IOTC–2017–WPTT19–42
Received: 22 September 2017

PRELIMINARY STOCK STRUCTURE STUDY OF SKIPJACK TUNA (Katsuwonus pelamis) FROM SOUTH JAVA USING OTOLITH SHAPE ANALYSIS

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ABSTRACT:

Skipjack tuna (*Katsuwonus pelamis*) regarded as cosmopolitan species and distributed vastly along the Indian Ocean south of Java, Bali and Nusa Tenggara. Known to have high exploitation by various fishing gear but yet it always managed as a single stock, not because of scientific evidence but merely based on “a scientific assumption”, so that vulnerable to subject of overfishing. The objective of this study was to find alternative tool for identifying the stock structure based on the otolith shape. Otolith samples were collected from four areas, namely: Binuangeun, Sadeng, Prigi and Labuhan Lombok during April, August, and September 2016. The otolith shape was reconstructed using outline analysis with discrete wavelet transformation technique. Multivariate statistic with cluster analysis using canonical analysis of principal (CAP) and ANOVA-like permutation test were also implemented to determine the signification among populations. The result showed that skipjack’s otolith shape was varied from one and another, especially in rostrum. But it wasn’t statistically different among regions ($p>0.001$), which means a single stock for skipjack was defined in the Indian Ocean (Indonesian territory of FMA 573). This study also proved that otolith shape can be useful marker tool to identify stock structure for management purpose.

KEYWORDS: otolith shape, stock structure, skipjack tuna, ShapeR

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INTRODUCTION

The stock concept is one of the most fundamental in fishery management (Hussy *et al.,* 2016). Failure to take stock mixing into account in fisheries management, particularly when the stocks provide a signification differences in productivity, may lead to sub-optimal exploitation and ultimately over-fishing of some stock components (Begg et al., 1999; Heath et al., 2014). Therefore, efficient stock discrimination methods are needed. Proper identification of population structure is of primary importance for successful management and conservation of marine fishery resources.

Nowadays, science develops very dramatically, so more and more analytical techniques have been used to identify fish stocks in marine waters. However, such methods are expensive and labor intensive, such as molecular genetics and otolith chemistry, and samples are subject to contamination when collected by field-based fishery sampling programs (Shepard et al., 2010). Thus, provides an opportunity for researchers to develop more efficient analytical techniques to identify stock structure, especially highly migratory species, such as skipjack tuna (*Katsuwonus pelamis*) which known as cosmopolitan species and distributed in tropical and sub tropical waters (Arai et al., 2005).

Among the methods used to identify stocks, the study of the morphological characteristics of otoliths has been considered an efficient tool for fish stock identification (Campana & Neilson, 1985). Otolith shape analysis has recently been widely used with success in stock identification of various marine fish species, including small pelagic fish such as anchovy (Bacha et al., 2014), Atlantic herring (Burke et al., 2008), and horse mackerel (Stransky et al., 2008). The aim of this study was to perform otolith-shape analysis to investigate the population structure of the skipjack tuna, which is still rarely implemented to assess marine fisheries in Indonesia.

MATERIAL AND METHODS

Sampling and Sample Handling

Skipjack tuna were sampled from four landing ports along southern Java and Nusa Tenggara during April, August and September 2016, such as Binuangeun, Sadeng, Prigi, dan Labuhan Lombok (details are summarized in Figure 1 dan Table 1). The sites were selected in order to cover as much as possible the whole geographical range of the species. Biological data also collected including fork length (cm, nearest 1 cm), total weight in gram (nearest 1 gram), sex and maturity visually and histology according to Effendie (1979) and Davis *et al.*,(1999). Otoliths, in particular sagittae, were extracted using *open the hatch method* (Secor *et al.*, 1992) and collected using fine tweezer, washed and cleaned in distilled water, and then dried in room temperature. Only the whole and complete otolith (left and right and no damage) were used in this study.

