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## The Use of Otolith Morphometrics in Determining the Size and Species Identification of Eight Mullet (Mugiliformes: Mugilidae) from Malaysia

(Kegunaan Otolit Morfometrik untuk Menentukan Saiz dan Pengenalpastian Spesies bagi Lapan Belanak (Mugiliformes: Mugilidae) dari Malaysia)

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### ABSTRACT

*Sagittal otolith morphometric measurements from Malaysian Mugilidae species were selected to investigate their possible role in species identification, due to the Mugilidae species' morphological similarities, and age determination. Fish standard length (cm), otolith length ( $\mu\text{m}$ ), width ( $\mu\text{m}$ ) and mass (g) measurements were taken from eight species: Chelon macrolepis, C. melinopterus, C. subviridis, Ellochelon vaigiensis, Moolgarda cunnesius, M. seheli, Mugil cephalus and Valamugil engeli. Otolith aspect ratio,  $O_{AS}$  (otolith length divided by width), was calculated and compared between species. The four homogenous groups based on their  $O_{AS}$  were C. melinopterus (mean=1.65) and V. engeli (1.66) and M. cunnesius (1.89) and E. vaigiensis (1.89); M. seheli (2.08), C. macrolepis (2.14) and M. cephalus (2.17); and the latter two with C. subviridis (2.43). The relationships between fish standard length and otolith length/mass showed positive correlations for both, with otolith length providing the stronger correlation ( $r_s = 0.897, P < 0.001$ ) than otolith mass ( $r_s = 0.795, P < 0.001$ ). It is concluded that the more morphologically similar species have similar otolith aspect ratios, related to head shape; however, otolith shape is also affected by a variety of other environmental factors that have to be taken account of.*

*Keywords: Mugilidae; otolith; sagittal; taxonomy*

### ABSTRAK

*Ukuran morfometri sagital otolit daripada spesies Mugilidae Malaysia telah dipilih untuk kajian kemungkinan peranannya dalam penentuan spesies yang disebabkan oleh persamaan morfologi spesies Mugilidae dan penentuan umur. Pengukuran piawaian panjang ikan (cm), panjang otolit ( $\mu\text{m}$ ), lebar ( $\mu\text{m}$ ) dan berat (g) telah diambil daripada lapan spesies: Chelon macrolepis, C. melanopterus, C. subviridis, Ellochelon vaigiensis, Moolgarda cunnesius, M. seheli, Mugil cephalus dan Valamugil engeli. Nisbah aspek otolit,  $O_{AS}$  (panjang otolit dibahagikan dengan lebar), dikira dan dibandingkan antara spesies. Empat kumpulan homogen berdasarkan  $O_{AS}$  mereka adalah C. melanopterus (min = 1.65) dan V. engeli (1.66) dan M. cunnesius (1.89) dan E. vaigiensis (1.89); M. seheli (2.08), C. macrolepis (2.14) dan M. cephalus (2.17); dan kedua-dua akhir dengan C. subviridis (2.43). Hubungan antara piawaian panjang ikan dan panjang otolit/berat menunjukkan korelasi positif bagi kedua-duanya, dengan panjang otolit memberikan hubungan yang lebih kukuh ( $r_s = 0.897, P < 0.001$ ) berbanding berat otolit ( $r_s = 0.795, P < 0.001$ ). Dirumuskan bahawa lebih morfologi spesies yang serupa mempunyai nisbah aspek otolit yang sama, yang berkaitan dengan bentuk kepala; namun, bentuk otolit juga dipengaruhi oleh pelbagai faktor persekitaran lain yang perlu diambil kira.*

*Kata kunci: Mugilidae; otolit; sagital; taksonomi*

### INTRODUCTION

Otoliths are calcareous structures (Popper & Lu 2000) found in the inner ear of fishes (Campana 2004). There are three pairs of otoliths; sagittae, asterisci and lapilli, found in three otolithic end organs; the saccule, lagena and utricle, respectively (Popper & Lu 2000). Their functions are involved in balance and hearing as mechanoreceptors (Popper et al. 2005).

