Thesis for the Degree of Master of Fisheries Science

Stock Identification of the Indian Mackerel (*Rastrelliger kanagurta*) in the Southern Java-Bali Waters by Otolith Shape Analysis

by

Arief Wujdi

KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

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인도네시아 남부 자바 - 발리 해역에 서식하는 고등어 (*Rastrelliger kanagurta*) 의 이석 형태에 따른 계군 분리

Advisor: Prof. Oh Chul Woong

by

Arief Wujdi

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Approved by:

(Chairman) Prof. Kim Hyun-Woo

(Member) Dr. Na Jong-Hun

(Member) Prof. Oh Chul-Woong

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Table of Contents

List of Figures

List of Tables

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Abstract

The Indian mackerel, *Rastrelliger kanagurta*, is a commercially valuable fish across the Indonesia archipelagic waters. The species was managed and assessed under a Fisheries Management Areas (FMAs) management concept, that establish based on the similarity of environmental characteristics. Despite its important role in the fisheries industry, information regarding stock structures for management and conservation purposes within a single FMA is still not known. This study aimed to identify the Indian mackerel stock by otolith shape variability along the southern coast of Java-Bali. Otolith samples were collected in 2016 and 2018 from four fishing ports: Palabuhanratu, Pacitan, Muncar, and

Kedonganan. Otolith outline was reconstructed by using ten levels and 64 Wavelet shape coefficients. The average of otolith shape for each location was visualized in x- and y-axis matrix overlaid to 0-360° angle correspond to morphological features of the otolith. Statistical analysis using a series of ANOVA-like permutation test followed by a cluster analysis using Canonical Analysis of Principal Coordinates (CAP), and Linear Discriminant Analysis (LDA) was applied to differentiate otolith variation between locations. The results showed significant differences in otolith shape, especially in four otolith morphological parts, namely the excisura major, antirostum, pararostrum, and postrostrum. Also, the variation of otolith shape was significantly different between localities (p=0.001). The LDA using cross-validation procedures correctly classified individual samples back to their original group with a success rate was ranged from 44.26 to 82.61%. These results indicate the existence of two major groups of *R. kanagurta* contributed to the stock that can have implications for providing more precisely of stock assessment and its management scenario for sustainable fisheries.

Keywords: Indian mackerel, otolith shape, stock structure, southern Java-Bali

1. Introduction

The Indian mackerel, *Rastrelliger kanagurta*, is a highly migratory epipelagic species which preys mainly on phytoplankton and zooplankton (Hulkoti *et al*., 2013; Solanki *et al*., 2005). Its distribution ranged along the region of Indo-Pacific, primarily from Southern Africa, India, Indo-Malay Islands, Northern Australian up to Indo-Pacific islands, off eastern China, and Ryukyu Islands (Collette and Nauen, 1983). Its abundance was significantly influenced by sea surface temperature and chlorophyll-a concentration (Nurdin *et al*., 2015). In Indonesia, it also plays an important role to the protein supply and economic income due to its abundance, with the annual landing contributes to 4% from the total catch of small pelagic fish. The species mainly caught by using purse seine and gill net with annual catch has been declined in recent years, from 109,974 tons in 2013 to 78,631 tons in 2016 (DGCF, 2017). Currently, the species is assessed and managed based on 11 distinct fisheries management areas (FMAs) as the main fisheries management regime of Indonesia fisheries by the establishment of the Decree of the Minister of Marine Affairs and Fisheries No. PER.01/MEN/2009 in 2019 (Fig. 1). Subdivision of 11 FMAs was established based on the similarity of the oceanography characteristic and dynamics in surrounding waters, so-called eco-region (RIMF, 2014; Marini *et al*., 2017). Fish stock inhabiting within a single FMA were supposed to be manage as a single stock, assuming merely eco-region concept, rather than the biological stock unit concept (Wujdi *et al*., 2017). An understanding of the stock structure is essential for constructing proper guidelines to manage marine fish resources effectively. However, stock and population are frequently understood as a similar thing, which can lead to confusion in the use of these two terminologies. "A fish population is defined as a group of individuals of the same species or subspecies that are spatially, genetically, or demographically separated from other groups" (Wells and Richmond, 1995). "A population will have a unique set of dynamics (e.g., recruitment, growth, and mortality) that influence its current and future status" (Wells and Richmond 1995). While, stock is defined as a population or part of a population in which all members have specific characteristics that cannot be inherited and are most influenced by the environmental factors (Effendie, 1979). Cadrin *et al.* (2013) also define a stock as a population or a meta-population or a component of a population that generally treated as discrete units with respect to assessment and management reasons. Each stock is exploited separately, or the catch is assigned to the original source of stock. Hence, stock is more related to abundance and management perspective of fishery resources, so it has implications for the fishery.

One of the main difficulties to management is defining whether the catches, biological characteristics, and monitoring of fisheries data are contributed from a mixture from several different stocks or a single panmictic stock (Cadrin *et al.* 2013). The reliability when assessing fish stock was challenged by the high uncertain and complexity of stock structure and allocation of resources (Waldman, 2005). It is caused by inadequate information of larval dispersal, migrations, and mixing fisheries between adjacent areas (Bergenius *et al*., 2006; Teacher *et al*., 2013), as the impact of environmental factors such as surface currents, fronts, and upwelling occurrence (Bembo *et al*., 1996; Patarnello *et al*., 2007). Besides, birth and spawning origin also contribute to the stock structure (Natoli *et al*., 2005; Svedäng *et al*., 2007). Hence, the misconception of management measures that ignore the knowledge of stock structures and bio-complexity can lead to over-exploited fisheries, primarily when the stocks differ in productivity (Begg *et al*., 1999; Heath *et al*., 2014). Also, it can lead to lowered genetic diversity (Smith *et al*., 1991), and this is more relevant when fishing activities combined with alterations on marine habitats at the same time as the impact of climate variability. Nowadays, such an understanding is of massive concern to Indonesia's fisheries management authority to establish appropriate management actions and harvest strategies for the fishery in its archipelagic waters.

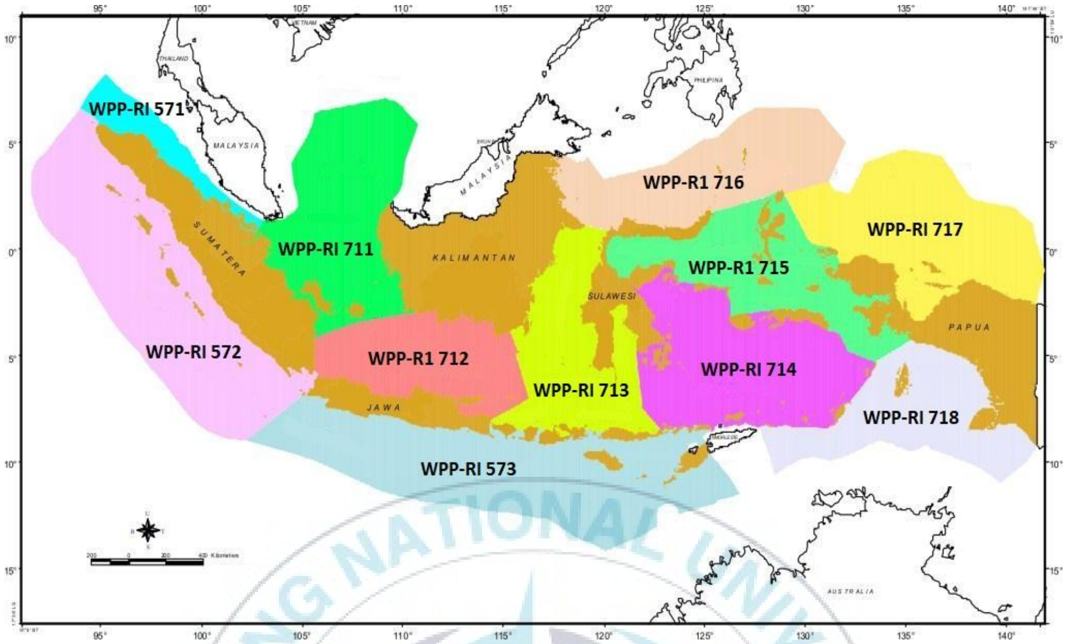

Fig. 1. The eleven of fisheries management areas (FMAs) in Indonesia (Sources: The Decree of the Minister of Marine Affairs and Fisheries No. PER.01/MEN/2009)

Despite its commercial importance, only several investigations were carried out on stock identification of Indian mackerel. Recently, investigation on the biological aspect of the Indian mackerel were most concerned in India, Andaman, and Nicobar waters as part of the Bay of Bengal Large Marine Ecosystem (BOBLME) initiative. Previous studies are mostly focused on the population dynamics (Bhendarkar *et al*., 2014) and feeding behavior (Hulkoti *et al.,* 2013; Das *et al*., 2016). By using genetic approaches, several studies also revealed the high connectivity of Indian mackerel populations around the India peninsula (Sukumaran *et al.,* 2017), and around Indo-Malaya region (Akib *et al*., 2015). Other studies also reported regional differences within Indonesia archipelagic waters alone, especially bio-population dynamic and biology reproductive (Arrafi *et al*., 2016; Hariati and Fauzi, 2011; Oktaviani *et al*., 2014), and genetic population between different FMAs (Zamroni *et al*., 2017; Zamroni *et al*., 2016).

