Ageing fish with Fourier-transform near infrared spectroscopy: theory and case study using otoliths from white grunt (*Haemulon plumierii*) in the U.S. South Atlantic

Envejecimiento de peces con espectroscopia de infrarrojo cercano por transformada de Fourier: teoría y estudio de caso utilizando otolitos de ronco blanco (*Haemulon plumierii*) en el Atlántico sur de U.S.

Vieillissement de poissons avec spectroscopie proche infrarouge à transformée de Fourier : théorie et étude de cas utilisant des otolithes de grognement blanc (*Haemulon plumierii*) dans l'Atlantique Sud des U.S.

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EXTENDED ABSTRACT

Fish ages are an essential component for the assessment of stock status. Age data are used for estimating longevity, cohort tracking, and understanding population dynamics of commercially and recreationally valuable species. Otoliths are the most widely utilized hard part in fish ageing. Traditional ageing methods can be time consuming, requiring sample handling, sectioning, and visually counting the incremental annuli using microscopes. The Southeast Fisheries Science Center collectively receives up to 85,000 hard parts each year. The process of ageing requires a large investment in time and staff resources to provide data for annual stock assessments. The use of advanced technology could increase production ageing if it can maintain comparable ageing accuracy to traditional methods. Currently, scientists from NOAA Fisheries are evaluating the feasibility of using Fourier Transform Near-Infrared (FT-NIR) spectroscopy to accurately predict fish ages.

FT-NIR spectroscopy was first utilized in the pharmaceutical, chemical, and agricultural industries, but has recently been applied to fish ageing (Helser et al. 2019). This method is non-destructive to the sample, generating a "spectral signature" of absorbance measurements by sending near-infrared light through a sample and exciting the sample's chemical bonds. This spectral signature quantifies the presence, and concentration, of organic bonds, displayed as peaks at specific wavelengths. The spectral peaks relate to the chemical composition of the sample. The spectral signatures can be converted to fish age estimates, but FT-NIR spectroscopy is a secondary measurement technique. It relies on a primary measurement technique (traditionally derived ageing) to convert spectra to the primary measurement (age).

When pursuing the development of spectral models for age prediction at the NOAA Beaufort Lab, white grunt was determined to be an ideal candidate species. White grunt is a reef-associated coastal fish found in the western Atlantic between the Chesapeake Bay, south through the Gulf of Mexico and Caribbean and along the eastern coast of Brazil. Previous studies have identified differences among different geographical stocks. Fish from southeast Florida grow much slower and attain smaller sizes than fish collected off the Carolinas (North and South Carolina; Potts & Manooch 2001). Fish from the Gulf coast of Florida reach comparable size-at-age to fish from the Atlantic coast of Florida, yet both are smaller-at-age than fish from the Carolinas (Murie and Parkyn 2005). Studies have identified 4 genetically distinct stocks of white grunt located in (1) the Carolinas, (2) southeast Florida and Florida Keys, (3) the eastern Gulf of Mexico, and (4) the Caribbean Sea (O'Donnell et al. 2019). White grunt are considered an important species in U.S. commercial and recreational fisheries (Potts 2000), however, there are no current size or trip regulations in either fishery. White grunt could be susceptible to overfishing if management is inappropriate. To date there has not been a stock assessment using current age-based methods. Additionally, future assessments have been delayed because of uncertainty in stock definition. The application of FT-NIR spectroscopy to the ageing of white grunt in this study could supply efficient and accurate age data to support a timely assessment.

The objective of this study was to use FT-NIR spectroscopy to age two genetically distinct stocks of white grunt from the U.S. Atlantic: the Carolinas and Florida. This involves (1) the development and application of standardized practices for FT-NIR spectroscopy tested across various NOAA labs, (2) scanning of known-age otoliths and creation of two different cross validated calibration partial least square models for both the Carolinas and Florida stocks of white grunt and (3) application of those calibration models to test validation sets to assess accuracy in predicting age of unknown otoliths.

For this study, 1,390 Atlantic white grunt otoliths were weighed, scanned on a TANGO-R spectrometer (Bruker),

sectioned on the Hillquist (thin sectioning machine), and aged under a microscope. To build the calibration model, spectral data of a representative set of known age otolith scans were used to build a partial least squares (PLS) regression model for both the Carolinas and Florida stocks using a chemometric software called OPUS (Bruker). A second set of otoliths of known age were scanned to determine how well the model performs, test validation. Once the performance of the model is confirmed, then otoliths of unknown age can be scanned to estimate their ages in an age production setting.

Similar to previous studies, the morphometric data of the fish in this study revealed white grunt from the Carolinas grew to larger sizes, older ages, and possessed heavier otoliths than the Florida white grunt. Preliminary results from the calibration model development show better correlation of spectral signature and predicted age for the Carolinas stock than the Florida stock. For the Carolinas PLS model, the R2 was 90.57 and the Root Mean Square Error-Cross-Validation (RMSECV) was 1.13 which means the model predictions were within 1.13 years from the reference age. The Residual Percentage Difference (RPD) was over 3, reflecting a good model fit. Next, a test validation will be done to assess prediction accuracy of 'unknown' age samples. For the Florida PLS model, the R2 was 86.64 and the RPD was less than the optimal 3. This model had difficulties predicting age, potentially because of the plateaued relationship between otolith weight and age in Florida fish. Modifications to this model will be needed before it is ready for test validation. Optimistically, 0-1 and 13+ year old otoliths will be incorporated into each stock's calibration model and a second age reader will be adopted to create consensus reference age reads. Overall, we observed a moderate correlation between (1) otolith absorbance and age assignment and (2) reference age and predicted age, but there are still improvements to be done. Further research is needed to understand why the technology predicts ages well for the Carolinas stock but not the Florida stock. FT-NIR spectroscopy promises to be an accurate predictive ageing method, but requires additional research, technological support, financial investment, and consistent model upkeep.

KEYWORDS: Ageing, FT-NIR spectroscopy, White grunt

LITERATURED CITED

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