Otoliths also have an important role in many different fields of scientific study such as: Age determination (Cailliet et al. 2001; Campana 2001; Wells et al. 2013); analysis of otolith chemical properties to distinguish

fish life histories (Tournois et al. 2013; Veinott et al. 2012); diet analysis for cetacean, birds, pinnipeds and piscivorous fish (Campana 2004; Radhakrishnan et al. 2010; Sweeney & Harvey 2011; Wenzel et al. 2013) and archaeological studies, to determine past fish community structure, sea temperatures and age distribution (Andrus et al. 2002; Lin et al. 2013; Reichenbacher et al. 2007).

Most importantly for the current study, otolith morphology is species specific, most notably in the sagittal otoliths (Popper et al. 2005). Morphology ranges from ellipsoidal to more complex shapes, with protrusions and invaginations (Campana 2004) and has been used in

many studies for species identification (Bani et al. 2013; Lord et al. 2011; Sadighzadeh et al. 2014; Tuset et al. 2006), notably in fisheries stock assessments (de Vries et al. 2002; Turan 2006). However, for accurate species identification, the production of a reference collection of otoliths from known species is required to generate enough comparative material to produce accurate descriptions of morphological characteristics (Campana 2001; Lin et al. 2013).

The current study investigates the use of otolith morphometrics rather than morphology. Measuring basic otolith dimensions has been found to be faster and more objective than morphological descriptions, especially for age determination (Matić-Skoko et al. 2011) and takes less time, equipment and skill (Fossen et al. 2003; Isermann et al. 2003). Several studies have already found a significant relationship between fish size and otolith length, as well as mass (Dub et al. 2013; Harvey et al. 2000; Lin et al. 2013), but these are species specific (Steward et al. 2009).

Fishes from the family Mugilidae, commonly known as mullets, are generally euryhaline and found in coastal marine and estuarine waters (Carpenter & Niem 1999). Approximately twelve species can be found in Malaysia, but several are similar morphologically so can be difficult to differentiate taxonomically. If the use of otolith morphometrics for identification to species level could be applied to Mugilidae species, it would provide an additional technique for identification.

The objective of the study was therefore to investigate the morphometric traits of otoliths in relation to mugilid fish species and size to see if they could be used as a classification tool for the Mugilidae.

## MATERIALS AND METHODS

### SAMPLING

The samples were collected monthly from February 2012 to August 2013 from various sites (Appendix 1) around Peninsular Malaysia using a variety of methods: Directly collecting samples by fishing using gill nets, trawls, large enclosure traps and collection in tidal pools. Specimens were also bought from local markets. Additionally, some samples were collected from Sabah, East Malaysia.

Ten Mugilidae species were collected ( $n = 597$ ): *Chelon macrolepis* ( $n = 63$ ), *C. melinopterus* ( $n = 28$ ), *C. planiceps* ( $n = 2$ ), *C. subviridis* ( $n = 355$ ), *Ellochelon vaigiensis* ( $n = 29$ ), *Moolgarda cunnesius* ( $n = 11$ ), *M. seheli* ( $n = 34$ ), *Mugil cephalus* ( $n = 65$ ), *Valamugil engeli* ( $n = 9$ ) and *V. speigleri* ( $n = 1$ ). Due to the small sample size, *C. planiceps* and *V. speigleri* were excluded. The samples were fixed in 10% formalin and then transferred into 70% ethanol solution for long term storage. The samples collected nearer the period of otolith removal and processing were frozen instead of being preserved.

Fish samples were then weighed to the nearest 0.01 g and body measurements were taken to the nearest 0.1 cm:

total length (TL), standard length (SL), eye diameter (ED), head length - longest point from tip of snout to back of operculum (HL), the length from the tip of the snout to the origin of the first dorsal fin ( $D_1$ ), the length from the tip of the snout to the origin of the second dorsal fin ( $D_2$ ), the length from the origin of the first dorsal fin to the ventral edge of the fish (MBH).