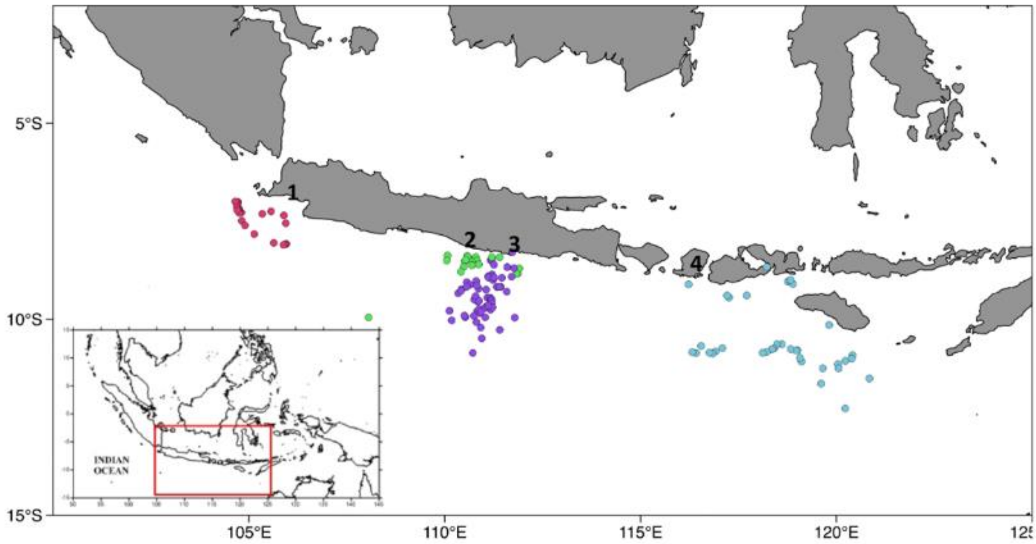


Figure 1. Sampling location for skipjack tuna (*K. pelamis*) otoliths : (1) Binuangeun, (2) Sadeng, (3) Prigi, dan (4) Labuhan Lombok. The dots were representing fishing ground where Binuangeun (red), Sadeng (green), Prigi (purple), and Labuhan Lombok (blue)

Image acquisition and shape analysis

The otoliths photographed with AxioCam 5MP camera connected to Carl Zeiss Stemi 2000C stereo microscope. The microscope magnification was adjusted to the same level i.e 6,5 times. The otoliths were placed on a microscope plate on dark background with the sulcus acusticus facing down (Figure 2a). High contrast images were obtained using transmitted light, resulting two dimensional objects with a dark background transformed to grey-scale on *jpg format. Manipulation on contrast and brightness of the image also implemented using ImageJ and GIMP2 are open source software on http://rsbweb.nih.gov/ij/ and http://gimp.org/downloads respectively.

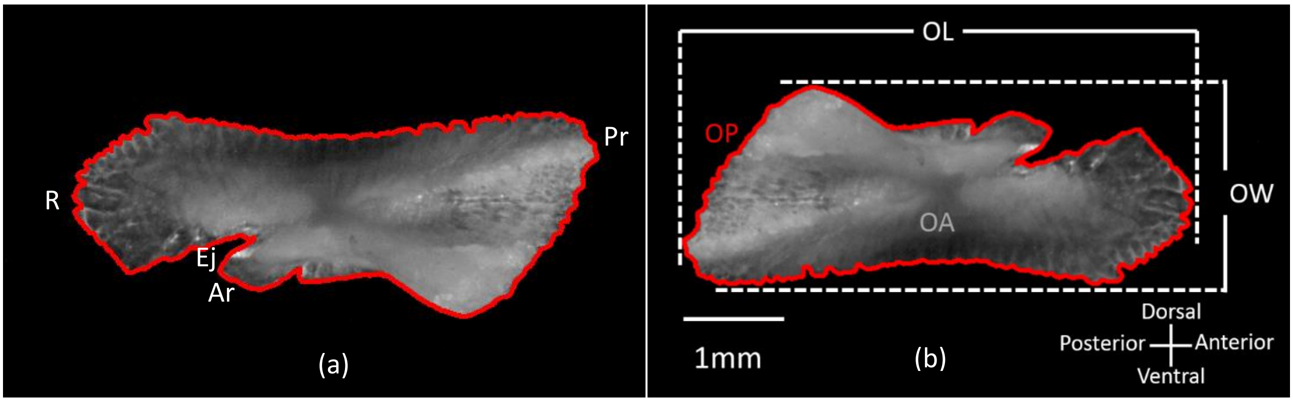
Table 1. Data collection of analysed otolith samples of skipjack tuna (K. pelamis) caught from small-scale tuna fisheries

