Otolith size and shape index of mackerel scad *Decapterus macarellus* (Cuvier, 1833) from Manado Bay and Kema Bay, North Sulawesi, Indonesia

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Abstract. This study aims to examine the difference in the otolith size and shape of mackerel scad *Decapterus macarellus* between sex or sampling sites in North Sulawesi. The otoliths of *D. macarellus* were studied for sagitta otolith pair samples of 63 males and 63 females from Manado Bay (Sulawesi Sea) and 32 males and 32 females from Kema Bay (Molucca Sea), North Sulawesi. These sagitta otolith images were interpreted using ImageJ tool to describe the otolith size (length, width, perimeter, and area), then we calculated the shape index (form factor, roundness, circularity, rectangularity, ellipticity and aspect ratio or length-width ratio). No significant difference was found in otolith length between left and right otoliths of *D. macarellus* from the two bays. Significant differences were found in otolith width, perimeter and area between left and right otoliths of *D. macarellus* from Manado Bay. The shape index showed significant differences in rectangularity, ellipticity, and aspect ratio as well. Therefore, the otolith size and shape of left-right sides from Manado Bay were asymmetrical. Significant differences were also found in otolith width and area between left and right otoliths of *D. macarellus* from Kema Bay, but the otolith shape index of the samples from Kema Bay was not significantly different. The left and the right otolith of samples from Kema Bay were symmetrical in shape but they are asymmetrical in size. No significant differences were found in otolith sizes between males and females of *D. macarellus* from both Manado Bay and Kema Bay. The otolith shape index of Manado Bay samples showed no significant difference between male and female, whereas Kema Bay samples showed significantly different shape index between male and female in roundness and rectangularity. Furthermore, significant differences were detected in otolith sizes and otolith shape index, except in rectangularity, between samples of *D. macarellus* from Manado Bay and from Kema Bay. The regressions of total length against otolith sizes (otolith length and otolith width of *D. macarellus* from both bays follow a power function. The growth patterns showed allometric growth in total length–otolith length and total length–otolith width relationships of *D. macarellus* from Manado Bay and isometric growth in both relationships from Kema Bay. At the similar total length, otolith length in *D. macarellus* from Manado Bay was longer and wider than that from Kema Bay. The observed differences in otolith sizes and shape index and regression lines of total length-otolith length and total length-otolith width between sampling sites show an adaptation to the environmental conditions and could inform that samples of *D. macarellus* from both bays came from different fish stocks.
Key Words: size, shape index, otolith sagitta, *Decapterus macarellus*, Manado Bay, Kema Bay.

Introduction. Mackerel scad *Decapterus macarellus*, locally called malalugis, is a fish species living in schools (Suwarso & Zamroni 2014) and supports pelagic fisheries. The fish are available along the year and used for human consumption and tuna bait. This fish production from fishing fisheries is the highest after tuna (Ministry of Marine Affairs and Fisheries 2013; Witono & Wirdana 2012). They migrate in large schools and are caught in Sulawesi Sea and Molucca Sea (Mamuaya 2007). Studies on *Decapterus* sp. have been done a lot, but the otolith study, especially in *D. macarellus*, is still very scarce (Mamuaya et al 2017; Manginsela et al 2017).

Otolith or statoconium or otoconium or statolith is known as a product of biomineralization in fish body due to effect of endogenous and exogenous factors and interaction of both (Young et al 2012). In fisheries science, otolith is mostly used to estimate the fish age as supporting information in fish stock management (Green et al 2009; William et al 2013). However, its benefit development shows that the otolith size and shape study enables it to be an indicator of fish stock discrimination (Hüssy et al 2016; Mapp et al 2017) and study of population structure (Adelir-Alves et al 2019; Moreira et al 2019).

The otolith study on various marine fishes in tropical regions, especially Indonesia, has not been significantly conducted. Wright et al (1990) have firstly reported it on *Stolephorus heterolobus* in Java Sea, then on several species of eels (*Anguilla* spp.) around Sulawesi waters and others (Arai et al 2000, 2003; Sugeha et al 2001; Kuroki et al 2005; Lee et al 2008; Chino & Arai 2010), bluefin tuna (*Thunnus maccoyii*) around Bali waters (Shiao et al 2009; William et al 2013; Farley et al 2014), Chinese herring (*Tenualosa toil*) around Sumatera waters (Milton & Cheney 2001), red snapper (*Lutjanus erythropterus*) around Nusa Tenggara waters (Fry & Milton 2009), Bali sardinella (*Sardinella lemuru*) around Bali waters (Wujdi et al 2016), skipjack tuna (*Katsuwonus pelamis*) in the southern waters of Java and the Sulawesi Sea (Wujdi et al 2017, 2018; Mogea et al 2019), and *Selar crumenophthalmus* in Manado Bay (Bahri et al 2018).

