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A preliminary analysis for age estimation of yellowfin tuna using near-infrared spectroscopy

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Kei Okamoto, Keisuke Satoh and Takashi Kimiya

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Kei Okamoto[1], Keisuke Satoh[1] and Takashi Kimiya[2]

[1]Fisheries Resources Institute, Fisheries Research and Education Agency, Yokohama, Japan.

[2]Marine Fisheries Research and Development Center, Fisheries Research and Education Agency, Yokohama, Japan.

Abstract

Near infrared spectroscopy (NIRS) is a non-destructive optical spectroscopy technique that has recently been applied to age estimation of fish species, including the Pacific cod and snappers. Age-length relationships of yellowfin tuna (*Thunnus albacares*) in the Western and Central Pacific Ocean have been estimated using various results such as otolith age-ring reading, tagging release and recaptures, and modal changes in length composition, but there is a need to develop more objective and simplified age estimation methods. In this study, the NIRS measurements were performed on otoliths of yellowfin smelt reared for several years from the young of year in the southern part of Japan. As a result, the NIRS data showed trends that were likely to be separated by age and size class by principal component analysis. Based on these data, we will construct an age estimation model to examine its applicability to age assessment of wild yellowfin tuna in the future.

Introduction

Age determination methods and growth model of the WCPO yellowfin tuna

In the stock assessment of yellowfin tuna in the Western and Central Pacific Ocean (WCPO), various biological parameters such as growth, maturity, natural mortality, and migration have been subject to frequent data updates and improvements in various estimation methods and models under the philosophy of best available scientific knowledge, but there are still some uncertainties, such as growth curve (Magnusson et al. 2023; Punt et al. 2023). Growth curves have been estimated by accumulating age data when a fish reached certain body size using various results such as otolith annual ring decipherment, tagged fish release and recaptures, and modal changes in length composition. Among these age assessment and estimation methods, the method based on otolith age annuli reading is widely used regardless of fish species, but it is one of the costliest elements in the fishery stock assessment process in terms of both cost and time. Traditionally, processing otoliths for age determination requires encapsulation in resin, thin sectioning, mounting of sections on slides, and counting growth zones with multiple readers to generate age estimates, with the total time required averaging several hours per specimen. In addition, reading methods, experience, and skill of the reader may bias the reading results (Campana 2001). Age determination of yellowfin has been conducted

using age traits (otoliths, vertebrae, dorsal fin spines, and scales (Aikawa and Kato 1938; Farley et al. 2018; Nose et al. 1957; Yabuta et al. 1960)) and using body length composition (Hennemuth 1961). In recent years, age assessment by bomb radiocarbon has also been used (Andrews et al. 2024). These have been shown to have a significant impact on stock assessment results in the WCPO yellowfin tuna (Punt et al. 2023), and the growth curve used for each stock assessment conducted periodically in the WCPFC is different, and the validation of age assessment methods (Farley et al. 2019) and the objective age estimation methods are still seeking. Thus, the methods that have been implemented to date are limited in terms of labor, time, and cost, the number of specimens and recaptures used for age assessment is limited (although a significant number have still been analyzed), and the data used for analysis may be biased. Therefore, if an age estimation method that is simpler, objective, quicker, and less costly can be developed, it may be applicable to a larger number of individuals and a broader population covering a wider range of areas.

The rationale and application of Near Infrared Spectroscopy (NIRS)

Near infrared spectroscopy is a non-destructive spectroscopic technique that has been used in agriculture and medicine for decades (Reich 2005) and more recently in wildlife biology (Passerotti et al. 2020; Vance et al. 2016). NIRS obtains spectral data by exposing a sample to light in the near infrared (NIR) region (wavelength 800-2500 nm (wavenumber 12500-4000 cm^{-1})) and observing the interaction of this light with the sample at each wavelength (or wavenumber). This indicates the presence and amount of organic chemical bonds in the sample, namely -CH, -OH, -NH, and -SH (Murray and Williams, 1987; Williams, 2008). In biological applications, spectral data on absorbance taken from various species have been correlated with variables such as mosquito age (Mayagaya et al., 2009), frog sex (Vance et al., 2014), and mammalian fecal content (Tolleson et al., 2005), and diagnostic tools have been developed to predict these indicators based solely on spectral data.