Location	***Gear***	***Sampling time***	***Number of fish***	**Size (cmFL)**	***Weight (gram)***
Binuangeun (BIN)	Handline, Purse Seine	Apr, Aug, Sep	56	37,5-58,0	
Sadeng (SAD)	Handline, Purse Seine	Aug, Sep	53	34,0-54,0	
Prigi (PRI)	Handline	Sep	37	35,0-51,0	
Labuhan Lombok (LOM)	Handline	Sep	80	41,0-63,5	

Otolith shape, in terms of outlines of otoliths, was collected from the digital images (Figure 2a) with functions written in the programme R and using the ShapeR package (Libungan & Palsson, 2015). Otolith shape measurement consisted from morphometry data including otolith length (O_L), width (O_W), perimeter (O_P), and Area (O_A) as described as Figure 2b. To remove size-induced bias, otoliths were normalized so that the otolith area would be equal in all otoliths by dividing the co-ordinates of each otolith with the square root of the otolith area.

Statistical analysis

Shape differences among populations were evaluated visually by plotting the average otolith shape for each stock by using means of the reconstructed outlines of the normalized Wavelet coeffcients. Independent Wavelet shape coeffcients were obtained by conducting a discrete Wavelet transform on the equally spaced radii using the wavethresh package in R (Nason, 2012) already included in ShapeR package. To estimate which areas and coeffcients on the outline contributed most to the difference between populations, mean shape coeffcients and their standar deviaton were plotted as x and y coordinate matrix against the angle which representing otolith morphology nomenclature according to http://isis.cmima.csic.es/aforo/oto-glo.jsp including *Rostrum* (R), *Antirostrum* (Ar), *Postrostrum* (Pr), and *Excisura Major* (Ej).



Remarks: Figure 2a. Ootlith morphology nomenclature: Rostrum (R), Antirostrum (Ar), Postrostrum (Pr), dan Excisura Major (Ej);
Figure 2b. measurement axes of otolith morphometry: otolith length (O_L), otolith width (O_W), otolith perimeter (O_P), dan otolith area (O_A)

Figure 2. (a) Otolith position for outline analysis and its morphological nomenclature; (b) measurement axes for otolith morphometry of skipjack tuna (*K. pelamis*). The result of otolith outline extraction using "ShapeR" package shown as the red line to determine variation within and among populations.

Canonical analysis of principal coordinates (CAP) based on wavelet coeffcients was used to determine differences between populations (Anderson & Willis, 2003) using the capscale function in the vegan package in R (Oksanen et al., 2013). An ANOVA-like permutation test (using 1000 level of permutations) also implemented to assess the significant difference among populations.

RESULT

Average shape of otoliths differed among the four populations, in particular on outer outline represented morphology of otolith, such as postrostrum (Pr), rostrum (R), antirostrum (Ar), and excisura major (Ej). The postrostrum (Pr), are the region represented at angles from 0 to 45 degree, while rostrum (R) at angles 160 to 200 degree, the excisura major (Ej) at angles 220 to 230, and antirostrum (Ar) at angles 230 to 250, show also the variation visually (Figure 3).