Otolith study does not merely aim to get information on fish aging and fish growth but also to identify or discriminate the fish stock. According to Campana & Casselman (1993), the size and the shape of otolith are also beneficial to use as stock identity indicator for varied growth rate among fish stocks. There is very significant difference in the otolith shape among many of fish samples, but it is also different with age, sex, and age class as well. This study is aimed at examining whether there is difference in the otolith size and shape of *D. macarellus* between sex or sampling sites in North Sulawesi.

Material and Method. This study was carried out from May 2017 to December 2018. Mackerel scads *D. macarellus* were obtained from fishermen's catches in Manado Bay and Kema Bay (Figure 1). The fish were caught with vertical multiple hook-handline and mini purse seine. They were proportionally taken to represent the exploited fish body size. Data collected included sex and total length (TL).

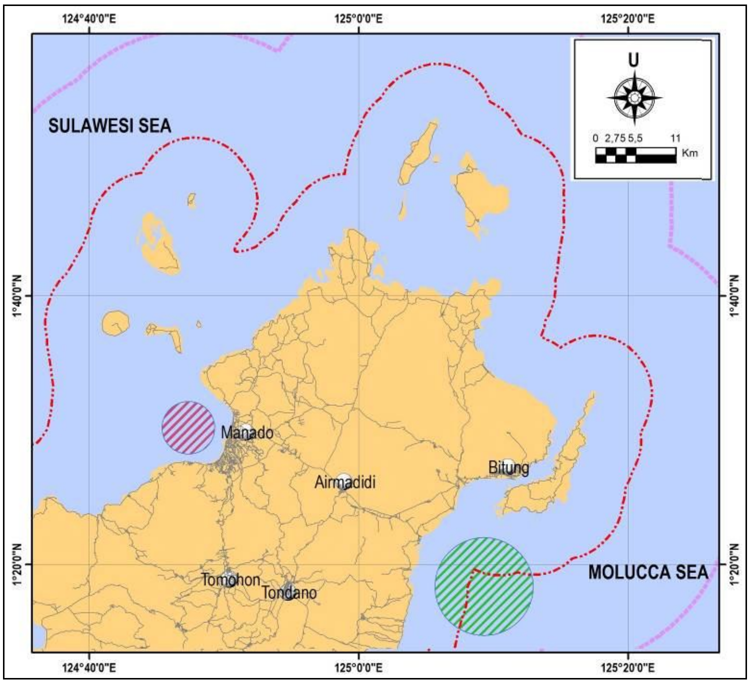

Figure 1. Sampling sites: Manado Bay (red spot) and Kema Bay (green spot).

The otoliths of *D. macarellus*, especially ‘sagitta‘, were obtained by transversally dissecting the fish head, taking the brain, removing the otolith from the ‘sacculus’ capsule, immersed in 5% H_2O_2 (hydrogen peroxide) solution for 12 hours, washed in distilled water, and dried up by dropping 75% alcohol. The dry intact and clean otoliths were then observed under a SZX7-DP21 Olympus stereophotographic microscope to have the otolith image. Furthermore, the otolith image was analyzed using the ImageJ software to determine the otolith dimensions, length (OL), width (OW), area (OA), and perimeter (OP) (Figure 2). Based on the otolith dimension, six otolith shape indices were determined (Wujdi et al 2016; Ladroit et al 2017), Form-Factor (FF) = $(4\pi AO)/OP^2$, Roundness (RO) = $(4OA)/(\Pi OL^2)$, Circularity (CI) = OP^2/OA, Rectangularity (RE) = $OA/(OL*OW)$, Ellipticity (EL) = OL- OW/OL + OW and Aspect Ratio (AR) = OL/OW.