An investigation of the Indian mackerel stock structure within similar FMA, i.e., in the southern Java-Bali (FMA-573) was not known. The FMA regime in fisheries management of Indonesia may not be appropriate for migratory species (Bailey *et al*., 2016). In fact, biological stock unit-based subdivision could be more applicable (Begg *et al*., 1999; Nishida *et al*., 1998), mainly when the mixed stock fishery was defined with different productivity between populations (Heath *et al*., 2014; Hussy *et al*., 2016; Libungan *et al*., 2015a). To describe a proper allocation in mixed fisheries case within FMA so that the depletion of the stock can be avoided, an efficient tool is urgently needed for stock identification purposes. To date, evaluating morphological characteristic including otolith shape is the most applied for stock separation worldwide during decades (Campana and Casselman, 1993; Cardinale *et al*., 2004; Stransky *et al*., 2008; Turan, 2006; Bacha *et al*., 2014; Ider *et al*., 2017), due to easy in procedures, inexpensive, time-efficient, and otolith archives are available frequently. Otolith shape and morphology features are species-specific (Campana and Casselman, 1993). Also, otoliths are the result of the continuous increment of calcium carbonate ($CaCO_3$) material and are not reabsorbed, thus providing a permanent record describing individual growth with corresponding to the environmental variability (Campana and Neilson, 1985). Hence, the otolith shape technique was well-established as a powerful tool to evaluate the fish stock as part of stock assessment purposes. Otolith shape analysis has been used successfully to discriminate among fish populations which are broadly distributed in different ocean basin such as tuna (Brophy *et al*., 2016; Duncan *et al*., 2018), swordfish (Mahé *et al*., 2016), and various species of mackerel (Castonguay *et al*., 1991; DeVries *et al*., 2002; Moreira *et al*., 2019; Stransky *et al*., 2008; Turan, 2006; Vasconcelos *et al*., 2018).

The main objective of the present study was to evaluate the variability of otolith shape as a methodology to identify the stock structures of the Indian mackerel within FMA that potential mixed fisheries were defined. The stock identification was examined statistically since management actions rely on precise estimates of each location's contribution within the stock. The otolith shape reconstruction was performed by utilizing a discrete Wavelet reconstruction to determine whether variation in shape may have implications for fishery management.

2. Material and Methods

2.1. Sample Collection

A total of 159 Indian mackerel (*Rastrelliger kanagurta*) were sampled from landing ports at four localities along the south coast of Java and Bali (Palabuhanratu, Pacitan, Muncar, and Kedonganan) between 2016 and 2018 (Fig. 2). Samples were sourced directly from fishing vessels right after the unloading process at fish auction place within the fishing port area and were hand-picked to achieve as best quality (in terms of fish condition) as possible. An interview was conducted to obtain the information on the boat and fishing gear specification, fishing tactics, and fishing ground during fishing activities. Samples were caught by artisanal fishery using gillnet and boat lift net, which both operated for a one-day trip within 12 miles away from the coastal line. These also indicated that samples were caught from surrounding waters representing the local port-landing base.

Biological parameters were recorded during the field sampling, including fork length (FL) and body weight (W) of each fish to the nearest 0.1 cm and 0.1 g, respectively. Sex was determined by visual inspection of gonads of male and female. The sagittal otoliths were removed by using up through the gill method (Secor *et al*., 1992), then washed in distilled water, stored in labeled plastic tubes (BEEM RB001 size 00) and dried in the room temperature. Due to the otolith forms as fragile and tiny, some otoliths were collected as broken or missing, so they were excluded for otolith shape analysis. Weighing of otolith mass (O_M) also conducted towards 147 pairs of whole otoliths (left and right) to the nearest 0.0001 g by a digital balance (Sartorius CPA224S). Fish sampling details are summarized in Table 1.

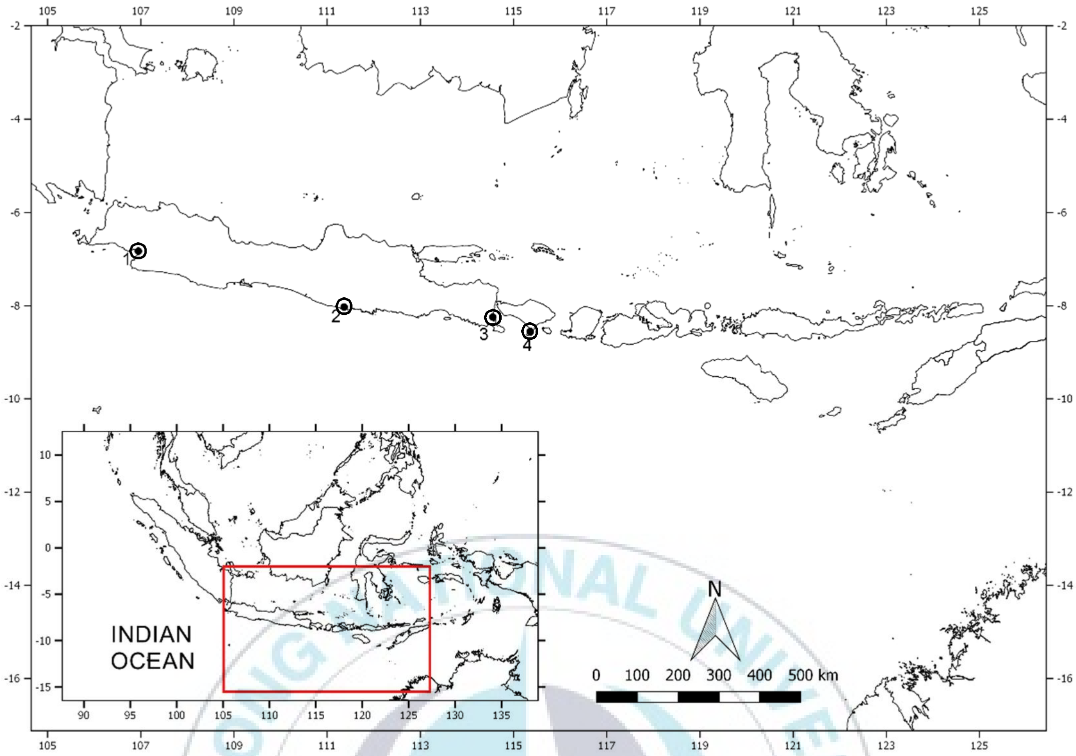

Fig. 2. Sampling location for the Indian mackerel (*Rastrelliger kanagurta*) along the southern Java-Bali coastal waters: 1. Palabuhanratu (PR), 2. Pacitan (CT), 3. Muncar (MC), and 4. Kedonganan (KD).

Table 1. Summary of otolith samples of the Indian mackerel (*Rastrelliger kanagurta*) that used in otolith shape analysis.