Further investigation of the mean shape of otolith shows that at the postrostrum part, population Labuhan Lombok (LOM) has the longest distance to centroid, while the others population had relatively same. At the rostrum, population Binuangeun (BIN) and Prigi (PRI) had the longest and closest to centroid. Then, from the excisura major point of view, population BIN furthest away from centroid, otherwise LOM has closest to centroid. Meanwhile, at the Antirostrum (Ar), four populations had relatively same distance.

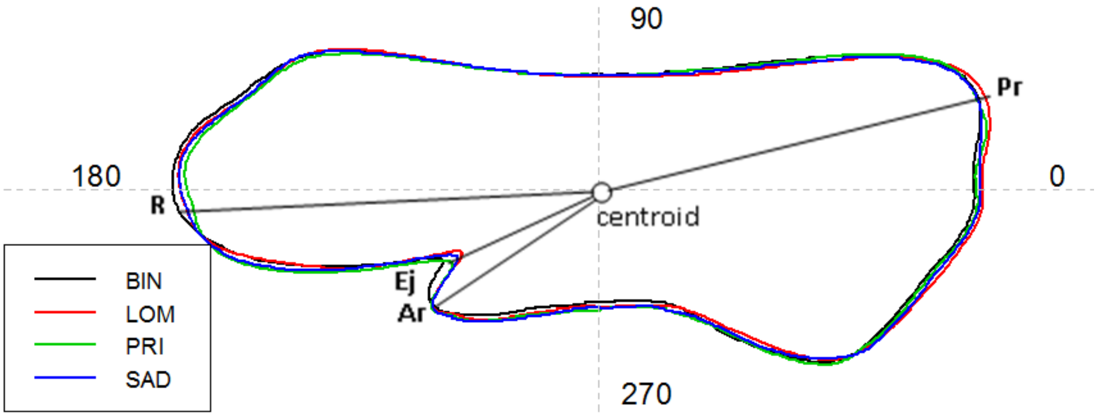


Figure 3. Mean otolith shape based on Wavelet reconstruction integrated with angle in degrees (^{o}) as unit

The variation also confirmed by the mean of wavelet coefficient plotted on each coordinates. The wavelet coefficient has the highest at 192 to 204 degree represented the rostrum part (Figure 4). So, it means rostrum was the most varied among populations.

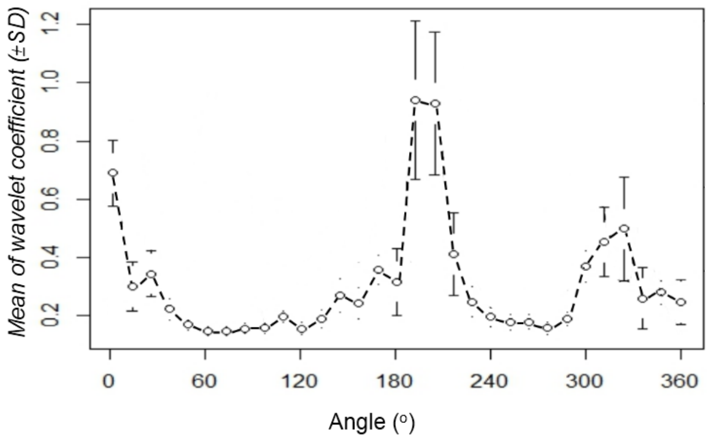


Figure 4. Mean ± standar deviation of Wavelet coefficient for all combined otoliths integrated with angle in degrees (°) as unit

Variation on the otolith shape also were examined among populations using multivariate test the canonical analysis of principal coordinates (CAP). The first discriminating axis explained 66.1% of the variation between population, meanwhile the second axis explained 22.9%. According to the first axis, population from BIN different from the rest, otherwise population SAD, PRI and LOM were close from each other, even SAD and LOM are overlapped. While examining the differentiation along the second axis, the population from PRI is different from BIN, SAD, and LOM (Figure 5). Variation of otolith shape was also performed by ANOVA-like permutation test (n = 1000). It shows there were not significantly different among populations (P> 0,001) as presented in Table 2.