Applications and methods of NIRS for age assessment of fish

Rapid age estimation of fish using NIRS scans of whole samples with age determination character (e.g. otoliths) has been applied previously (Wedding et al., 2014; Rigby et al., 2014; Robins et al., 2015; Helser et al., 2019; Passerotti et al., 2020). To apply NIRS technology to fish age estimation, a calibration model that uses spectral data as explanatory variables and age as the response variable is developed using multivariate regression analysis, such as partial least squares (PLS) regression. Next, the model is evaluated using another set of spectral data which is obtained from a sample of known age. This process produces a linearly correlated model that predicts the age of the fish based on a fast scan of the whole otolith (typically a few minutes). It is expected that the calibration model will capture as much age-related spectral variability as possible so that its subsequent predictive ability will be robust. Both internal cross-validation and external validation using a separate otolith test set will be

used to evaluate the predictive ability of the calibration model (Williams, 2008). Although otolith samples of known age are required to use this method, there is currently uncertainty in the results of age estimation of yellowfin tuna collected in the wild WCPO using conventional methods. It is also necessary to confirm the effects of otolith storage conditions, the method of otolith placement at the time of measurement, the left and right sides of the otolith, and the damage status of the otolith (Robins et al., 2015), but no information is available for yellowfin tuna.

In this study, we conducted NIRS measurements of otoliths from young WCPO yellowfin tuna with known approximate daily age and used the resulting spectral data to investigate their relationship to age information, with a view to developing an age estimation model in the future.

Materials and method

Field sampling and tank experiment

In this study, otoliths of yellowfin tuna caught in the waters around the Amami Islands (27°01' to 28°46' N and 128°07' to 130°06' E) and reared in outdoor sea cages (20 m and 18 m in diameter each) at the Amami Field Station of the Fisheries Technology Institute (28°09'N, 129°15'E) were used. The fish were measured FL, identified by dart tags or PIT tags, reared, and then otoliths were removed from the dead fish. These yellowfin tunas were started rearing in October 2020, May 2021, and May 2022, taken ashore within a few days of death, frozen, stored and removed otoliths. The otoliths were refrozen, cleaned, dried, and stored at room temperature in 2-mL tubes. To keep the conditions constant, the samples were dried in a thermostatic chamber at 40℃ overnight before NIRS measurements. Note that complete, undamaged otoliths were used for NIRS measurements.

NIRS Spectral data collection

Spectral data were collected by reflectance mode using an XDS Monochromator Type XM-1000 (Metrohm, Herisasu, Switzerland). The whole otolith was placed in the center of the sample window with the rostral axis horizontal to the sample window, with the sulcus touching the sample window surface, and two different orientations of the rostrum were measured at 90-degree intervals (back and right). For each otolith, a total of 32 spectral scans were acquired every 0.5 nm along the entire NIR region (wavelength: 800-2500 nm), and the scans were averaged to produce a representative spectrum for each sample. Each spectrum took approximately 60 seconds to create. The raw spectral data obtained was used for data reduction and visualization by principal component analysis (PCA). Spectral data analysis was performed using R (R core team 2022). Note that data from 1150 to 2350 nm were used to perform the PCA.

Data analysis

The daily age at start rearing was estimated by applying the starting FL to one of the growth models

used in the WCPO yellowfin tuna stock assessment (Vincent et al. 2020). The daily age at death was determined by adding the number of reared days. When visualizing the data after the PCA was performed, the daily age at death data were truncated to the 10th decimal place and grouped by 100 days. The PC1 and PC2 plots in PCA were grouped by cohort by FL (cohorts per 20 cm), daily age (cohorts per 100 days), and age (cohorts per 0 and 1 year), respectively.

