An ode to ear stones: A summary of activities around IPWoFA 22

The Indo-Pacific Workshop on Fish Ageing 2022 (IPWoFA22) kicked off at the end of November 2022, and included 28 participants from 13 institutions and 7 countries. The workshop was organised as part of a "Funding with Intent" project to generate epigenetic clocks² for a suite of commercially important deep-water snapper species, and support fish ageing capacity across the region via otolith reading or epigenetic methods.

That is a lot to unpack! Here is the long story. Fish ageing is an important element of fishery management because the age structure of a fish stock has major implications for its sustainability. For example, if a species has a life cycle whereby it becomes disproportionately more reproductively successful with age, it is important to ensure that the oldest fish in a stock are not selectively depleted by fishing efforts. Therefore, fisheries management requires an accurate way to assess the age of fish.

The most common and traditionally enshrined way to age a fish is by counting the growth rings that form on little "ear stones" located on either side of the fish's head, known as otoliths. The concept is the same as counting tree rings; throughout its life, a fish lays down translucent or opaque material at the edge of the otolith, depending on environmental conditions, creating daily and yearly growth rings. Extract the otolith, count the rings, and you will know how many winters the fish has seen. In species that do not lay down nice, clear growth rings (e.g. tropical species that do not experience significant seasonal environmental shifts), otolith chemistry can also be used to infer age. The sexiest version of this type of analysis is bomb radiocarbon otolith ageing. Specifically, the atomic bombs that exploded in the 1940s and beyond fundamentally changed the ratio of carbon isotopes in the atmosphere and, consequently, in fish otoliths. By measuring the carbon isotope ratio along the length of an otolith, it is possible to calibrate the absolute values and change over time with the historical atmospheric levels in order to estimate the year a fish was born and when it died.

Otoliths have been the gold standard for fish ageing for decades. There are some limitations, however, most notably that it does not work in some species, and that a fish must be dead in order to extract its otoliths. Another option is using length vs age growth curves that plot the length of a fish against its (otolith-validated) age, and then apply the resulting pattern to a non-aged fish. It would be great to need nothing but a fish's length to know its age, but individual variation in growth and growth cessation after a certain age can introduce considerable inaccuracy in the resulting estimations.

Enter epigenetic ageing. The basic concept is that any living organism has DNA that begins degrading as soon as it is born, particularly by the increased methylation of key areas in the genome. For those of you who are molecular biology and chemistry enthusiasts: methylation happens when an

extra methyl group (CH₃) attaches to the phosphate groups that make up the backbone of DNA molecules, and results in reduced expression of certain genes. The relative amount of methylation of an individual's DNA can, therefore, be calibrated to its age, with some technical discussion of biological age vs chronological age. The same applies to some humans who are more vital at age 70 than others who are 60. Developing epigenetic clocks also requires a validated ageing method (i.e. otolith reading) in order to make the initial calibration. However, after a lot of prerequisites and upfront work, epigenetic ageing allows for reasonably precise, cheap and non-lethal ageing of an organism.

All of the above was covered in IPWoFA22. The fundamental goal was to draw together fisheries scientists, fisheries professionals and geneticists (who sometimes happen to work on fish) from across the Indo-Pacific region to showcase and share knowledge on current and emerging fish ageing methods, and to demonstrate why accurate age estimates are important for stock assessments. At the workshop, there was also plenty of shop talk among fish agers from around the Pacific and beyond, and practical lab time where everyone who cared to could practice otolith extraction (and, later, ring counting), genetic sampling using biopsy punch tools, and even an off-the-cuff demonstration of extracting a fish eye lens (which is another organ that can be used for age validation).

The workshop covered an ambitious amount of material, and no one walked away without some expansion of their knowledge base. Ultimately, it is hoped that IPWoFA22 and its outcomes will help foster an Indo-Pacific network that is skilled in the latest fish ageing techniques, and with the capacity to coordinate and apply these techniques in future work across the region. Cheers to IPWoFA22, its organisers, and to the many collaborations in fish ageing to come!

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 $^{^{2}}$ An epigenetic clock is a tool to estimate the age of an organism based on biophysical properties of its DNA.