Localities	Sampling time	Total sample	Fishing Gear	Fork length (cm) Mean ± s.d.	Weight (g) Mean ± s.d.
Palabuhanratu (PR)	April 2018	38	Boat lift net	18.5 ± 2.2	96.4 ± 33.5
Pacitan (CT)	April 2018	23	Gillnet	21.3 ± 2.3	154.7 ± 70.7
Muncar (MC)	August 2016	54	Boat lift net	22.6 ± 1.7	202.5 ± 54.3
Kedonganan (KD)	July 2018	44	Gillnet	20.3 ± 4.9	169.9 ± 133.5

2.2. Otolith Shape Analysis

Each otolith was photographed under a stereomicroscope (Leica M50), which connected with Leica IC80 HD digital camera. The microscope magnification was adjusted to ensure the same magnification (6.3 times) for all otoliths. Images were calibrated by the camera software program Leica Application Suite and stored in jpg-format. The otolith was placed on a dark background, rostrum pointed to the left, with the distal surface upwards and sulcus facing downwards (Fig. 3), to minimize the effect of distortion when normalization process (Jemaa *et al*., 2015; Vasconcelos *et al*., 2018), then a clear delineation obtain for reconstructing the otolith outline. Manipulation on contrast, brightness, and transformation to greyscale mode was implemented for each image by ImageJ software (https://imagej.nih.gov/ij/).

Otolith images were analyzed into the R software (R Core Team, 2016), using the ShapeR package followed the systematic procedure and description (Libungan and Palsson, 2015) to determine otolith morphometry parameters including otolith length (O_L, in mm), otolith width (O_W, in mm), otolith area (O_A, in mm^2), and otolith perimeter (O_P, in mm). The definition of those otolith morphometry parameters was adopted from previous studies (Aguera and Brophy, 2011; Zischke *et al*., 2016). The shape of otolith was determined for each digital image of otolith by the Conte function (Claude, 2008) in the pixmap package (Bivand *et al*., 2011). The shape from individual otolith was documented in a matrix of x and y-axis and 0 to 360 degrees coordinates to visualize the outline according to otolith morphological features described as the nomenclature such as rostrum (R), antirostum (Ar), excisura major (Ej), postrostrum (Pr), pararostrum (Pa) presented in previous literature (Smale *et al*., 1995; Tuset *et al*., 2008). Otoliths were standardized then resulted in an equal otolith area for all otoliths visualization by dividing the coordinates of each otolith with the square root of the area of the otolith. The space between centroid to the otolith outline, as defined as radii, were derived equally using the regular-radius function (Claude, 2008). Otolith shape reconstructed based on the Wavelet shape coefficient, whose advantage for revealing more accurate comparison of single morphological landmarks, which are most contributing to the disparity between locations. The wavelet transformation was chosen in this study rather than elliptical Fourier harmonics transformation since it is the most excellent way for differentiating of stocks (Libungan *et al*., 2015a; Sadighzadeh *et al*., 2014a). Unlike a Fourier transform provides function in the form of sines and cosines which is local in frequency, the Wavelet transform is localized in both time and frequency (Gencay *et al*., 2001). Therefore, the Wavelet transform provides more detail for approximating the edges than the Fourier transform (Graps, 1995 *in* Libungan *et al*., 2015a). The Wavelet transformation was applied in previous study for evaluating the stock based on the intraspecific variation of otolith outline (Libungan *et al*., 2015a; Libungan *et al*., 2015b; Tuset *et al*., 2019).

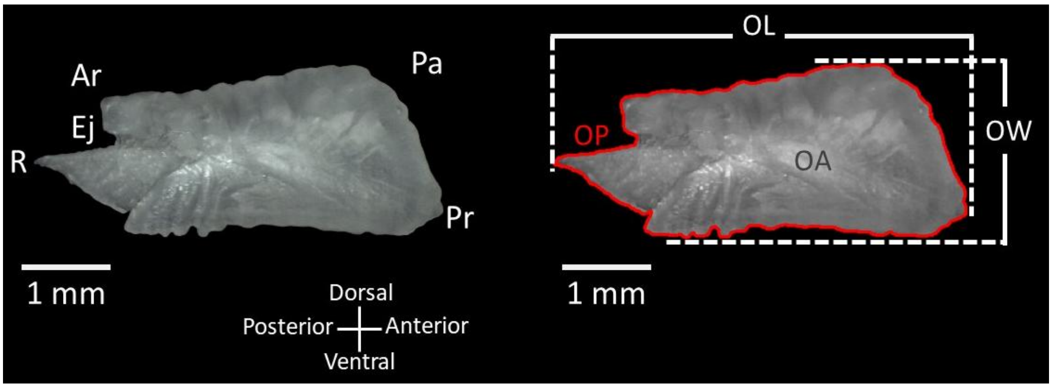

Fig. 3. The position of otolith for outline analysis with its morphological nomenclature, namely; (R) Rostrum, (Ar) Antirostrum, (Pr) Postrostrum, (Pa) Pararostrum, and (Ej) Excisura Major. Remarks: Delineated of otolith outline by using the ShapeR package shown as the red line to determine variation otolith morphometry among locations, including otolith length (O_L), otolith width (O_W), otolith perimeter (O_P), and otolith area (O_A).

The ten Wavelet levels were used in the present study result in 64 Wavelet shape coefficients (Fig. 4). Then, a total of 98.5% successful classification rate or error rate of 1.5% was obtained (Libungan *et al*., 2015a) by applying a discrete transformation on radii using the Wavethresh package (Nason, 2012). A correction was performed on the four wavelet shape coefficients to eliminate a signification of interaction between otolith shape coefficients and allometric-size effect following the regression method (Lleonart *et al*., 2000), meaning those coefficients would not be included from the analysis (Aguera and Brophy, 2011; Begg *et al*., 2001). After applying a normalization technique, a total of 60 standardized Wavelet shape coefficients were remained and normalized. Thus, an equal area of reconstructed otolith was obtained after normalization (Bacha *et al*., 2014; Libungan *et al*., 2015a; Libungan and Palsson, 2015; Zischke *et al*., 2016).

2.3. Statistical analysis

A univariate statistic test using a paired t-test was applied to determine the significant differences of O_L, O_W, and O_M parameters between the left and right otoliths. The mean of otolith shape from each location was evaluated to visualize the variation of the otolith shape corresponding to main otolith morphological features. Shape variations were plotted based on 60 standardized Wavelet coefficients against the angle using the gplots package (Warnes *et al*., 2015). A multivariate series Canonical Analysis of Principal Coordinates (CAP) and ANOVA-like permutation test using 1000 permutations were also implemented on the mean of standardized Wavelet coefficients to investigate the differences in shape among locations using the capscale function included in the vegan package (Oksanen *et al*., 2013). The CAP and ANOVA-like permutation also employed in pairwise mode between two localities to test for otolith shape variations between locations. Individual classification of the otolith back to their geographical origin was examined by a Linear Discriminant Analysis (LDA) on the standardized Wavelet coefficients using the ipred and lda function within the MASS package in R (Peters and Hothorn, 2015; Ripley *et al*., 2014). The LDA is a classification procedure utilized to differentiate between individuals who have been grouped based on their respective original groups. The classification of success rate was assessed using a cross-validation process between localities alternately using the CV=TRUE argument to examine how precise otolith shape could classify between two locations back to their sample origin. All statistical analyses were accomplished using the R statistic software (R Core Team, 2016).

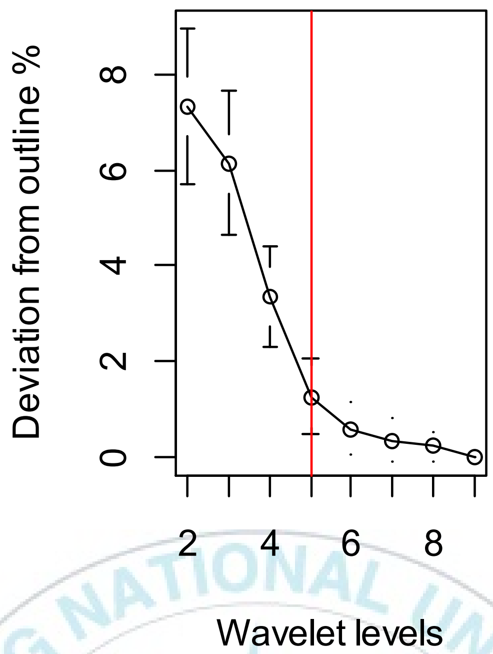

Fig. 4. The quality of the outline reconstruction using Wavelet. The red vertical line indicates the level of Wavelet required for resulting a 98.5% precision and 1.5% error rate of the otolith reconstruction.