Results

The 122 yellowfin tunas used in this study had a FL of 32.9-57.0 cm at the beginning of rearing (Fig. 1) and were estimated to be 184-383 days old. The fish were then reared in the sea cage for 0-512 days and had a FL of 31.9-82.4 cm at the time of death. These yellowfin tuna otoliths were measured NIR absorbance spectra were obtained for each individual (Fig. 2). PCA of the obtained spectral data showed that the first two principal components (PC1 and PC2) explained 99.5% of the spectral variation among otoliths (Fig.3). The PC1 and PC2 scores were grouped by FL, daily age, and annual age cohorts, and although there was some overlap between cohorts, each cohort could be clearly classified in all cases. The wavelengths with the highest contribution in PCA were 1150.5-1168 nm, 1261.5-1269 nm, and 1272-1279.5 nm for PC1, and 2340.5-2343 nm for PC2.

Discussion

In this study, NIRS measurements were made on yellowfin tuna otoliths and spectral data were obtained for the first time. Based on the NIR spectral data of the entire yellowfin otolith, it was found that the spectra differed according to age and body size. PCA showed a clear difference between 0- and 1-year-old fish when annual age was used as an indicator, indicating results that seem to be discriminable, similar to fish species that have previously been reported to be capable of age estimation. As a more detailed scale, each cohort, grouped by 100-day in daily age, had significant overlap and was difficult to classify. This may be due in part to the limited number of specimens and the fact that the dispersion of individuals comprising the 100-day break was not uniform among the 0-100, 101-200, and 201-300 day groups. In addition, this report is a preliminary analysis; otoliths from fish older than 2 years have not been analyzed, and the number of individuals analyzed from young of year fish is small. However, even at this point, we believe that our results are useful for constructing an age estimation model, and we expect that further data will be accumulated in the future by increasing the sample size and adding otoliths from older fish.

The wavelengths at 1150.5-1168 nm, 1261.5-1269 nm, 1272-1279.5 nm, and 2340.5-2343 nm, where the contribution in PC1 and PC2 of PCA was high, are the absorption bands of functional groups such as -CH, -CH2, and -CH3. Thus, it was suggested that the content of these groups may vary depending on the age and body size of yellowfin tuna.

In this study, NIRS data obtained from otoliths of 0- and 1-year-old WCPO yellowfin tuna allowed

us to discriminate between both age groups. Generally, when building age estimation models using otolith NIRS data, PLS analysis is performed using each PC score obtained by PCA (Wedding et al., 2014; Rigby et al., 2014, 2016; Robins et al., 2015; Helser et al., 2019; Passerotti et al., 2020). In the WCPO, this species is considered to have a life span of about 15 years (Andrews et al. 2024), and the data in this study were limited to young fish and the number of data was also limited. Therefore, it is expected that more otoliths from various sizes will be analyzed in the future to estimate ages of this species as well as investigating the mechanisms of age determination from the NIRS data, that is also not well understood in previous similar studies on other fish species (Passerotti et al. 2020).

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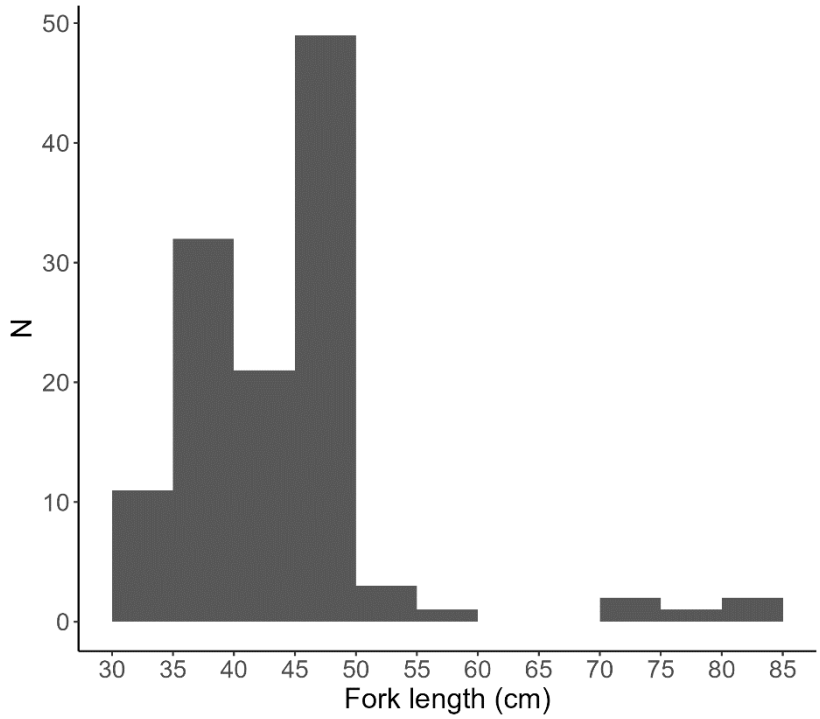

