# Tick tock tuna stock: The advent of the epigenetic clock as an aging tool for fisheries stock assessments

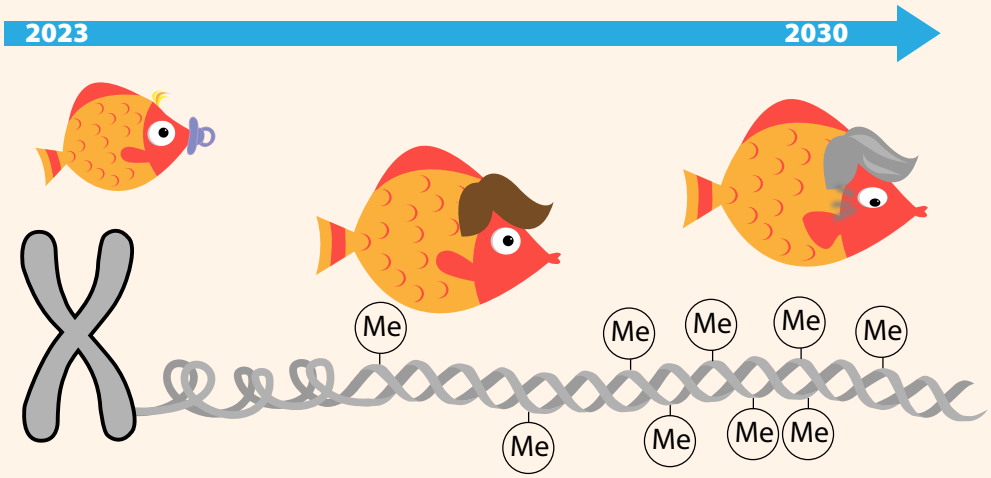
The genomics field is pushing its way into all sorts of research topics. It's possible that you've heard of the use of mitochondrial DNA barcoding to confirm the species of a sample when it isn't visibly evident, such as when using processed fish products (Pollack et al. 2018). Or perhaps you've seen studies predicting the impacts of climate change based on the standing amount of genetic variation in a population of concern (e.g. Capblancq et al. 2020). Or, here's a cool one: did you know that (epi)genomics is now increasingly being used to estimate the age of specimens? The concept is called an epigenetic clock, and it can improve everything, from flagging risk of age-related diseases in humans to growth estimates in tuna stock assessments.

The theory of the epigenetic clock is quite elegant. As individuals age, their DNA ages with them. By quantifying changes in the structure of DNA that occur at a predictable rate over time, it only takes some simple algebra to calculate how long those changes have been accruing and, therefore, how long the individual has been alive.

In slightly more detail, genetic aging occurs in various ways, including epigenetically. Epigenetic aging is distinct from the more commonly known way that DNA changes over time, in which the genome sequence itself is damaged or otherwise mutates in ways that can lead to protein malfunction and eventually to an individual's decline. In contrast, epigenetic processes are those involving the structure of the molecule, and do not affect the actual DNA sequence. The epigenetic mechanism of aging occurs when additional methyl groups bind to the phosphate backbone of a DNA molecule at key areas called CpG sites, where a cytosine precedes a guanine in the genetic code. The presence of the extra methyl groups makes it harder for enzymes to attach to the DNA and start the process of making proteins. Less protein synthesis begets less cell productivity begets less overall vivacity in the organism and more chances to develop dysregulation diseases like cancer. In fact, part of what makes epigenetic methylation such a powerful tool for estimating the age of an organism is that it directly quantifies one of the major mechanisms of aging, rather than providing a proxy.

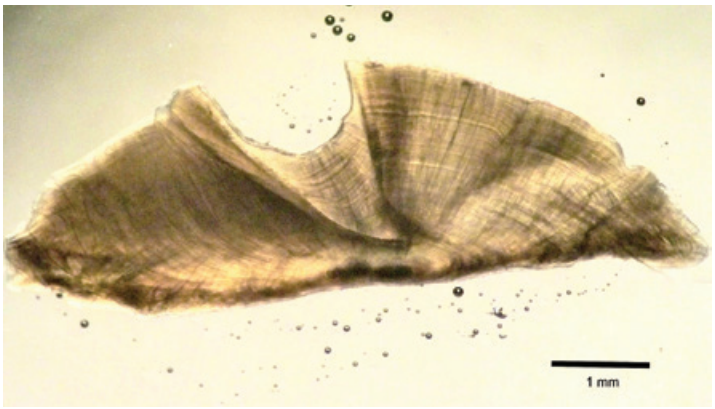
Another powerful benefit of observing epigenetic methylation (and of the epigenetic clock that measures it) is its efficiency of data collection. Given some up-front lab work to select the most informative subset of the genome to observe, and some statistical work to quantify the relationship between known age and the amount of methylation at those selected key points along the genome (which is similar to the amount of effort that goes into any age-versus-predictor curve), it becomes possible to estimate an organism's age from a highly targeted bisulphate sequencing genomic assay – the genomics equivalent of claiming something is "plug and play". Once the methodology is in place for a species, the only limit on the number of specimens that can be aged in parallel is the sequencing capacities of the laboratory.