Difference in otolith size and shape index between the left and the right ones was analyzed using pair t-test, while the difference in the otolith size and shape between sampling sites and between sex was tested with t-test. The relationship of otolith size against the fish TL was estimated using equation $Y = a X^b$, where Y is otolith size (OL and OW) and X is fish total length (TL), a is intercept, and b is slope. Parameters a and b were calculated using Least Squares method after the data had been transformed to natural logarithm as $\ln Y = \ln a + b \ln X$. The linearity and the goodness of fit were analyzed using ANOVA, whereas the regression line comparison used analysis of covariance (ANCOVA) (Scherrer 1984; Draper & Smith 1998; Zar 2010). To examine whether b equals to the theoretical value of b = 1 (for the relationship of OL and OW), t-test was applied. If $t_{calc.}$ is bigger than $t_{tab.}$, the growth pattern is allometric or the otolith growth increment does not go along with the body length increment whereas if it is the opposite, the growth pattern is isometric or the otolith size increment is in line with the body length increment. Similar to the comparisons of otolith morphometric parameters between sexes and sampling sites, the relationships of otolith length and width with total length of fishes were determined using left otolith values for all individuals.

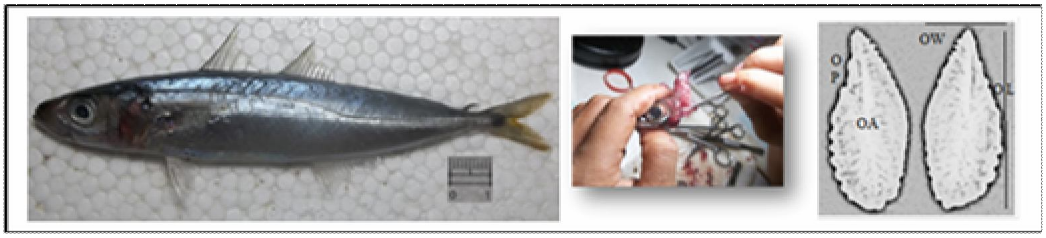

Figure 2. Mackerel scad *Decapterus macarellus* and otolith (sagitta) size, length (OL), width (OW), perimeter (OP), and area (OA).

Results and Discussion

Fish total length. Samples of *D. macarellus* were collected as many as 126 individuals from Manado Bay with a range (mean ± standard deviation) of 190-255 mm TL (228.09±14.38 mm) and 64 individuals from Kema Bay with a range of 142-265 mm TL (234.98±15.67 mm). The computed p-value of t-test was less than 0.05 showing a statistically significant difference between the means of TL of two sampling sites (Manado Bay and Kema Bay), in which the individuals from Kema Bay were longer than that from Manado Bay. Gumanao et al (2016) found a size range of 95-245 mm SL (or 110-303 mm TL) of *D. macarellus* from Davao Gulf (Philippine). Masuda et al (1984) found that male *D. macarellus* could reach maximum size of 300 mm TL, while Jiménez Prado & Béarez (2004) found that common size of the caught species was 300 mm TL and could reach maximum size of 460 mm TL. The distribution of TL of *D. macarellus* samples from Manado Bay and Kema Bay is presented in Figure 3.

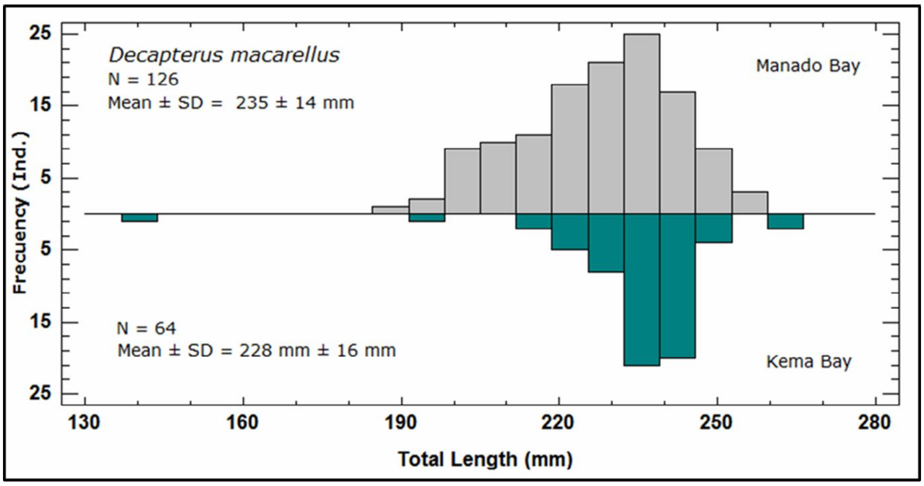

Figure 3. Total length distribution of *D. macarellus* samples from Manado Bay and Kema Bay.

