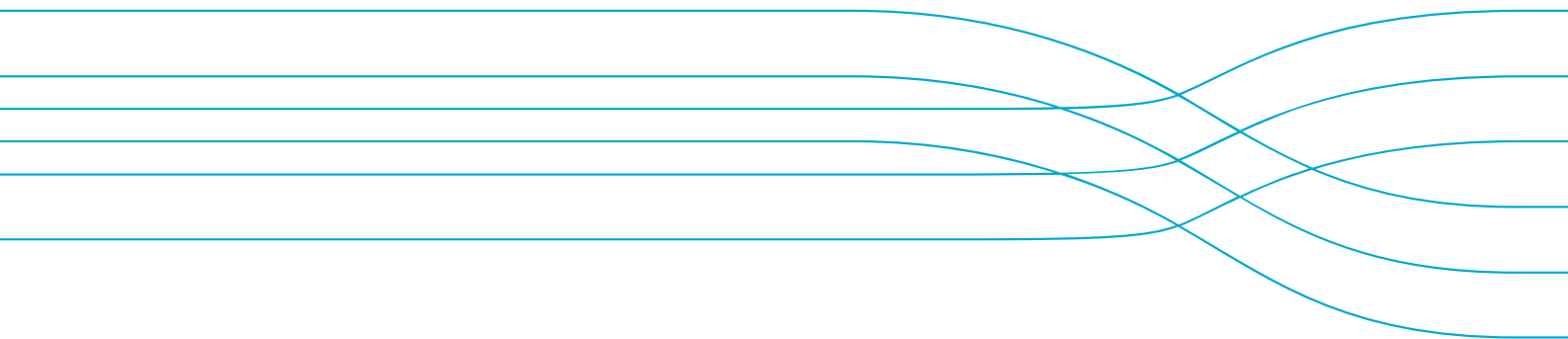




Rapid epigenetic age estimation for southern bluefin tuna

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1 Abstract

In fisheries, age is often estimated by counting growth increments in otoliths or other hardparts, which can become costly and time consuming when collecting and processing a large number of samples. Recently, it has been shown age can be estimated from DNA in a wide variety of vertebrate species. In this project, we propose to use muscle tissue from known age¹ southern bluefin tuna (SBT) to develop a DNA based age estimation method. A DNA based age estimation method has the potential to reduce the cost and time to determine age in a large number of samples of SBT. A rapid age estimator provides the ability to more readily estimate growth rates, catch-at-age and other life-history parameter required to assess the status of a stock.

2 Background

Animal age is fundamental for a broad range of research questions relating to population biology. It can be used to estimate many age-specific demographics of populations including growth rates and age structure. However, there is not a practical and non-invasive method to estimate age of most wild animals.

DNA methylation, an epigenetic modification to DNA has been shown to be predictive of age in wide variety of animals (De Paoli-Iseppi et al., 2017). Previous studies have used blood, saliva, and skin tissue to develop a non-lethal method to estimate age in many animals, including but not limited to Humpback whales (Polanowski et al., 2014), Gray Wolves (Thompson et al., 2017) and Bechstein's Bats (Wright et al., 2018).

We aim to develop this methodology of age estimation by DNA into a wide variety of fish. We have recently shown lifespan can be predicted using the DNA in vertebrates including fish (Mayne et al., 2019). This demonstrates DNA in fish has age associated properties. The limiting factor to extending age estimation methods into wild animals is the cost to DNA sequencing. However as described below we have developed a rapid targeted approach to DNA sequencing, thereby reducing the cost of age estimation. The reduction in costs and time for age estimation can improve the accuracy of other models relating to population biology. The work described in this project has the potential to significantly impact many aspects of wildlife management as well as industries including fisheries.

3 Age estimation in fish

Fish age is usually estimated using counts of annual growth increments in hardparts such as otoliths (Campana 2001). There are several limitations to this such as being lethal and time-

¹ Otolith-based age estimated with high confidence

consuming. An alternative method to estimate age would need to be non-invasive, rapid, and cost effective. We are currently developing non-invasive rapid assays using DNA methylation from accessible animal tissue such as blood, saliva, and skin tissue.

To date, we have developed a targeted, cost effective approach to DNA age estimation method in zebrafish. Our model in zebrafish is accurate to 3.2 weeks or 4.1% of lifespan and approximate costs of between \$5-10 per sample. Zebrafish were an ideal fish species for age estimation as their DNA is highly conserved in a broad variety of fish species. By comparing the DNA in zebrafish to other fish species we have identified age associated markers in a broad variety of fish. We are currently applying these age associated markers in other fish species including the Australian Lungfish, Mary River Cod, and Atlantic Salmon. Our overall aim is to develop DNA based age estimation in a wide variety of fish species for better wildlife management.

4 Application to SBT

Since 2003, the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) agreed that all SBT fisheries should collect and analyse hardparts (otoliths) to characterise the age distribution of their catch. Given that sashimi-grade fish are very valuable, collecting large numbers of otoliths can be difficult and time consuming. The successful development of a rapid epigenetic age estimation method would substantially improve our ability to get representative age data for all fisheries, as it would only require the collection of a tissue sample, not the extraction of otoliths, which requires much less time and expertise and with little or no risk of reducing the commercial value of the fish.

The first step of the project involves selecting 120 fish with high confidence age estimates with an equal male:female ratio. Approximately 15mg of fin tissue will be used for DNA extraction. DNA will be sequenced for age associated sites as previously identified in our zebrafish study. The performance of the model will be assessed by the correlation and the absolute error rate between the age from otoliths and the estimated age from DNA. Once the model has been generated we will report back to CCSBT ESC on results of our initial trial work.

If the trial is found to be successful it may be used as an alternative to age estimation in SBT when otoliths cannot be obtained.

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