3. Result and Discussion

3.1. Otolith Morphometric

Fish that had an intact pair of otoliths (left and right) were used in this initial analysis to test whether there was a significant difference in otolith length (O_L), otolith width (O_W), and otolith mass (O_M) between left and right otoliths. Therefore, only 147 of 159 pair of otoliths were included for the morphometric test. The mean values of O_L, O_W, and O_M were measured as 4.57±0.77 mm, 2.02±0.36 mm, and 0.0047±0.0025 gram respectively (Fig. 5). The results from the paired t-tests showed that no differences were detected using paired t-tests between the left and right side when morphometry of otoliths examined, including otolith length (O_L), otolith width (O_W), and otolith mass (O_M) described as Table 2. The difference between left and right otoliths could be significant for some species and should always be evaluated (Ider *et al*., 2017). Given these results, only left-side otolith from each fish was selected for use in the shape analysis. The lack of significant on differences between the left and right otolith is coherent with the previous observation that the otolith pair of Indian mackerel are mirror images of each other and symmetrical (Jawad *et al*., 2011), especially when its size ranged from 221 to 260 mm (Al-Mamry *et al*., 2015).

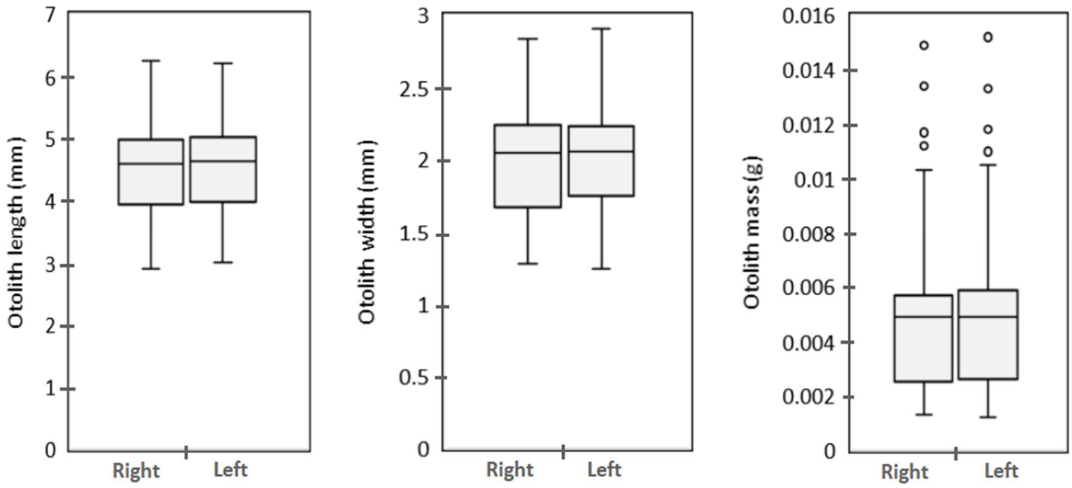

Fig. 5. Parallel boxplots of the three otolith parameters measurement, such as otolith length (O_L), width (O_W), and mass (O_M).

Table 2. A paired t-test results on the right and left side otolith of the Indian mackerel.

Morphometry	Mean different	df	t	p
O_L (mm)	0.02832	146	-0.998	0.3195
O_W (mm)	0.004279	146	-0.5904	0.5558
O_M (g)	0.0000388	146	-1.4453	0.1505

3.2. Stock Identification by Otolith Shape

A total of 159 Indian mackerel otoliths were analyzed to examine whether there were any differences in otolith shape between locations. The mean size of Muncar (MC) otoliths was the highest compared to the mean from rest locations such as Palabuhanratu (PR), Pacitan (CT), and Kedonganan (KD) (Table 3). The reconstructed outlines of the mean Wavelet coefficient were plotted as an overlay picture with 0-360° angle to visualize modifications in average otolith shapes between locations (Fig. 6). The average of otolith shape varied among the four localities. The variation among the four investigated sampling sites can be detected in four morphology areas, at 40°, 160, 170°, and 340° angle of the otolith outline which corresponds roughly to the pararostrum (Pa), antirostrum (Ar), excisura major (Ej), and postrostrum (Pr) part respectively. Further examination at the excisura major reveals that samples from KD and MC had overlapped with each other. Also, their outline had the farthest distance from centroid compared with CT and PR. At the antirostrum, KD appeared as the most variation to the centroid followed by MC, PR, and CT. Thus, two groups are defined by observing the otolith shape from the excisura major and antirostrum point of view. At the pararostrum, MC has the most variation with the farthest distance while KD, CT, and PR have closer to the centroid. These conditions also similar at the postrostrum, whereas MC has the most distant from the centroid.

Table 3. The value of mean ± standard deviation for otolith parameters from the Indian mackerel collected in Palabuhanratu (PR), Pacitan (CT), Muncar (MC), and Kedonganan (KD).

Variables	Mean ± sd			
	PR	CT	MC	KD
Otolith area (mm^2)	5.430 ± 1.272	6.571 ± 1.344	7.563 ± 1.262	6.172 ± 3.07
Otolith length (mm)	4.131 ± 0.504	4.549 ± 0.484	4.967 ± 0.419	4.344 ± 1.083
Otolith width (mm)	1.842 ± 0.266	2.038 ± 0.239	2.199 ± 0.208	1.909 ± 0.54
Otolith perimeter (mm)	10.334 ± 1.463	11.466 ± 1.437	12.303 ± 1.199	10.823 ± 3.170

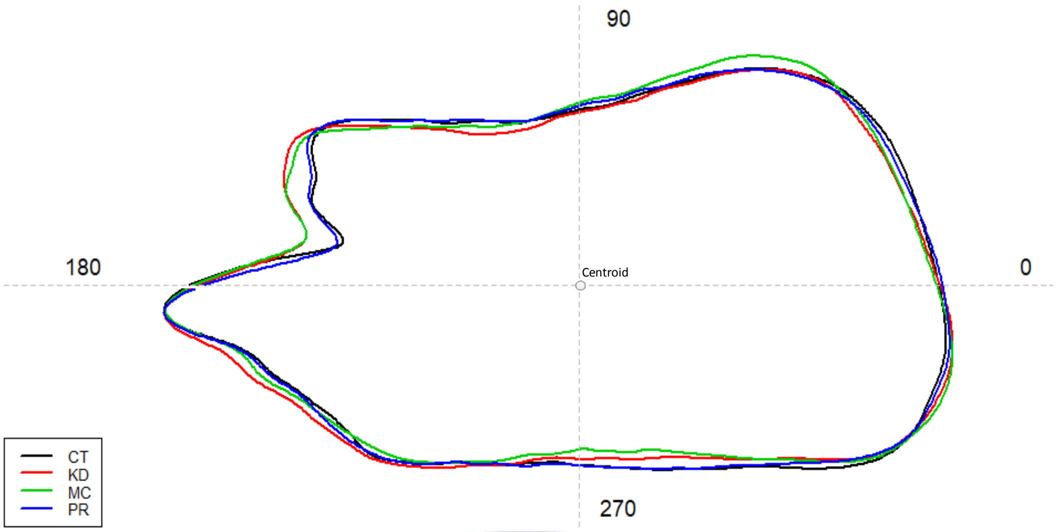

Fig. 6. The average otolith shape based on the Wavelet reconstruction of the Indian mackerel among four localities. Remarks: numbers correspond to angles in degrees (°) based on polar coordinates (see Fig.7). The point obtained by the crossed-two coordinate axis is the central point of polar coordinates, which defined as the centroid.

The variation of otolith shape also can be reaffirmed by plotting the mean value of the Wavelet coefficient against angle. It showed that the most varied of Wavelet coefficient was found at 170° angle corresponding to in the excisura major part. Then, it followed by areas at 160°, 40°, and 340° angle corresponding to the antirostrum, pararostrum, and postrostrum area, respectively (Fig. 7). The variation in the current study is similar with previous study conducted by Libungan *et al.* (2015), where the excisura major was detected as the most varied among herring populations in the Northern Atlantic region.

Through Wavelet transformation demonstrated its suitability to reconstruct otolith outline as four morphological features were detected as most contribute towards the overall variation among localities. Variations in otolith shape on several morphological parts between species may also reflect differences in the physiological functions of balance and hearing, and such variations can have adaptive implications (Brophy *et al*., 2016). There are linkages between intra- and inter-specific variants in otolith shape and feeding behavior and swimming performance (Kishida *et al*., 2011), habitat preferences (Volpedo and Fuchs, 2010; Volpedo *et al*., 2008), and trophic niche (Lombarte *et al*., 2010). For instance, benthic fishes were lived in the bottom layer of water, characteristically have rounded, thick, and wide otoliths, while pelagic species commonly have elongated otoliths with a well-developed rostrum (Bani *et al*., 2013; Gauldie and Crampton, 2002; Volpedo and Echeverría, 2003). However, it is not yet known what physiological functions are affected by variations in the excisura major of sagitta, as does sulcus acusticus which affects on the hearing ability of fish (Popper *et al.,* 2005).