Size and shape index of otolith. The size of otolith pair of *D. macarellus* from Manado Bay and Kema Bay is shown in Table 1. No significant difference was found in OL between left and right otoliths of *D. macarellus* from Manado Bay and Kema Bay. Significant differences were found in OW, OP and OA between left and right otoliths of *D. macarellus* from Manado Bay. The shape index showed significant differences in RE, EL, and AR as well. Therefore, the otolith size and shape of left-right sides from Manado Bay are asymmetrical. Significant differences were found in OW and OA between left and right otoliths of *D. macarellus* from Kema Bay, but the otolith shape indexes of the samples from Kema Bay are not significantly different. The left and the right otolith of samples from Kema Bay are symmetrical in shape but they are asymmetrical in size. The significant difference in OW between the left and right otoliths of *D. macarellus* from Manado Bay gives a significant difference between the left and right sizes of either OP and OA, or the shape index, RE, EL and AR. As for samples from Kema Bay, the significant difference in OW between the left and right otolith of the fish only affects OA and there is no effect on the shape index. For samples from Manado Bay, left OW is greater than right OW, while for samples from Kema Bay, right OW is greater than left OW.

The comparisons of otolith morphometric parameters between left and right otoliths of fishes was a major aspect of studies conducted on fish otoliths. In a study of *Katsuwonus pelamis* in Sulawesi Sea, there were no significant differences between the left and right otoliths in OL, OP, FF, RS, but asymmetric influence was very significant in OW, OA, CI, RE, EL and AR (Mogea et al 2019). Similarly, no significant differences between left and right otoliths were recorded in the otolith sizes and the otolith shape indices of *Selar crumenophthalmus* in Manado Bay, except in OL, RS, EL and AR of female fish in August 2017 and in OL, OW and OP of male fish in November 2017 (Bahri et al 2018). In a study of carangids species in Persian Gulf, no significant differences were found in OL, OW, OP and OA between left and right otoliths of two species *Carangoides chrysophrys* and *C. malabaricus*, but a significant difference was found in OA between left and right otoliths of *C. coeruleus pinnatus*. In shape index, no significant difference was found between left and right otoliths of the three *Carangoides* species (Fashandi et al 2019).

Table 1

Mean size and standard deviation and shape index of otolith pair of mackerel scad, *D. macarellus* from Manado Bay and Kema Bay

Size and shape index	*Mean±SD of left otolith (mm)*	*Mean±SD of right otolith (mm)*	*t-test*	
			t-calc	*P*
Manado Bay (N = 126 ind; N♂ = 63 ind, N♀ = 63 ind)				
Size				
Otolith length (OL)	5.906±0.375	5.879±0.402	1.831	0.069^{ns}
Otolith width (OW)	2.588±0.100	2.521±0.109	14.957	0.000**
Otolith perimeter (OP)	16.129±1.090	16.012±1.168	2.419	0.017*
Otolith area (OA)	9.832±0.826	9.682±0.879	8.265	0.000**
Shape index				
Form factor (FF)	0.477±0.035	0.477±0.039	0.038	0.970^{ns}
Roundness (RS)	0.360±0.026	0.358±0.025	1.539	0.126^{ns}
Circularity (CI)	26.512±2.130	26.541±2.220	-0.185	0.853^{ns}
Rectangularity (RE)	0.643 ±0.015	0.653±0.018	-7.975	0.000**
Ellipticity (EL)	0.390±0.024	0.399±0.021	-6.683	0.000**
Aspect ratio (AR)	2.282±0.128	2.331±0.116	-6.616	0.000**
Kema Bay (64 ind; N♂ = 32 ind, N♀ = 32 ind)				
Size				
Otolith length (OL)	5.398±0.409	5.424±0.430	-1.990	0.051^{ns}
Otolith width (OW)	2.226±0.183	2.252±0.179	-2.741	0.008**
Otolith perimeter (OP)	15.108±1.747	15.165±1.671	-0.772	0.443^{ns}
Otolith area (OA)	8.084±1.072	8.208±1.129	-2.950	0.004**
Shape index				
Form factor (FF)	0.452±0.070	0.454±0.060	-0.306	0.761^{ns}
Roundness (RS)	0.353±0.032	0.355±0.029	-0.820	0.415^{ns}
Circularity (CI)	28.605±5.498	28.294±4.500	1.002	0.320^{ns}
Rectangularity (RE)	0.671±0.019	0. 669±0.020	0.829	0.410^{ns}
Ellipticity (EL)	0.416±0.032	0.413±0.029	1.509	0.136^{ns}
Aspect ratio (AR)	2.433±0.185	2.413±0.168	1.696	0.095^{ns}

Notes: SD = standard deviation; ns = non significant; * = significant; ** = highly significant.