Fish were then identified to species level using morphological traits and body length ratios taken from the FAO Species Identification Guide for Fishery Purposes by Carpenter and Niem (1999).

### OTOLITH PREPARATION AND ANALYSIS

Sagittal otoliths were removed, as were the large pieces of remaining tissue, using tweezers, before being placed in water filled eppendorfs to soak overnight. If tissue still remained after this, otoliths were either left to soak in eppendorfs filled with a 1% solution of potassium hydroxide overnight or a 3% solution of potassium hydroxide for 5 h before being washed in water. Otoliths were then dried overnight before being photographed using the Olympus SZX10 (Japan) Trinocular Stereomicroscope at 6.3 $\times$  magnification with an attached Olympus DP25 camera (Japan) equipped with the imaging system cell<sup>^</sup>a. An image was taken of the interior and exterior of both the left and right otoliths. Using the same imaging software, measurements ( $\mu\text{m}$ ) on the exterior side were taken of otolith length – the longest distance between the most anterior and posterior points - ( $O_L$ ) and otolith width – the longest distance between the ventral and dorsal edges - ( $O_w$ ), with the measurements for  $O_L$  and  $O_w$  perpendicular to each other. Otoliths were then weighed to the nearest 0.001 g – otolith mass - ( $O_M$ ) (Figure 1).

### STATISTICAL ANALYSIS

Otoliths that had broken or fragmented during the removal and cleaning process were excluded from statistical analysis.  $O_L$  values for the different species were then compared between the left and right otoliths. Data for *C. melinopterus*, *E. vaigiensis*, *M. cunnesius*, *M. cephalus* and *V. engeli* conformed to normal distribution (Kolmogorov-Smirnov,  $P > 0.05$ ). For these species a paired t-test was then conducted and all were found to have no statistically significant difference between left and right  $O_L$  values: *C. melinopterus* ( $t = -0.52$ ,  $df = 37$ ,  $P > 0.05$ ), *E. vaigiensis* ( $t = -0.37$ ,  $df = 13$ ,  $P > 0.05$ ), *M. cunnesius* ( $t = -0.01$ ,  $df = 7$ ,  $P > 0.05$ ), *M. cephalus* ( $t = 0.71$ ,  $df = 55$ ,  $P > 0.05$ ) and *V. engeli* ( $t = 0.72$ ,  $df = 4$ ,  $P > 0.05$ ). For the remaining species that did not conform to normal distribution (Kolmogorov-Smirnov,  $P < 0.05$ ), a Wilcoxon Matched Pairs test was conducted. *C. subviridis* ( $Z = 22393.0$ ,  $n = 280$ ,  $P > 0.05$ ), *C. macrolepis* ( $Z = 543.0$ ,  $n = 45$ ,  $P > 0.05$ ) and *M. seheli* ( $Z = 164.0$ ,  $n = 23$ ,  $P > 0.05$ ) were all found to have no statistical difference between left and right  $O_L$  values. Therefore in all following statistical analysis only the values for the left otolith were used to