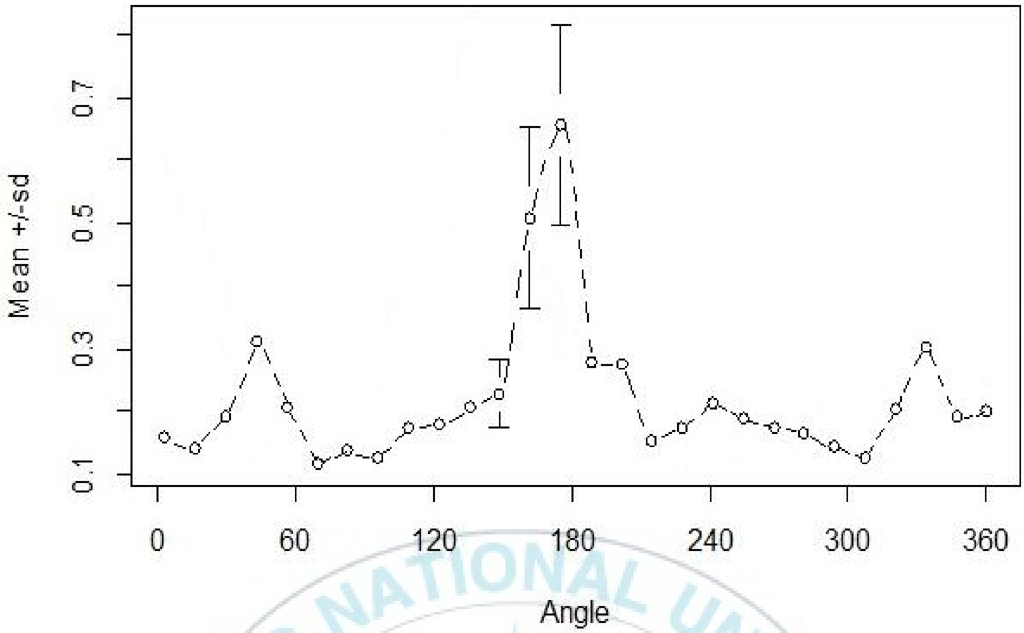

Fig. 7. The mean value and standard deviation of the Wavelet coefficients from reconstructed outline of otolith shape of the Indian mackerel connected to the directional angle (°) of the otolith centroid.

Variations were detected on the otolith shape of the Indian mackerel from four localities by using the clustering statistic of the canonical analysis of principal (CAP) shown as Fig. 8. The first discriminating axis described 66.8% of the disparity between localities. Based on the first discriminating axis, the samples from CT and PR are similarly indicated by an overlap of the mean ± standard error value of the canonical score of the Wavelet coefficient. The CAP analysis also showed a clear separation between sample from CT and-or PR with the rest locations, where the mean CT and PR located in different quadrant compare to KD and MC. The second discriminating axis of CAP explained 27.6% of variations. On the first axis, KD and MC appear imminently, but they differed at the second axis. The difference of otolith shape between the four localities of Indian mackerel also was detected by ANOVA-like permutation test using 1000 permutations (Table 4). The comparison was made using pairwise analysis between two locations to identify the source of the significant difference. The result showed that a significant difference was detected between KD vs MC, MC vs PR, MC vs CT, KD vs PR, and KD vs CT ($p=0.001$) while only PR and CT showed a non-significant different ($p=0.233$).

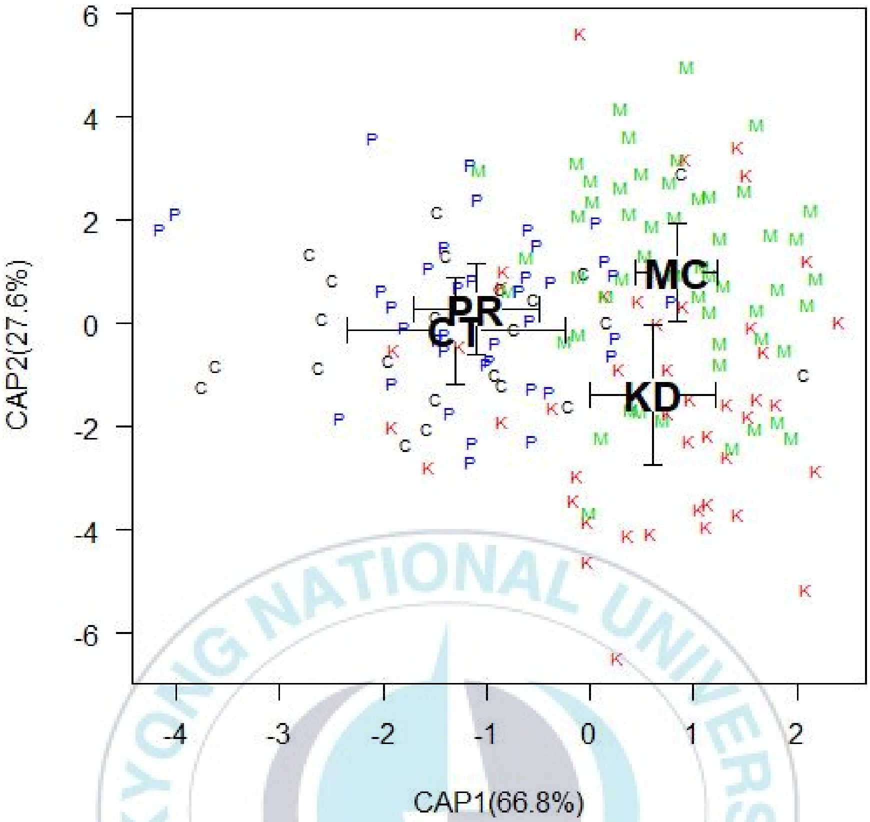

Fig. 8. The cluster analysis plots from the otolith reconstruction of the *Rastrelliger kanagurta* using Wavelet coefficients, calculated by Canonical analysis of Principal Coordinates (CAP). Remarks: the two canonical scores, CAP1 and CAP2, are used to categorize the axes. The bigger black letters represent the average canonical scores for each location, PR=Palabuhanratu; CT=Pacitan; MC=Muncar; KD=Kedonganan. The small letters (P, C, M, K) interpreted the first letter of each study area name. The interval surrounding the average canonical scores present one standard error (mean ± 1 standard error).

Table 4. The comparison of otolith shape of the Indian mackerel from four localities using ANOVA-like permutation test

Comparison	df	Sum of Square	F	P	Residual df	Residual SS
All localities	3	15.81	7.704	0.001***	155	106.020
PR vs CT	1	1.163	1.244	0.233	59	55.151
PR vs KD	1	9.543	9.355	0.001***	80	81.608
PR vs MC	1	12.418	13.898	0.001***	90	80.417
CT vs MC	1	10.158	11.444	0.001***	75	66.572
CT vs KD	1	8.609	8.258	0.001***	65	67.763
MC vs KD	1	6.558	6.767	0.001***	96	93.029

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

A further analysis was conducted using cluster analysis of CAP with a pairwise procedure between two locations to investigate whether any variation of otolith shape between study areas. The cluster plot describes the different positions of the CAP scores (Fig. 9). The CAP scores are more concentrated in CAP1 for the variations in otolith shape of Indian mackerel from four localities. In this study, the consistent differences in otolith shape between locations can be captured using the CAP of the Wavelet coefficients generated from otolith outlines. The CAP provides a more robust analysis if there is similarity in terms of the number of variables and observations (Anderson and Willis, 2003).

The LDA test can classify individuals of *Rastrelliger kanagurta* back to their sample origin based on the Wavelet coefficient. The misclassification error and total correct percentage were explained by pairwise between two locations. The highest misclassification error was estimated between two related sites between PR and CT (0.5082), while the total correct percentage was lowest (44.26%). On the contrary, the two sampling sites namely between PR and MC had the lowest error rate and highest total correct, were calculated as 0.1413 and 82.61% respectively (Table 5). By filtering only two locations using pairwise procedure, gave us a clear evidence of the separation between study areas. The highest successful classification rate was obtained between PR in the westward and MC in the eastward, which was distanced more than 600 nautical miles. These results agreed with previous study where classification values above 75% are generally considered acceptable in terms of stock discrimination (Friedland and Reddin, 1994). These results may indicate a limited movement of the most eastward and westward area of study. While, the classification rate between PR-CT, CT-MC, CT-KD, and MC-KD was lower than 75%, therefore a possibility movement was indicated between those localities.