The comparisons of otolith sizes and shape index between male and female samples of *D. macarellus* are presented in Table 2. No significant differences were found in otolith sizes between males and females of *D. macarellus* from both Manado Bay and Kema Bay. The otolith shape index of Manado Bay samples does not also show significant difference between male and female, whereas Kema Bay samples have significantly different shape index between male and female in RS and RE. In general, otolith size and otolith shape index of *D. macarellus* cannot be used for sex differentiation in this species. In other words, this species does not show sexual dimorphism in otolith size or shape, except in RS and RE for samples from Kema Bay.

There were no significant differences in the size and shape index of the otolith between male and female of *K. pelamis* in Sulawesi Sea (Mogea et al 2019). Likewise, the otolith sizes and shape index did not differ between sexes of *S. crumenophthalmus* in Manado Bay (Bahri et al 2018). In the study conducted by Fashandi et al (2019) on three species of *Carangoides* in Persian Gulf, it was found that significant differences were detected in OL, OW and OA of the left otoliths and OW of the right otoliths between male and female of *C. malabaricus*. However, no significant differences were found between male and female of *C. chrysophrys* and *C. coeruleus pinnatus.*

Table 2
Sagitta otolith size and shape index of male-female of *D. macarellus* from Manado Bay and Kema Bay

Size and shape	*Female Mean±SD mm)*	*Male Mean±SD (mm)*	*t-test*	
			t_{cal}.	*P*
Manado Bay (N = 126 ind; N♂ = 63 ind, N♀ = 63 ind)				
Size				
Otolith length (OL)	5.913±0.369	5.898±0.428	0.223^{ns}	0.824
Otolith width (OW)	2.592±0.096	2.584±0.105	0.424^{ns}	0.671
Otolith perimeter (OP)	16.189±1.083	16.068±1.103	0.625^{ns}	0.532
Otolith area (OA)	9.851±0.805	9.814±0.852	0.248^{ns}	0.803
Shape index				
Form factor (FF)	0.474±0.035	0.480±0.036	0.850^{ns}	0.396
Roundness (RS)	0.359±0.027	0.360±0.027	0.061^{ns}	0.951
Circularity (CI)	26.665±0.363	26.358±0.352	0.807^{ns}	0421
Rectangularity (RE)	4.324±0.363	4.303±0.354	0.758^{ns}	0.309
Ellipticity (EL)	8.065±0.438	8.043±0.464	0.277^{ns}	0.781
Aspect ratio (AR)	2.282±0.130	2.283±0.126)	0.030^{ns}	0.975
Kema Bay (64 ind; N♂ = 32 ind, N♀ = 32 ind)				
Size				
Otolith length (OL)	5.388±0.304	5.409±0.247	0.207^{ns}	0.837
Otolith width (OW)	2.262±0.134	2.190±0.182	1.596^{ns}	0.117
Otolith perimeter (OP)	15.203±1.111	15.012±1.965	0.435^{ns}	0.665
Otolith area (OA)	8.277±0.721	7.891±1.040	1.451^{ns}	0.153
Shape index				
Form factor (FF)	0.455±0.063	0.450±0.076	0.317^{ns}	0.753
Roundness (RS)	0.364±0.001	0.342±0.001	2.823**	0.006
Circularity (CI)	28.185±4.299	29.025±0.363	4.940^{ns}	0.545
Rectangularity (RE)	0.679±0.016	0.661±0.017	4.069**	0.000
Ellipticity (EL)	0.408±0.030	0.423±0.031	1.920^{ns}	0.059
Aspect ratio (AR)	2.389±0.176	2.478±0.176	1.961^{ns}	0.054

Notes: SD = standard deviation; ns = non significant; ** = highly significant.

The comparisons of otolith sizes and shape index between sampling sites of *D. macarellus* are presented in Table 3. Significant differences were detected in otolith sizes and otolith shape index, except in RE, between samples of *D. macarellus* from Manado Bay and from Kema Bay. Even though TL of *D. macarellus* from Kema Bay is longer than that from Manado Bay, their otolith sizes OL, OW, OP and OA are smaller than from Manado Bay. Otolith sizes and shape index have been used in several studies for fish stock separation or discrimination (Campana & Casselman 1993; Stransky et al 2008; Ferguson et al 2011; Hüssy et al 2016; Mapp et al 2017; Vasconcelos et al 2017).