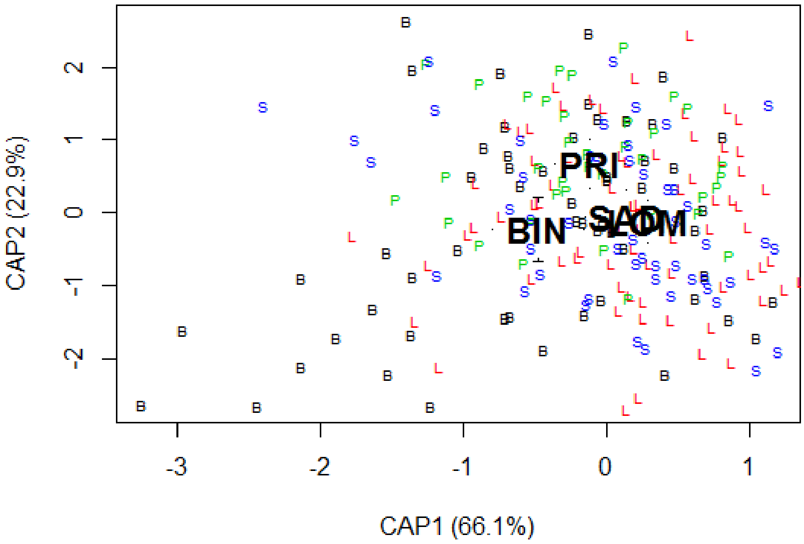


Figure 5. Canonical score of wavelet coefficients on discriminating axis 1 and 2 for each skipjack tuna population (K. pelamis)

Table 2.The result of ANOVA-like permutation test of the otolith shape among four skipjack tuna populations (K. pelamis).

Metode	df	Var	F-value	P
Semua populasi				
Model	3	0.06063	2.3247	0.002
Residual	222	1.93007		

DISCUSSION

Variation of otolith shape, especially on rostrum, from 4 populations were not significantly different statistically. It means there was single stock of skipjack tuna appeared in southern Java and Nusa tenggara waters. Variation of otolith shape impacted by combination between genetic diversity (Cardinale et al., 2004) and environment factor (Campana & Casselman, 1993; Campana & Neilson, 1985), while environmental factor may lead the differentiation such as water temperature and food availability (Vignon, 2012; Cardinale *et al.*, 2004).

Referring to skipjack tuna characteristic as highly migratory species, there was suspected a movement of the fish following the current movement. Although, this has not been proven by tagging. Kunarso et al., (2011), reported that oceanographic conditions and primary productivity characterized by increasing sea surface temperatures and chlorophyll-a, namely upwelling. Upwelling has evolved in southern Bali in June and then moves to the west to West Java in October. Eventually, abundance of skipjack is influenced by increasing chlorophyll-a and sea surface temperature (Mugo et al., 2010; Setiawan et al., 2013; Setiyawan et al., 2013).

In conclusion, otolith shape can be used as an effective tool for identifying stock structure. These results mirror some other studies, where otolith shape also proven effectively for anchovy *Engraulis enrasicolus*, (Bacha *et al.,* 2014; Zengin *et al.,*2015), herring *Clupea harengus* (Burke *et al.,*2008; Libungan *et al.,* 2015), horse mackerel *Trachurus trachurus* (Stransky, 2008), northern atlantic mackerel *Scombrus scombrus* (Turan, 2006), and red snapper *Lutjanus johnii* (Sadighzadeh *et al.*, 2014). Eventually, this result can be used as entry point for other analysis to strengthen stock investigation of skipjack tuna to decide proper harvest strategy for sustainability.

ACKNOWLEDGEMENT

This research is funded by national budget of Research Institute for Tuna Fisheries (RITF) in 2016. The authors would like to thanks to Dr. Lisa Anne Libungan from Department of Life and Environmental Science, University of Iceland, Reykjavik, Iceland who develop “ShapeR” package for R statistical software program and valuable inputs for paper preparation.

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