The stock structure of Indian mackerel around the southern coast of the Java-Bali was undetermined before this study. So far, there is an assumption that all fish species were caught from the same FMA within EEZ are identified as a single stock, causing no strong initiation to distinguish the stock structure using a shred of evidence scientifically. The present study demonstrated that *Rastrelliger kanagurta* in the southern Java-Bali has variability in the shape of the otolith. Our findings may provide a new viewpoint on the stock structures of *Rastrelliger kanagurta* and underlined that the proposed concept of fisheries management scenario must be involving the variability of species characteristics information. These allow management measures to be carried out comprehensively to figure out the temporal and spatial dynamics of populations. Besides, these may help to describe how fish resources respond to natural and anthropogenic behaviors (Ward *et al*., 2016).

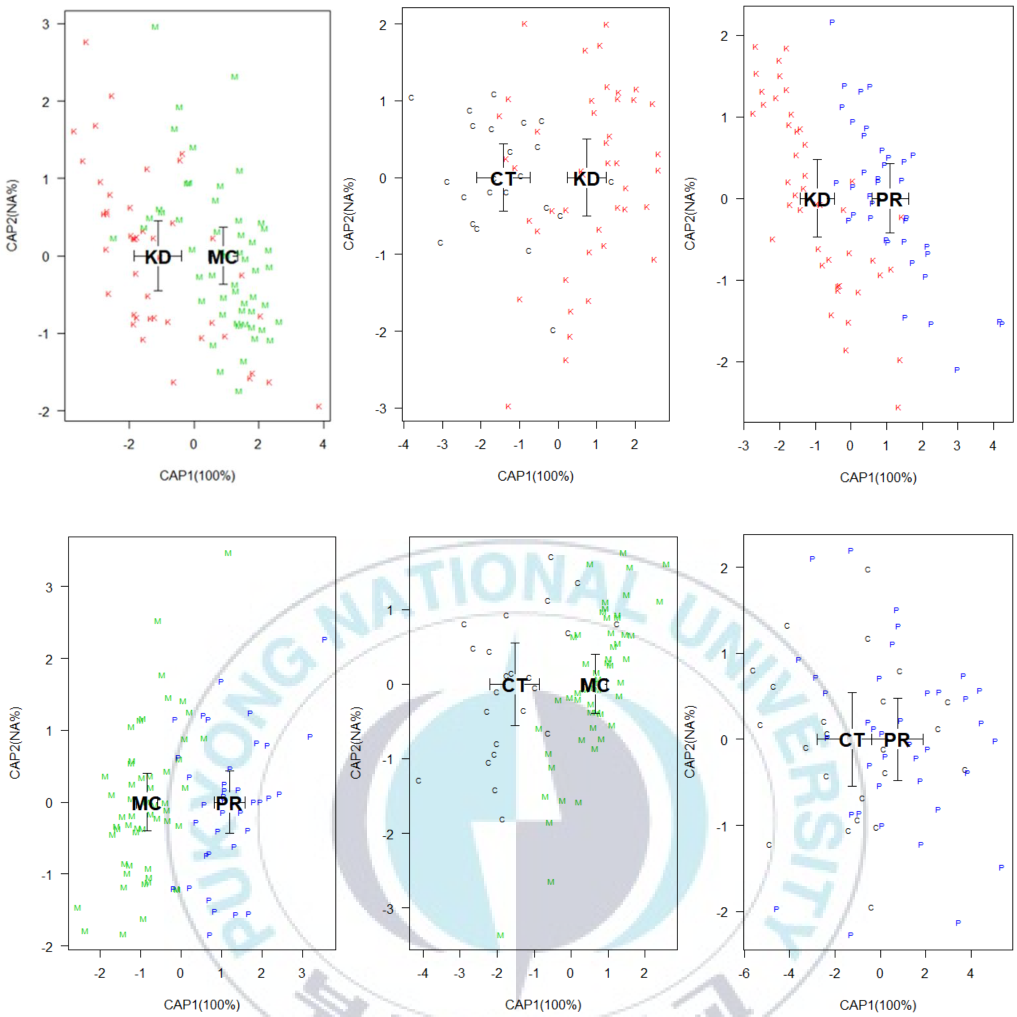

Fig. 9. The cluster analysis plots of reconstructed otolith of the Indian mackerel using pairwise procedure between two locations. Remarks: Palabuhanratu (PR), Pacitan (CT), Muncar (MC), and Kedonganan (KD).

Table 5. Summary of the Linear Discriminant Analysis for the four localities of the Indian mackerel based on wavelet coefficient

Pairwise locations	Linear Discriminant Analysis	
	Misclassification error	Total correct (%)
PR vs CT	0.5082	44.26%
PR vs KD	0.3415	71.95%
PR vs MC	0.1413	82.61%
CT vs MC	0.3247	72.72%
CT vs KD	0.4627	50.75%
MC vs KD	0.3776	57.14%

The present study revealed the distinctive characteristics of Indian mackerel otoliths along the southern coast of Java-Bali by evaluating variability on otolith shape. Otolith shape was understood to be influenced by an amalgamation of genetic and environmental factors (Campana and Casselman, 1993; Cardinale *et al*., 2004; Vignon, 2012; Vignon and Morat, 2010). The separation between samples from PR with KD and MC seemed to be affected by the oceanographic features around the southern coast of Java-Bali waters, which contribute to limited feeding migration, larval dispersal, and growth rate within species. Firstly, there was a seasonal pattern of upwelling occurrence that may develop from eastward to the westward, started from June until October along the southern coast of Java-Bali waters (Kunarso *et al*., 2011). Upwelling phenomena when the cold water mass moved up to the surface as markedly with a lower sea surface temperature (SST) and a high concentration of chlorophyll-a as the main indicator for primer productivity (Sartimbul *et al*., 2010; Suniada and Susilo, 2017; Varela *et al*., 2016), containing rich nutrition may led abundant of phytoplankton and zooplankton (Hendiarti *et al*., 2004; Sartimbul *et al*., 2010). An increased concentration of phytoplankton and zooplankton may impact the feeding migration of *Rastrelliger kanagurta* as a plankton feeder (Das *et al*., 2016; Hulkoti *et al*., 2013). However, the upwelling intensity was weakened when entering the west part of Java Island (Kuswardani and Qiao, 2014), which may lead to a potentially limited migratory of pelagic fish from eastward to westward. The upwelling intensity also fluctuated under varying environmental conditions such as the El Nino-Southern Oscillation (ENSO) and Indian Ocean Dipole (IOD) (Amri and Satria, 2013; Amri *et al*., 2015; Susanto and Marra, 2005), and monsoon wind e.g. northwest monsoon, transition I, southwest, and transition II in between Indonesia and Australia waters (Ilahude, 1975; Wyrtki, 1962; Sartimbul *et al*., 2010). Upwelling was occurred in high intensity in IOD positive and El-Nino period, indicated by colder water mass rising in the surface (Fig. 10). It may affect the movement and abundance of small pelagic fish (Hendiarti *et al*., 2005).