Table 3
Sagitta otolith size and shape index of *D. macarellus* from Manado Bay and Kema Bay

Size and shape	*Manado Bay Mean±SD (mm) (N = 126 ind)*	*Kema Bay Mean±SD (mm) (N = 64 ind)*	*t-test*	
			t-calc.	*P*
Size				
Otolith length (OL)	5.906±0.375	5.398±0.409	-8.552	0.000**
Otolith width (OW)	2.555±0.102	2.226±0.183	-13.399	0.000**
Otolith perimeter (OP)	16.129±1.090	15.108±1.747	-4.273	0.000**
Otolith area (OA)	9.832±0.826	8.084±1.072	-11.435	0.000**
Shape index				
Form factor (FF)	0.477±0.035	0.452±0.070	-2.648	0.010**
Roundness (RS)	0.360±0.026	0.353±0.032	-1.477	0.143^{ns}
Circularity (CI)	26.512±2.130	28.605±5.498	2.936	0.004**
Rectangularity (RE)	0.648±0.015	0.671±0.019	8.437	0.000**
Ellipticity (EL)	0.394±0.021	0.416±0.032	4.896	0.000**
Aspect ratio (AR)	2.307±0.115	2.433±0.185	5.002	0.000**

Notes: SD = standard deviation; ns = non significant; ** = highly significant.

Relationship between total length and otolith length and width. Regression between TL and OL and between TL and OW of *D. macarellus* from both bays follows a power (or multiplicative) function (Figure 4) or linear function after transformation to natural logarithm (Table 4).

