

Fourier Transform Near Infrared (FT-NIR) Spectroscopy as a Rapid and Transformative Method for Estimating Fish Age

Espectroscopia de infrarrojo cercano por transformada de Fourier (FT-NIR) como un rápido y método transformador para estimar la edad de los peces

La spectroscopie FT-NIR (transformée de Fourier dans le proche infrarouge) en tant que spectroscopie rapide et Méthode transformatrice d'estimation de l'âge des poissons

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EXTENDED ABSTRACT

National Marine Fisheries Service laboratories from five regions across the United States are investigating the utility of Fourier transform near infrared (FT-NIR) spectroscopy as a rapid and transformative method for estimating fish age. With increasing needs for more stock assessments to support the federal regional fishery management councils, more efficient alternatives to the conventional, labor-intensive, and time-consuming methods for providing age estimates are needed. FT-NIR spectroscopy is a well-established quality control method utilized by the food and agriculture, chemical, petrochemical, and pharmaceutical industries. Recently it has been applied to fish otoliths (Wedding et al. 2014; Helser et al. 2018; Passerotti et al. 2020), as well as shark (Rigby et al. 2016) and skate vertebrae (Arrington et al. 2021), as a novel method for estimating ages. Unlike traditional age processing methodologies, FT-NIR spectroscopy provides a rapid method to estimate fish age; however it is a secondary ageing approach. A critical component for successful implementation of FT-NIR spectroscopy relies on building predictive models, where age estimates assigned by an age reader, using traditional methods, provide the input ages that allow translation of scan outputs to ages. It requires little to no sample preparation and the average scan time is approximately one sample per minute. FT-NIR spectroscopy is a non-destructive method, which means that samples will be available for future research opportunities. Perhaps even more important is that this technology could move us closer to providing more real time data for stock assessments.

Fish otoliths, which continue to grow throughout the life of a fish, are composed of calcium carbonate (CaCO₃) deposited on a protein matrix. Successive growth increments are microscopically enumerated to estimate age. Preliminary results suggest that the spectral information most important for otoliths displays in the regions related to the molecular constituents (or functional groups) in proteins, such as carbon-hydrogen (C-H), oxygen-hydrogen (O-H), and nitrogen-hydrogen (N-H) groups. In general, spectral absorbance increases as fish age increases (Figure 1). For this study, FT-NIR spectroscopy was applied to otoliths from gray snapper, *Lutjanus griseus*, collected from the northern Gulf of Mexico to estimate fish age.

Otoliths from gray snapper collected across five years from the northern Gulf of Mexico were scanned with a Bruker Multi Purpose Analyzer II FT-NIR spectrometer. All otoliths were covered with a gold stamp surrounded by a rubber mold to reduce stray light. After the otoliths were scanned, they were sectioned using a Hillquist1 high speed thin section saw. Ages, hereafter referred to as reference ages, were then assigned by two independent age readers using traditional, microscopic ageing methods. Calibration models using partial least squares (PLS) regression were applied to the reference ages to predict an FT-NIR spectroscopy age. Once a calibration model was fit, this model was then applied to an external (i.e., test) set of traditionally aged otoliths to evaluate the predictability of the model to predict fish age. Model fit was analyzed using coefficient of determination (R²), root mean square error of cross validation (RMSECV), root mean square error of prediction (RMSEP), and a relative percentage difference (RPD). In general, an RPD > 3 is considered a good model. The chemometric software package OPUS1 (version 8.5, Bruker Optics) was used for data processing and generating PLS models. Data were preprocessed using first derivative and vector normalization.

A total of 1,419 otoliths from gray snapper were scanned from years 2016-2020. Reference ages ranged from 2 to 29 years. The calibration model (n = 420), which uses a leave one out cross validation method, had an R² = 92.03, RMSECV = 1.53, and an RPD = 3.54 (Figure 2). The test model (n = 999) had an R² = 89.71, RMSEP = 1.55, and RPD = 3.11 (Figure 2).

Overall, FT-NIR-predicted ages were in good agreement with traditional ages. For the calibration set, 92% of the variability in ages generated from the spectral data from gray snapper otoliths can be explained by the reference ages.