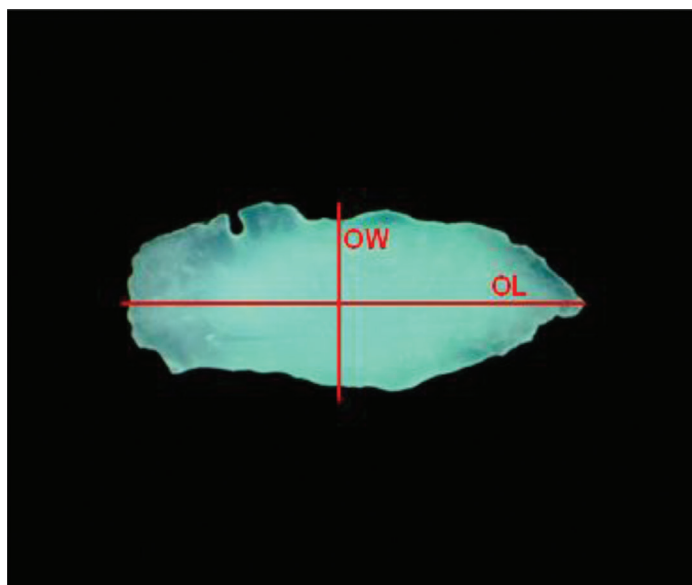


FIGURE 1. Positioning of otolith morphometrics measured (in  $\mu\text{m}$ ) from *Chelon subviridis*,  $O_L$  (otolith length) the longest distance between the most anterior and posterior points,  $O_W$  (otolith width) the longest distance between the ventral and dorsal edges

avoid pseudoreplication, leading to the following sample sizes: *C. macrolepis*,  $n = 53$ ; *C. melinopterus*,  $n = 22$ ; *C. subviridis*,  $n = 286$ ; *E. vaigiensis*,  $n = 19$ ; *M. cunnesius*,  $n = 7$ ; *M. seheli*,  $n = 27$ ; *M. cephalus*,  $n = 55$ ; and *V. engeli*,  $n = 5$ .

#### OTOLITH ASPECT RATIO

Otolith aspect ratio ( $O_{AS}$ ) was calculated by dividing  $O_L$  by  $O_W$ . Aspect ratio data was tested for normality and all species except *C. subviridis* conformed to normal distribution (Kolmogorov-Smirnov,  $P > 0.05$ ). There was no significant difference in variances across the eight species (Levene's test,  $L = 1.86$ ,  $P > 0.05$ ). Despite *C. subviridis* not having a normal distribution (Kolmogorov-Smirnov,  $P < 0.05$ ), an ANCOVA could still be conducted due to the large sample size ( $n = 281$ ). ANCOVA was performed to test for differences among species'  $O_{AS}$  and the effect of the size covariate SL.

#### OTOLITH LENGTH/ FISH SIZE

The relationship between  $O_L$  and fish standard length (SL) was examined. Both sets of data did not conform to normal distribution (Kolmogorov-Smirnov,  $P < 0.05$ ) and so Spearman's rank correlation coefficient was calculated along with regression ANOVA analysis.

#### OTOLITH MASS/ FISH SIZE

The relationship between  $O_M$  and fish SL was examined, both sets of data did not conform to normal distribution (Kolmogorov-Smirnov,  $P < 0.05$ ) and so Spearman's rank correlation coefficient was calculated along with regression ANOVA analysis.

## RESULTS AND DISCUSSION

#### OTOLITH ASPECT RATIO

*C. melinopterus* had the lowest aspect ratio of the eight species (mean  $\pm$  S.D. =  $1.65 \pm 0.13$ ), while *C. subviridis* had the highest ( $2.24 \pm 0.21$ ). *E. vaigiensis* had the highest standard deviation and therefore the highest variation between otolith measurements ( $1.89 \pm 0.29$ ) in Table 1.

Mean  $O_{AS}$  was found to range between 1.65 and 2.24 (Figure 2) and except for *E. vaigiensis* and *M. cunnesius* most species had a similar standard deviation of no more than 10% from the mean. There was a statistically significant difference in the left  $O_{AS}$  between species (ANCOVA,  $F = 23.74$ ,  $df = 7,465$ ,  $P < 0.001$ ). Fish size (SL) however had a significant effect on  $O_{AS}$  being positively correlated (ANCOVA,  $F = 303.68$ ,  $df = 1,465$ ,  $P < 0.001$ ).

*Post-hoc* Duncan multiple comparison ( $P = 0.05$ ) results are displayed in Tables 1 & 2. The four homogenous groups ( $P > 0.05$ ) based on their  $O_{AS}$  were *C. melinopterus* (mean = 1.65) and *V. engeli* (1.66); *M. cunnesius* (1.89) and *E. vaigiensis* (1.89); *M. seheli* (2.08), *C. macrolepis* (2.14) and *M. cephalus* (2.17); and the last two with *C. subviridis* (2.43). The results show no particular pattern in term of similarity among congeneric species, e.g. *Chelon* and *Moolgarda* species were separated in two homogenous groups.