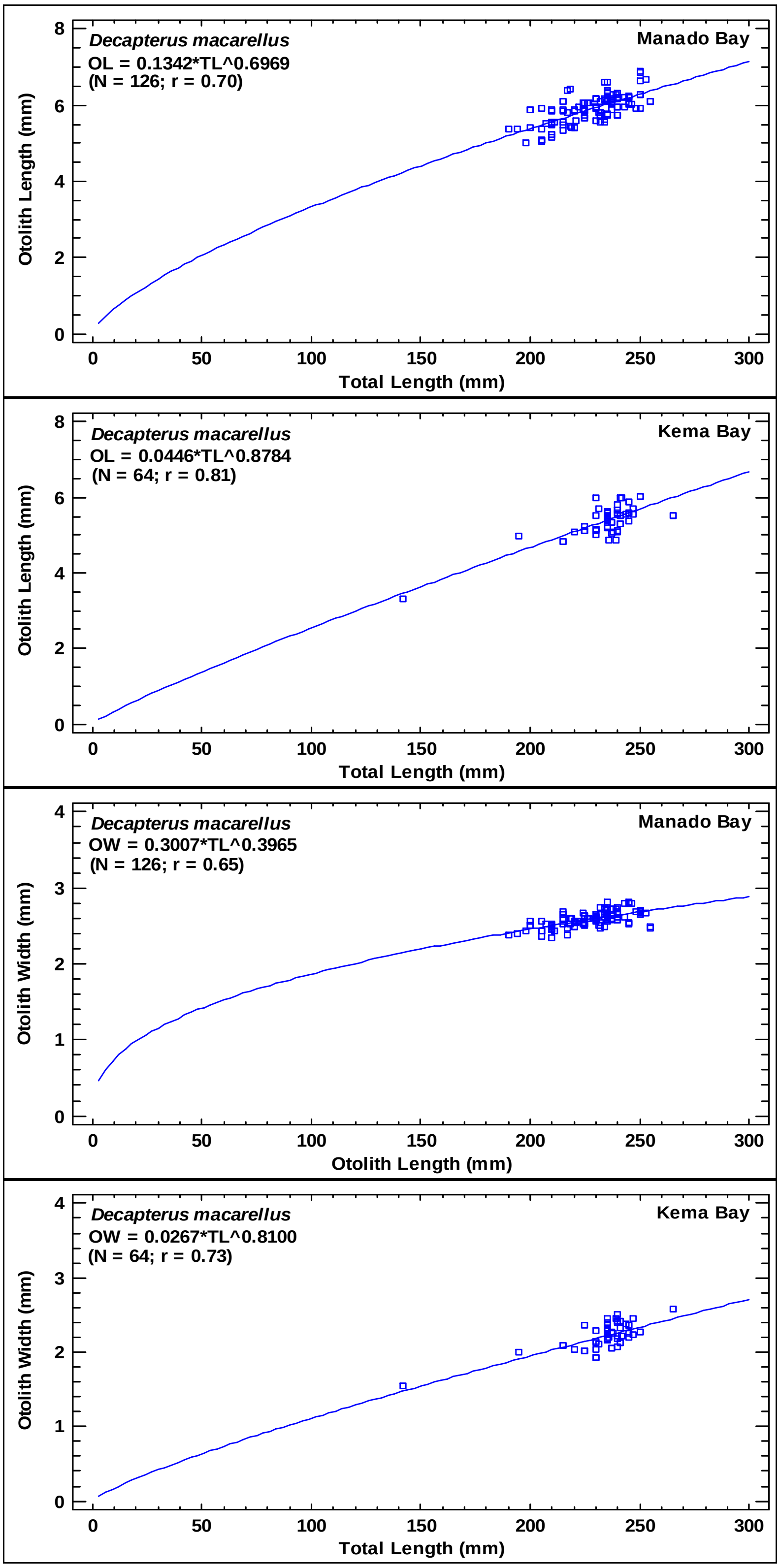

Figure 4. The power model of TL-OL relationship and TL-OW relationship of *D. macarellus* from Manado Bay and Kema Bay.

Table 4

Linear relationship of ln otolith length (ln(OL)) and ln otolith width (ln(OW)) with ln total length (ln(TL)) of *D. macarellus*

Model	*N*	*R^2*	*ANOVA*		*t-test (for b = 1)*			
		(%)	*F-ratio*	*P-value*	*S_b*	*t-calc.*	*t-tab*	*GP*
Manado Bay								
ln(OL) = -2.009 + 0.697*ln(TL)	126	48.43	116.44	0.000	0.065	4.694*	1.979	Allo.
ln(OW) = -1.202 + 0.396*ln(TL)	126	42.86	93.00	0.000	0.041	14.680*	1.979	Allo.
Kema Bay								
ln(OL) = -3.111 + 0.878*ln(TL)	64	65.90	119.83	0.000	0.080	1.515^{ns}	1.999	Iso.
ln(OW) = -3.623 + 0.810*ln(TL)	64	53.80	72.19	0.000	0.095	1.994^{ns}	1.999	Iso.

Notes: N = No. samples; s_b = standard error of b; GP = growth pattern; * = significant; ns = non significant.

Regression of the TL against the OL and the TL against the OW of *D. macarellus* of Manado Bay and Kema Bay indicated variations in slopes (b) and determination coefficients (R^2) in which *D. macarellus* from Kema Bay had higher R^2 than that from Manado Bay. Similarly, b values characterizing the growth pattern show allometric growth in TL–OL relationship and TL–OW relationship of *D. macarellus* from Manado Bay and isometric growth in both TL–OL and TL–OW relationships of *D. macarellus* from Kema Bay.

To compare the linear regression lines of ln(TL)–ln(OL) and ln(TL)–ln(OW) between sampling sites, ANCOVA was employed (Table 5). For the regression of ln(TL)–ln(OL), because the p-value for the slopes is greater or equal than 0.05 (F-ratio = 3.210; p = 0.075), there are not statistically significant differences between the slopes for the various values of sampling sites (Manado Bay and Kema Bay) at the 95% confidence level. Because the p-value for the intercepts is less than 0.05 (F-ratio = 234.69; p = 0.000) (F-ratio = 234.69; p = 0.000), there are statistically significant differences between the intercepts for the various values of sampling sites at the 95% confidence level. Figure 5 shows that at the similar TL, OL in *D. macarellus* from Manado Bay is longer than that from Kema Bay. The comparison of linear regression of ln(TL)–ln(OW) between both sampling sites shows significantly different slopes (F-ratio = 21.59; p = 0.000) and intercepts (F-ratio = 685.43; p = 0.000) indicating that *D. macarellus* of the similar TL from Manado Bay has wider otolith than that from Kema Bay (Table 5).