Fig.1. The FL distribution of yellowfin tuna used in this analysis.

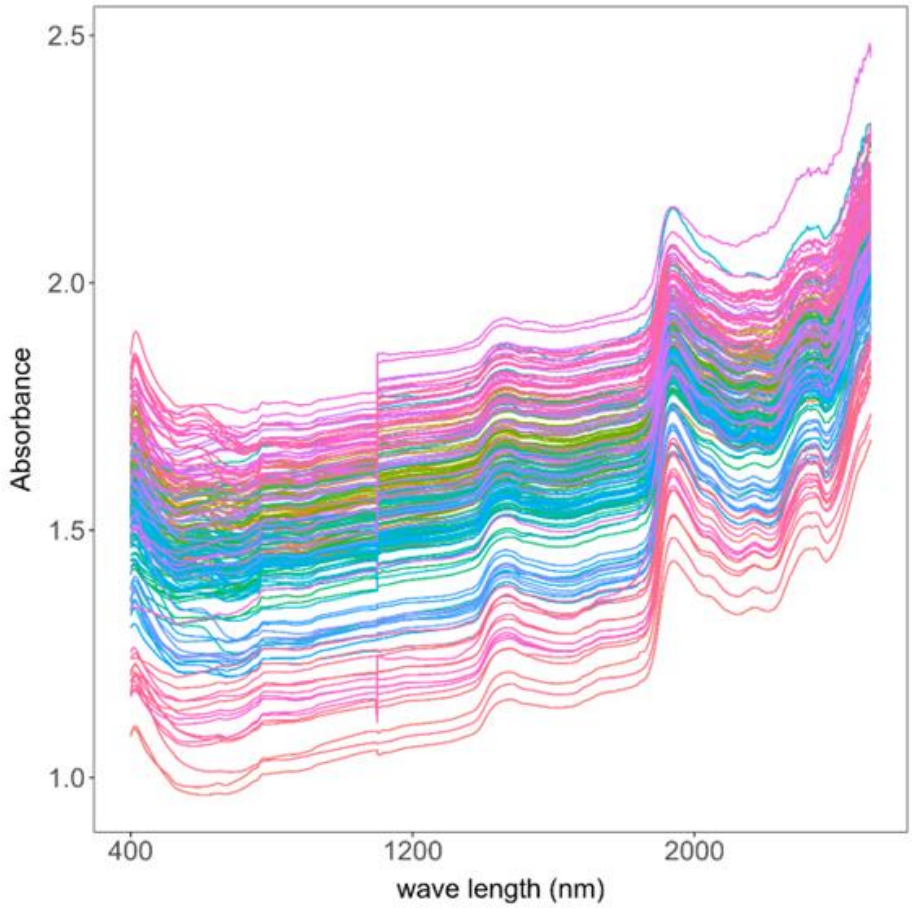

Fig.2. NIR absorbance spectral measured from yellowfin tuna otolith. Each line indicates NIR absorbance spectral data of each specimen.

