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# Rapid epigenetic age estimation for southern bluefin tuna

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## 1.1 Abstract

In fisheries, age is often predicted by counting increments in otoliths or other hard parts. Age prediction by otoliths can be costly and time consuming and potentially impractical in some operational situations. Recently, it has be shown in a wide variety of vertebrate species that age can be predicted using DNA. In this project, we propose to develop a DNA-based method to predict age from muscle tissue of southern bluefin tuna (SBT). Age prediction by DNA has the potential to reduce cost and time, making it advantageous with large sample sizes. Importantly, in the cases of SBT and other highly migratory pelagic species, it would increase the potential for large-scale collection of direct age data by reducing the logistic challenge of sample collection at sea relative to otoliths. This has the potential to substantially improve the spatial and temporal coverage of catch-at-age data by fleet. By being able to access and process large sample sizes provides the basis to readily estimate growth rates, catch-at-age, and other life-history parameter required to assess the status of a stock.

## 1.2 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 parameters of populations including growth rates, age structure, and rates of mortality. Unfortunately, there is no practical and non-lethal method to determine age for most wild animals. However, it has recently been shown that age can be predicted from DNA in a wide variety of animals [1].

DNA methylation, is an epigenetic modification in DNA and is commonly used as a molecular method for age prediction for humans and other vertebrates [2]. Most studies use DNA from blood, saliva, or skin tissue as a non-lethal method to predict age. The limiting factor extending this method to wild animals has been the cost of DNA sequencing. However, we have developed a cost-effective method making it possible to age large samples sizes of wild animals. This has the potential to improve wildlife management and industries including commercial fisheries.

## 1.3 Age estimation in fish

Fish age is usually estimated using counts of annual growth increments in hard parts, such as otoliths [3]. There are several limitations to this approach, such as being lethal, various logistic constraints on the collection of otoliths and otolith preparation, and multiple readings can be time-consuming. An alternative method to predict age would need to be non-invasive, rapid, and cost effective. Recently, we have developed a method to predict age from DNA in fin tissue for zebrafish, Australian lungfish, Murray cod, and Mary river cod [4,5]. The cost of the method is comparable with, and potentially less than, the cost of conventional age estimation using otoliths. Our overall aim is to apply the method to a broad variety of fish species for better wildlife management.

## 1.4 Application to SBT

Since 2003, the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) agreed that all SBT fisheries should collect and analyse hard parts (otoliths) to characterise the age distribution of their catch. Given that sashimi-grade fish are very valuable and often frozen at sea soon after capture, 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. It would also provide the basis for age estimation of live fish released as part of tagging programs.

To date, we have developed a DNA based method to predict the age of SBT. This method uses muscle tissue and was calibrated (89 samples) and tested (36 samples) with ages derived from otoliths ranging from 1 to 38 years. The method also uses DNA biomarkers that were derived from zebrafish (*Danio rerio*) [4]. By using DNA biomarkers from other fish species, we can potentially develop a universal assay for fish age prediction. Our method was found to have a median absolute error rate of 1.7 years (Figure 1). This work suggests DNA methylation in SBT is predictive of age consistent with our previous work with other fish species. There is also the potential to improve the model through additional DNA sequencing to identify other biomarkers of age that may refine the accuracy and precision of the method for SBT.

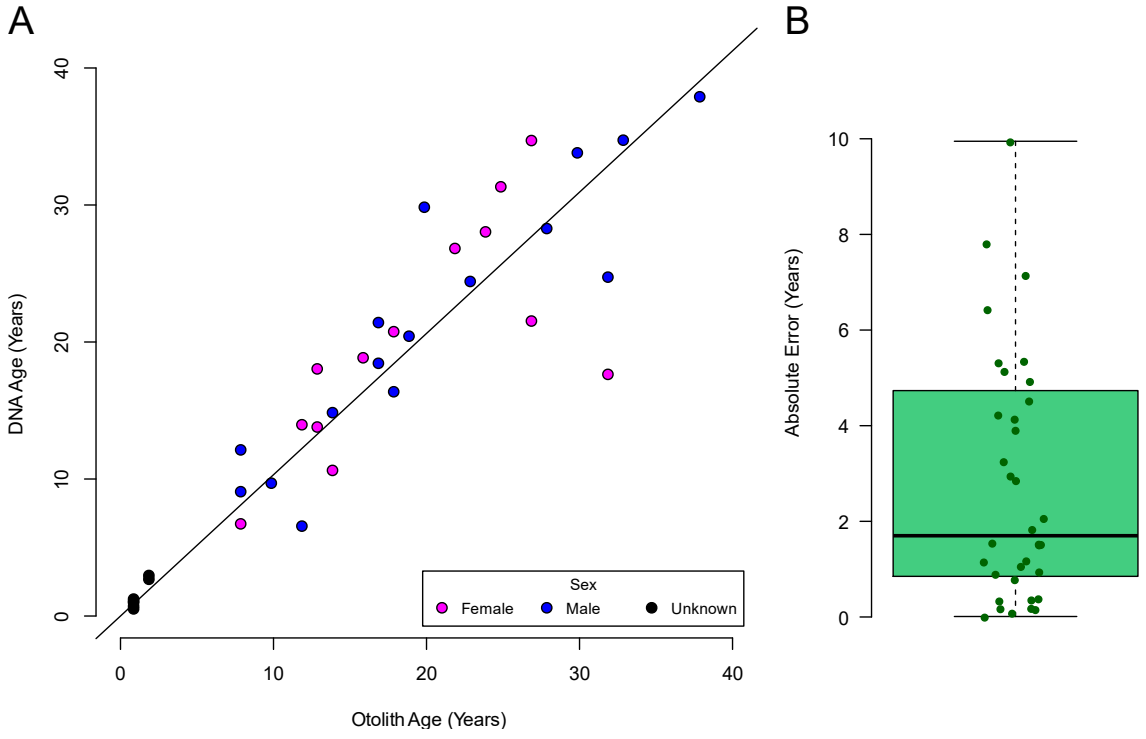


**Figure 1.** Performance of age prediction by DNA methylation in southern bluefin tuna. **A.** The correlation between the age derived from otoliths and predicted by DNA (Pearson correlation = 0.93, p-value = $1.23 \times 10^{-16}$). **B.** Absolute error rate between otolith age and DNA age (Median absolute error = 1.7 years).

## 1.5 Future directions

The initial results presented here highlights the potential to use DNA-based methods for age prediction for SBT. However, additional DNA sequencing can be potentially used to identify other candidate biomarkers in the SBT genome to improve the model. As demonstrated previously, sample size can influence the performance of the model [6]. Large sample sizes in combination with additional DNA sequencing may improve the model. However, given similar biomarkers and sample size have been used on other species, large improvements on the current model are not expected.

## 1.6 Acknowledgements

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