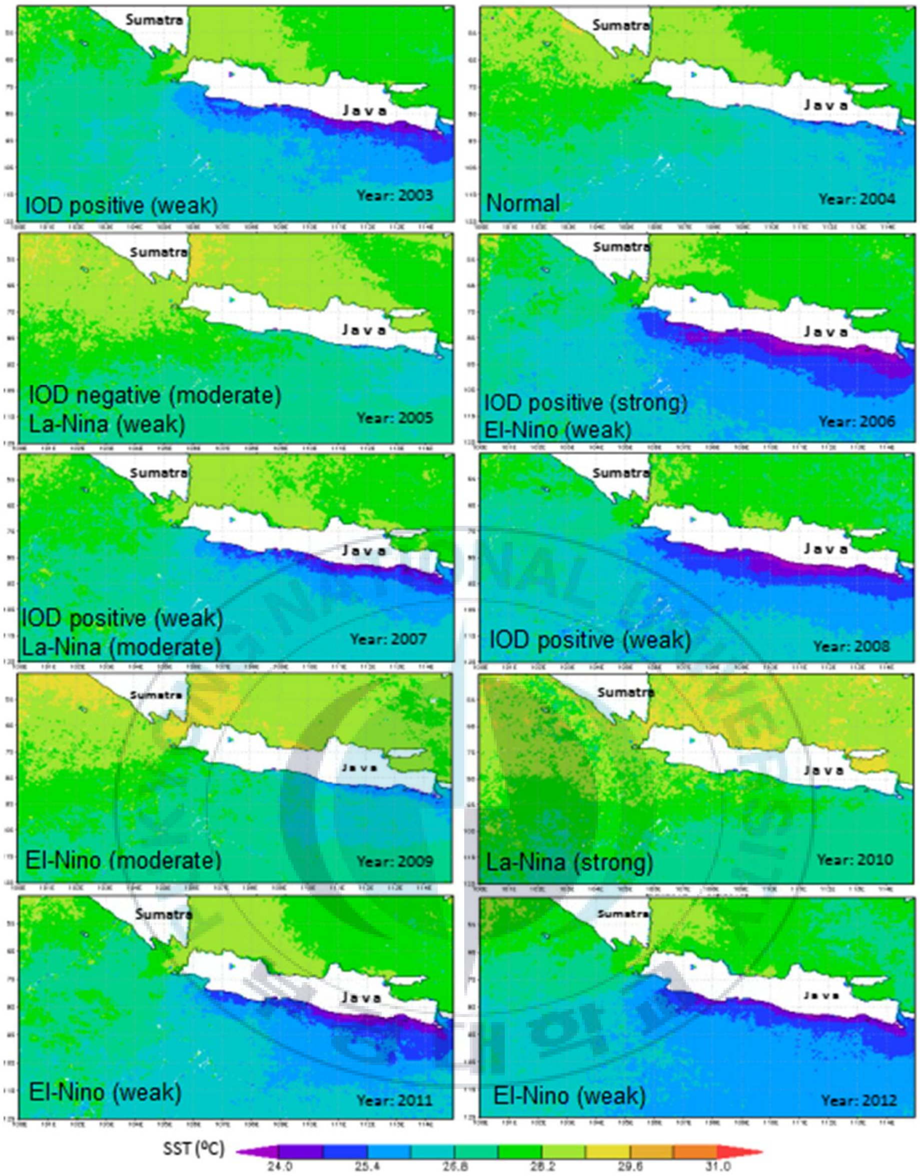

Fig. 10. The upwelling occurrence around the southern coast of Java-Bali related to sea surface temperature gradient (Source: Amri *et al*., 2015). Remarks: blue color showed a low SST indicating an upwelling has occurred in the area.

Secondly, sea surface currents also can play a vital function in larval dispersal of marine species. The water circulation of the Indian Ocean, particularly in the surface layer, is mainly under the influence of monsoon, also referred to as monsoon drift (Fig. 11). The South Equatorial Current (SEC) flowing to westward was dominated throughout the year (Ilahude, 1975; Wyrtki, 1961), while a limited coastal current called the Java Coastal Current (JCC) or Southern Java Current (SJC) according to (Peng *et al*., 2014; Qiu and Masumoto, 2011; Gingele *et al*., 2002) flowing to eastward along coastal area closed to Java Island during the northwest and the first transition monsoon from December to June (Ilahude, 1975). The JCC is tilted to penetrate toward the Bali Strait waters during the northwest monsoon season from December to April (Ilahude, 1975), which may lead to mixing distribution of fish larvae around the Bali Strait waters (MC-KD). The currents circulation is very meaningful to larval dispersion of the Indian mackerel, where CT appears as the mixing zone and coverable by fish from MC and KD location in the east and PR in the west, although this must be comparable by other result using tagging data. There were highlighted that environmental factors such as upwelling and surface current can have a significant effect on feeding behavior related to growth rate on specified species that may vary between areas.

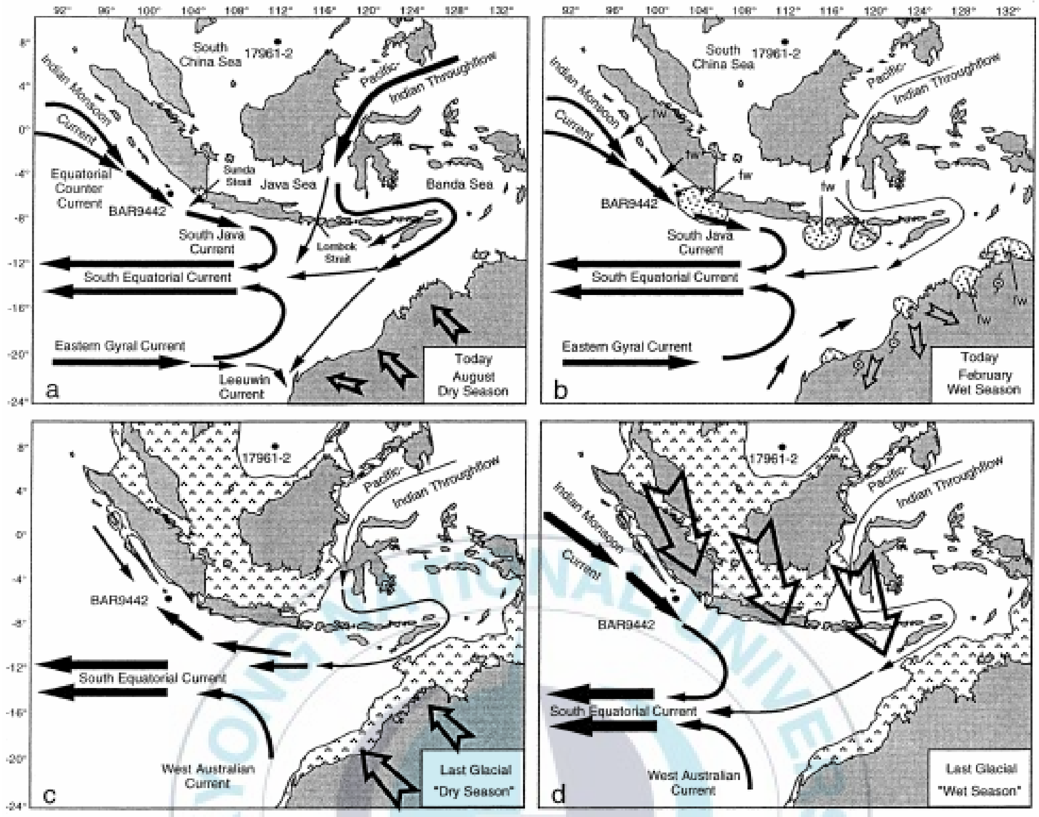

Fig. 11. The today's seasonal variation of current and wind compared to the last glacial period in the south coast of Java-Bali (Source: Gingele *et al*., 2002). Remarks: Currents movement indicated by thick and thin arrow which also shows the water circulation. While, the wind direction indicated by transparent arrow.

Other studies also highlighted that differences in otolith shape are most likely influenced by other oceanographic features, such as salinity and temperature, that experienced by the fish in different life stages (Sadighzadeh *et al*., 2014b). Differences in food availability may also have some influence (Libungan *et al*., 2015a).

Some researchers also highlighted the genetic factor may has an effect on otolith growth, from juvenile to adult stages, regulating the influences of exogenous factors such as the development of crystal formation and diet, and variations in protein deposition in otoliths (Gauldie and Nelson, 1990; Hüssy, 2008; Reichenbacher *et al*., 2009; Vignon and Morat, 2010). Due to limitation on the genetic studies of the *Rastrelliger kanagurta* in the southern Java-Bali waters, there were no information how genetic factors can affiliate on the otolith shape. However, several studies revealed the scenario how the monsoon cycle generates changes in oceanographic features, such as water current and salinity, which concur with the seasonal movement of adults and influence the dispersion of the larvae of pelagic fishes (Rohfritsch and Borsa, 2005). At the same time, water circulation can also be a limiting factor in fish migration, as is the availability of food indicated by upwelling occurrence (Hendiarti *et al*., 2005), so that fish populations will be geographically isolated. This tends to result in inbreeding which can affect the decrease in nucleotide diversity, asymmetrical sex ratio, and success in reproduction, then these are compounded by overfishing (Akib *et al.,* 2015; Munpholsri *et al.,* 2013). Consequently, it also influences on specific gene characteristics and growth rate that can cause otolith increments to be deposited differently (Feet *et al.,* 2002; Fox *et al.,* 2003), result in different of otolith phenotypic forms (Campana and Neilson, 1985; Gauldie and Nelson, 1990), as a reflection in different growth rate induced by environment variability (Cardinale *et al*., 2004; Vignon, 2015).