Table 5

ANCOVA of regression line of ln(OL) – ln(TL) and ln(OW) – ln(TL) of *D. macarellus* from Manado Bay and Kema Bay

Size	*Sample*	*ANCOVA*			
		Slope (b)		*Intercept (a)*	
		F-ratio	*P-value*	*F-ratio*	*P-value*
ln(OL) – ln(TL)	*D. macarellus* from Manado Bay and Kema Bay	3.210	0.075^{ns}	234.69	0.000**
ln(OW) – ln(TL)	*D. macarellus* from Manado Bay and Kema Bay	21.59	0.000**	685.43	0.000**

Notes: ns = non significant; ** = highly significant.

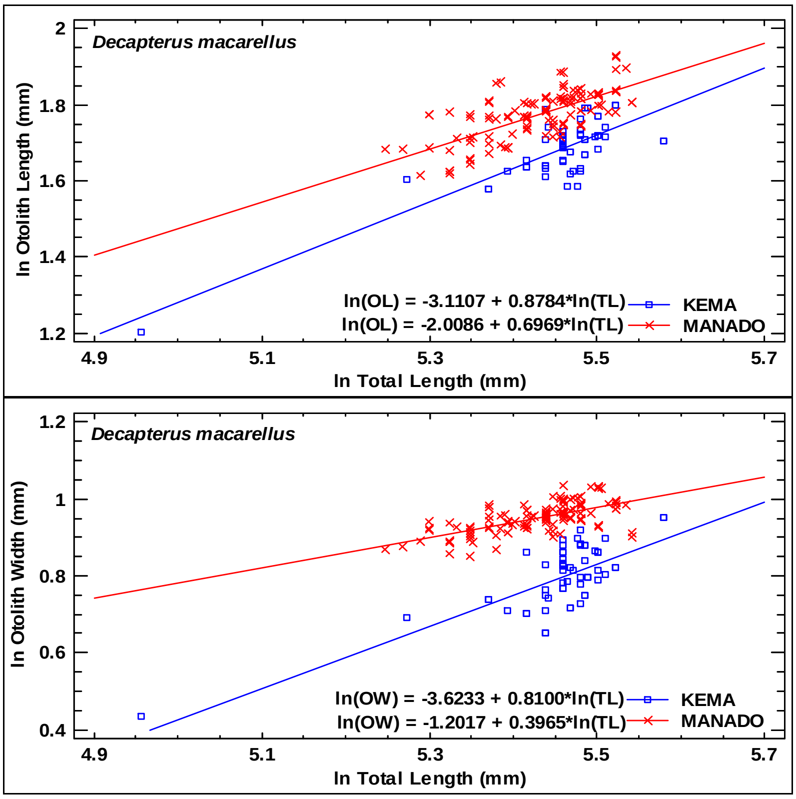

Figure 5. Regression line comparison of ln OL-ln TL and ln OW-ln TL of *D. macarellus* from Manado Bay and Kema Bay.

Conclusions. The otolith size and the shape index of *D. macarellus* from Manado Bay and Kema Bay were successfully characterized. No significant difference was found in otolith length between left and right otoliths of *D. macarellus* from the two bays. Significant differences were found in otolith width, perimeter and area between left and right otoliths of *D. macarellus* from Manado Bay. The shape index showed significant differences in rectangularity, ellipticity, and aspect ratio as well. Therefore, the otolith size and shape of left-right sides from Manado Bay were asymmetrical. Moreover, significant differences were found in otolith width and area between left and right otoliths of *D. macarellus* from Kema Bay, but the otolith shape indexes of the samples from this area were not significantly different. The left and the right otolith of samples from Kema Bay were symmetrical in shape but they are asymmetrical in size. No significant differences were found in otolith sizes between male and female *D. macarellus* from both Manado Bay and Kema Bay. The otolith shape index of Manado Bay samples showed no significant differences between male and female, whereas Kema Bay samples showed significantly different shape index between male and female in roundness and rectangularity. Significant differences were also detected in otolith sizes and otolith shape index, except in rectangularity, between samples of *D. macarellus* from Manado Bay and from Kema Bay. The regressions between total length and otolith sizes (otolith length and otolith width) of *D. macarellus* from both bays follows a power function. The growth patterns showed allometric growth in total length-otolith length and total length-otolith width relationships of *D. macarellus* from Manado Bay and isometric growth in both relationships from Kema Bay. At the similar total length, the otolith of *D. macarellus* from Manado Bay was longer and wider than that from Kema Bay. Differences in otolith sizes and shape index and TL-OL and TL-OW regressions between sampling sites could inform that samples of *D. macarellus* from both bays came from different fish stocks.

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