In contrast, the current gold standard for estimating the age of a fish is via otolith aging. The process requires cutting little ear stones out of each specimen (which requires opening




A simplified diagram of epigenetic aging. Additional methyl groups (Me) bind to the phosphate backbone of a DNA molecule, affecting the structure of the DNA molecule.

Extracting otoliths from a tuna is not a simple process and requires killing the fish. ©Malo Hosken, SPC




Section of an otolith (from a ruby snapper) under the microscope. ©SPC 2012

the fish's brain cavity), setting the delicate calcium structure in epoxy, slicing off a one millimetre thick cross-section, putting it under a microscope, and (hopefully) counting out the number of growth rings that can be used as a proxy for years lived. The process is labour intensive and impractical to automate, and the step of counting rings can be surprisingly subjective. Additionally, and very pertinent to many species of fishery interest in the Pacific, strictly tropical species do not lay down clear, seasonal growth bands on their otoliths because they do not experience strong seasonality or other regime shifts. Therefore, even the gold standard of fish aging is uninformative in species like skipjack tuna.

Granted, epigenetic clocks have their weaknesses as well. An epigenetic clock can only be as accurate as the data it is calibrated against. For almost all bony fish species, that is otolith data. Therefore, the uncertainty in otolith aging estimates is introduced into an epigenetic clock's confidence intervals. In addition, epigenetic clocks introduce their own uncertainty into calculations. In particular, methylation is linked to the biological age of an organism, rather than its chronological age. This limitation circles back to the fact that the clocks observe an actual mechanism of aging, which varies between individuals in response to genetic and environmental factors. For example, in humans, we might blame an earlier decline in quality of life on an individual's family history, poor diet, smoking habits, a sedentary lifestyle, or living in proximity to a pollution-emitting industry. Equivalent factors influence variations in fish. An epigenetic clock's direct observation of biological age is highly useful in some contexts (e.g. predicting an individual's "all-mortality" risk) but the accuracy and precision of absolute age estimates may be impacted if the training dataset is unrepresentative of the population in some way, such as through spatial or temporal bias, or if only some age classes are represented.

Regardless of these potential drawbacks, the popularity of epigenetic aging continues to increase. Epigenetic clocks have been designed across species, with a single clock now developed that is conserved across all tested mammals (Wang et al. 2020), and with human clocks so sensitive they can account for using different types of tissue (Voisin et al. 2020). There are also efforts to understand the conservation of methylation patterns between taxa, which will expedite

the development of assays for genomically uncharted species and those that cannot be independently calibrated (e.g. skipjack). The Pacific Community is currently working to develop epigenetic clocks for deep-water snappers, such as the flame snapper (*Etelis coruscans*) and albacore tuna (*Thunnus alalunga*). The tools will aid future fishery stock assessments by filling knowledge gaps about the age structure of snapper stocks, and by providing essential age information for an inaugural close-kin mark-recapture (CKMR) assessment of albacore tuna, which will estimate the absolute adult population size of the South Pacific stock.

The albacore study is a seminal example of how epigenetic clocks facilitate other types of research to circumvent logistical barriers. In order to give a confident result, CKMR will require age data and genomic sequences from 25,000–30,000 albacore specimens in just a few years. It is impractical, if not impossible, to age that many otoliths in the lifespan of the project. Furthermore, the study will sample commercially caught fish, meaning the cooperating fishermen may not appreciate the mutilation of their valuable catch in pursuit of an otolith. The only other option for aging is the length-at-age growth curve that will introduce a suboptimal amount of uncertainty into age data. In contrast, CKMR already requires the collection of a small amount of tissue for genetic purposes, which can be shared for epigenetic purposes. Without the option for aging by epigenetic clock, it would be difficult to apply CKMR to albacore tuna.

The benefits of epigenetic aging outweigh the shortcomings. Access to an epigenetic clock lifts a major logistical limitation on fisheries research by enabling the aging of hundreds of individuals in parallel using only a few cubic millimetres of muscle tissue that can be collected non-invasively and non-lethally. In the immediate future, it will become possible to apply CKMR to species with higher biomass, and to validate growth curves or age-at-maturity assumptions that significantly impact stock assessments. And the applications will only expand and diversify.

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