The otolith shape analysis, as presented here, provided an evidence that non-homogeneous stock of Indian mackerel was observed in southern coast of Java-Bali. The results from the present study indicate that the Indian mackerel from the southern coast of Java-Bali can be divided into two potential distinguishable groups. With all limitations, this study shows that otolith shape analysis can be utilized as a method to complement other population markers, such as parasites (Moore *et al*., 2019; Vasconcelos *et al*., 2017), genetics (Sukumaran *et al*., 2017), otolith stable isotopes (Moreira *et al*., 2017; Newman *et al*., 2010), body morphometric (Jayasankar *et al*., 2004; Sajina *et al*., 2011), and fatty acid markers (Sajina *et al*., 2015), in order to increase the accuracy of mixed-stock estimation. Many documented studies were expanding its coverage area regarding the presence of pelagic fish bio-complexity (Ruggeri *et al*., 2016; Ruzzante *et al*., 2006), where different populations within the region have been observed, but still managed as a single stock. To date, the integration of multi-technique analysis using genetic markers is currently the most recommended approach to apply in terms of evaluating stock structure between regions (Barton *et al*., 2018; Marengo *et al*., 2017; Taillebois *et al*., 2017). Otolith shape analysis is potentially a more cost-effective, speed, and easier method of addressing some stock structure questions, especially for a routine basis for fisheries management purposes. A holistic approach by combining different techniques should be taken in future studies to distinguish the stock structure of a species more powerfully than using a single technique

(Barton *et al*., 2018; Begg and Waldman, 1999; Taillebois *et al*., 2017). Particularly, when CT as the middle area shown in this study was discovered as the mixing area of Indian mackerel that its bio-complexity is necessary to be investigated. Therefore, combining the otolith shape method with other techniques would allow an outcome with a higher confidence level for providing fine-scale management policies that needed to ensure the resources will be well-managed. Also, in order to know the effects of changes in habitat on the Indian mackerel stock structure and distribution, environmental factors need to be included into account in any future strategy of research. These may consider relevant to develop appropriate management strategies in mixed fisheries related to climate variability, especially for stocks that were fished at the same time in adjacent waters.

4. Conclusion

The otolith shape of the Indian mackerel (*Rastrelliger kanagurta*) was examined to distinguish variations between different groups of fish which are inferred to indicate its stock structure around the southern coast of Java and Bali. The present study found that the shape of otoliths varies between the four different locations and can be used to identify the stock structure with a high rate of success. Differences in the shape of otoliths between four localities were detected, particularly in the morphological structure of the excisura major followed by antirostrum, postrostrum, and pararostrum. These allowed us to distinguish two major groups of Indian mackerel that contributed to the fishery in the southern coast of Java-Bali. The sample from Palabuhanratu and Pacitan were defined as the first group, showed a high degree of similarity. The second group consisted of Pacitan, Muncar, and Kedonganan were also similar, while between Muncar-Kedonganan are differed from Palabuhanratu. These results can be input for the current management of Indian mackerel fishery, which is managed based on the single stock in the southern coast Java-Bali. Also, future studies are necessary to take the species bio-complexity into account of assessing the fish stock, in particular when a mixed-stock fishery was defined in the specific area.

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Appendix

The script used for otolith shape analysis in this study using the ShapeR package in R statistical software (Libungan and Palsson, 2015)

New working directory, reading a csv file, and loading the shapeR package
```
>setwd("C:/Users/Desktop/shapeR-master")
>library(shapeR)
>shape <- shapeR("C:/Users/Desktop/shapeR-master/ShapeAnalysis", "mackerel.csv")
```

Detecting the outline of otolith from an image file as an object of research
```
>shape=detect.outline(shape,threshold = 0.2,write.outline.w.org = TRUE)
>shape=smoothout(shape,n=100)
>shape=generateShapeCoefficients(shape)
>shape=enrich.master.list(shape)
```

Removing the outline of otolith from a selected file
```
>shape = remove.outline(shape,"PR","4-BY-14-2")
```

Redetecting the outline of otolith after removal using additional command
```
>shape=detect.outline(shape,threshold = 0.2,write.outline.w.org = TRUE, mouse.click
  = TRUE)
```

Otolith parameters measurement
```
>getMeasurements(shape)
>tapply(getMeasurements(shape)$otolith.area,getMasterlist(shape)$pop,mean)
>tapply(getMeasurements(shape)$otolith.length,getMasterlist(shape)$pop,mean)
>tapply(getMeasurements(shape)$otolith.width,getMasterlist(shape)$pop,mean)
>tapply(getMeasurements(shape)$otolith.perimeter,getMasterlist(shape)$pop,mean)
```

Standardize the coefficients
```
>shape=stdCoefs(shape,classes = "pop","length_cm",bonferroni = FALSE)
```

Visualize Wavelet coefficients
```
>plotWaveletShape(shape,"pop",show.angle = TRUE,lwd = 2,lty=1)
```

Examine the mean shape
```
>plotWavelet(shape,level = 5,class.name = "pop",useStdcoef = TRUE)
>est.list = estimate.outline.reconstruction(shape)
>outline.reconstruction.plot(est.list, max.num.harmonics = 15)
```

Statistical analysis by the canonical analysis of principal (CAP) and ANOVA-like permutation using the vegan package

```
>library(vegan)
>cap.res=capscale(getStdWavelet(shape) ~ getMasterlist(shape)$pop)
>anova(cap.res,by = "terms",step = 1000)
>eig=eigenvals(cap.res,constrained=T)
>eig.ratio=eig/sum(eig)
>cluster.plot(scores(cap.res)$sites[,1:2],getMasterlist(shape)$pop, xlim =
  range(scores(cap.res)$sites[,1]), ylim = range(scores(cap.res)$sites[,2]), xlab =
  paste("CAP1(",round(eig.ratio[1]*100,1),"%)",sep=""),ylab=paste("CAP2(",round(eig
  .ratio[2]*100,1),"%)",sep=""),plotCI=TRUE,conf.level=0.95,las=1)
```

Classification of individuals from two localities

```
>shape=setFilter(shape,getMasterlist(shape,useFilter= FALSE)$pop%in%c("PR","CT"))
>table(getMasterlist(shape)$pop)
```

Linear Discriminant Analysis using the ipred, lda, and MASS packages respectively

```
>library(ipred)
>library(lda)
>mypredict.lda <- function(object,newdata)
+predict(object,newdata = newdata)$class
>stdw = getStdWavelet(shape)
>pop = factor(getMasterlist(shape)$pop)
>dd = data.frame(stdw = stdw,pop = pop)
>library(MASS)
>errorest(pop ~., data = dd, model = lda, estimator = "cv", predict =
  mypredict.lda,est.para = control.errorest(nboot = 1000))
>lda.res.w = lda(getStdWavelet(shape),getMasterlist(shape)$pop,CV=TRUE)
>ct.w = table(getMasterlist(shape)$pop,lda.res.w$class)
>diag(prop.table(ct.w,1))50
>sum(diag(prop.table(ct.w)))
```

Statistical analysis by the CAP and ANOVA-like permutation between 2 localities

```
>cap.res = capscale(getWavelet(shape) ~ getMasterlist(shape)$pop)
>anova(cap.res)
>eig=eigenvals(cap.res,constrained=T)
>eig.ratio=eig/sum(eig)
>cluster.plot(scores(cap.res)$sites[,1:2],getMasterlist(shape)$pop, xlim =
  range(scores(cap.res)$sites[,1]), ylim = range(scores(cap.res)$sites[,2]), xlab =
  paste("CAP1(",round(eig.ratio[1]*100,1),"%)",sep=""), ylab =
  paste("CAP2(",round(eig.ratio[2]*100,1),"%)",sep=""),plotCI=TRUE,conf.level=0.95,
  las=1)
```

Reset filter

```
>shape = setFilter(shape)
>table(getMasterlist(shape)$pop)
```

Save the RData file

```
>save(shape,file = "file-name.RData")
```