Calibration model performance slightly decreased when applied to the test set. Typically, gray snapper otoliths are one of the easier otoliths to age. However, age readers described the gray snapper otoliths used in this study as mostly difficult to age, which means ageing error in the reference ages may be contributing to a lower fit of the model. All in all, results from this study are promising for a paradigm shift from the typical, laborious, time-constraining process currently used to a more rapid, cost-effective method using FT-NIR spectroscopy to estimate fish age.

KEYWORDS: FT-NIR spectroscopy, otoliths, age, gray snapper

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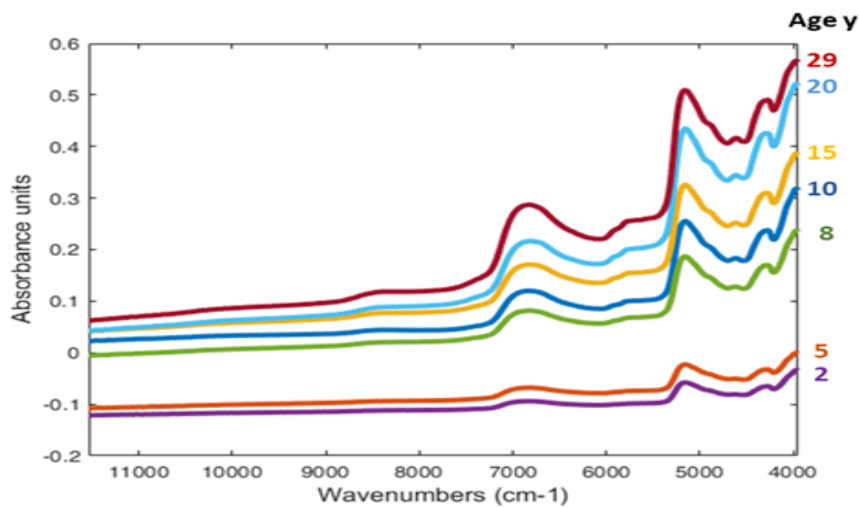


Figure 2. Average Fourier transform near infrared spectra of gray snapper otoliths for reference ages 2, 5, 8, 10, 15, 20, and 29 years.

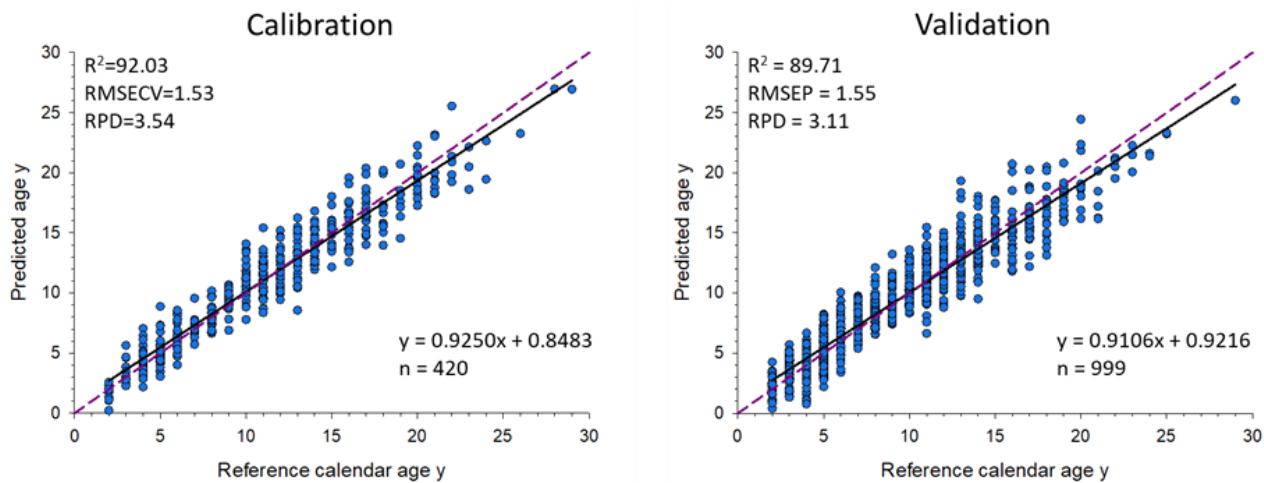


Figure 2. Results from partial least squares regression (solid line) for gray snapper calibration and test models. A 1:1 line is represented by the dashed line.

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