Development of a novel NIR method for estimating the age of tropical fish from otoliths

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Introduction

Reliable fish ageing methods are needed for age-based stock assessment and sustainable fisheries management. Fish ear bones (i.e. otoliths) are calcareous structures that have annual growth rings similar to tree-trunks. Otoliths are formed through an accretionary process, which results in alternated translucent summer growth and opaque winter growth rings or bandings termed annuli.[1] Conventional methods of fish age estimation are based on the examination and interpretation of these translucent or opaque growth rings in otoliths. Different fish species have otoliths of different shapes and sizes. Estimation of fish age by otolith increment analysis is a time-consuming, labour intensive and expensive process.[2] The technique is also highly subjective in the visual interpretation of growth bandings, particularly for many tropical fish species where large variations in otolith size and development result in a wide range of banding patterns. With these otoliths, it is usually necessary to section off a thin slice and resin mount before viewing the bands under a microscope.

Processing and interpretation errors of otoliths can result in age estimates that differ by as much as a factor of three among investigators.[3] As a result of various aging difficulties, only expert readers with a proven experience for a given species and a given stock can provide reliable age estimates.[1] This was demonstrated during a European workshop on plaice otolith reading held in 2003 at which inter-reader agreement rates varied from 40 to 95% depending on the reader's experience of fish samples whereas this rate was 85 to 95% for expert readers.[1]

At present, no rapid, objective and widely-applicable method exists to determine the age of fish. For routine ageing, the cost-benefit analysis of an automated system needs to balance the accuracy reached by the system compared to trained readers and the gain in terms of processing time. In this preliminary study, the potential of Fourier Transform near infrared (FT-NIR) spectroscopy was investigated as a tool to predict the age of saddletail snapper (*Lutjanus malabaricus*) from whole otoliths.

Materials and Methods

Otoliths from 100 *Lutjanus malabaricus* were obtained in 2009 from the Gulf of Carpentaria, Australia. Diffuse reflectance spectra of individual whole, dried otoliths were collected using the integrating sphere and sample wheel system on a Bruker MPA FT-NIR spectrophotometer (Bruker Optics, Ettlingen, Germany; operating software: OPUS™ version 6.5) in the 800–2780 nm range. All otolith samples were placed with the same orientation (convex down) in the sample vials in preparation for spectra capture. In obtaining each sample spectrum, 32 scans at a resolution of 8 cm^{-1} were collected and averaged.

The Unscrambler (version X 10.1; Camo, Oslo, Norway) was used to develop partial least squares (PLS) and multiple linear regression calibration models between the reference otolith age estimation of growth bands and the FT-NIR spectra. The reference age estimations of fish otoliths were obtained with the assistance of trained Queensland Fisheries staff from Southern Fisheries Centre, Deception Bay using the standard method of increment determination of otolith cross-sections.[3,4]

Full cross-validation was performed in the regression analysis and a number of different pre-treatments were investigated. Results obtained using a PLS model based on a combination of a 25 point Savitzky-Golay (SG) spectral smoothing (second order polynomial) and a first derivative transformation (25 point SG smoothing and second order polynomial) over a selected wavelength region (not shown) was found to give optimum results and are presented in this study. Model performance was based on the coefficient of determination (R^2), root mean square error of cross-validation (RMSECV) and bias while the standard

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deviation ratio (SDR = standard deviation of the data set / RMSECV) was used to determine the predictive ability of the calibrations.

Results and Discussion

The PLS calibration model results presented in this study yielded an R^2 of 0.94, RMSECV of 1.41 and an SDR of 4.2 for otolith age in the range from 1 to 21 years. The SDR is in excess of three so the prospect of age estimation is good.[5] The results were encouraging considering the preliminary nature of the trials and the error associated with the conventional visual interpretation of the otolith banding patterns for estimating age.

Table 1. Preliminary PLS calibration statistics for whole dried *Lutjanus malabaricus* otoliths.

Spectra (n)	Age range (years)	SD	LV	R^2	RMSECV	Bias	SDR
100	1 – 21	5.89	3	0.94	1.41	-0.0153	4.2

Note: LV = latent variables; SD = standard deviation.

Conclusion

This preliminary study has demonstrated the potential for FT-NIR spectroscopy to rapidly and non-destructively predict the age of *Lutjanus malabaricus* from whole excised otoliths. Further work is required to optimise this technology in relation to sample presentation, calibration model development, and speed of throughput for industry adoption. The development of robust calibration models will require training sets that cover variables such as summer and winter otolith growth, yearly differences, fish size, diversified age structure and location variables, and measurement conditions (sample handling and presentation). The successful development of NIR methods for innovative fish ageing would not only minimise the reading error associated with subjective visual interpretation but also substantially reduce the time and labour component of existing otolith preparation procedures. NIR spectroscopy could assist in sustainable fisheries management by providing a reliable and real time ageing method.

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