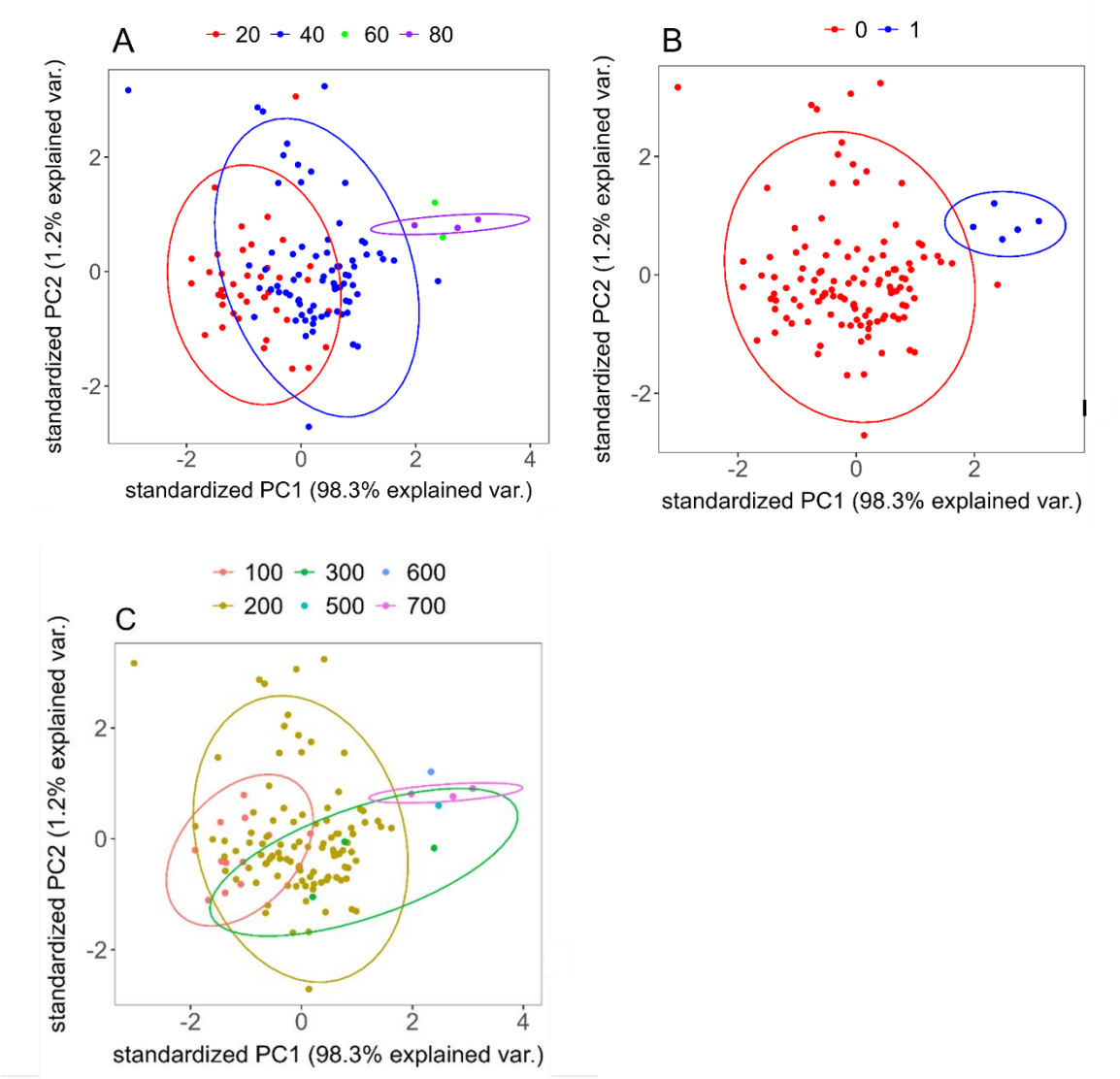

Fig.3. The score plots of PC1 and PC2 from PCA of NIR spectral data colored by FL cohort (A), annual age cohort (B), and daily age cohort (C).