#### OTOLITH LENGTH/ FISH SIZE

A statistically significant, strong, positive correlation was found between  $O_L$  ( $\mu\text{m}$ ) and SL (cm) for the eight Malaysian Mugilidae species (Spearman's rank correlation coefficient,  $r_s = 0.897$ ,  $n = 453$ ,  $P < 0.001$ ) (Figure 3). There was a significant relationship determined by method of

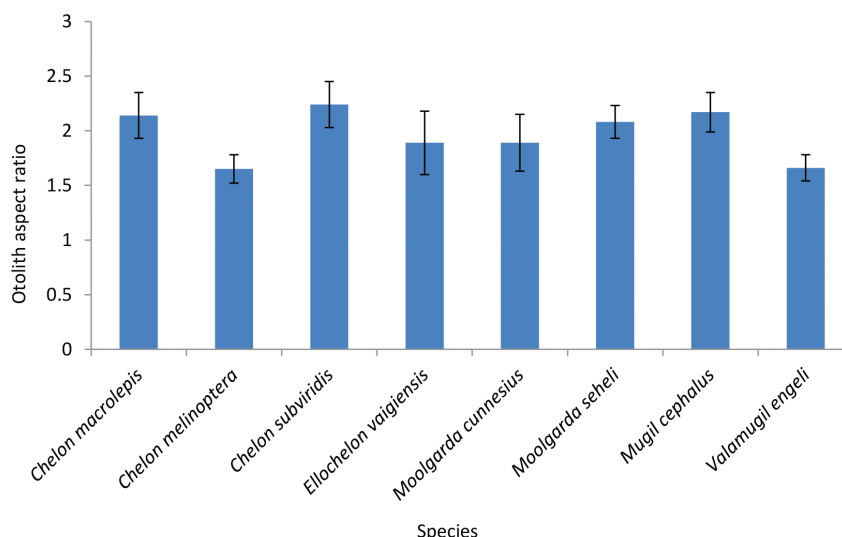


FIGURE 2. Mean left otolith aspect ratio (the length of the longest distance between the most anterior and posterior points divided by the length of the longest distance between the ventral and dorsal edges) for eight different Mugilidae species, presented with standard deviation

TABLE 1. *Post-hoc* Duncan test results from the comparison of the left otolith aspect ratio ( $O_{AS}$ ) from eight Mugilidae species, presented with species mean and standard deviation. Species in the same letter group are not significantly different from each other

Species	Grouping	$O_{AS}$ Mean $\pm$ S.D.
<i>Chelon macrolepis</i>	C D	2.14 $\pm$ 0.21
<i>Chelon melinopterus</i>	A	1.65 $\pm$ 0.13
<i>Chelon subviridis</i>	D	2.24 $\pm$ 0.21
<i>Ellochelon vaigiensis</i>	B	1.89 $\pm$ 0.29
<i>Moolgarda cunnesius</i>	B	1.89 $\pm$ 0.26
<i>Moolgarda seheli</i>	C	2.08 $\pm$ 0.15
<i>Mugil cephalus</i>	C D	2.17 $\pm$ 0.18
<i>Valamugil engeli</i>	A	1.66 $\pm$ 0.12

least squares regression between otolith length ( $\mu\text{m}$ ) (X variable) and standard length (cm) (Y variable) (regression ANOVA: Linear:  $F = 37.0781$ ,  $df = 1,451$ ,  $P < 0.001$ ). The equation for the line of fit through the points was: Standard length (cm) =  $9.254 \pm 0.9593$  otolith length ( $\mu\text{m}$ ), which accounted for 92.0% ( $R^2$ ) of the variation in the Y variable.

#### OTOLITH MASS/FISH SIZE

Figure 3 shows a weaker correlation with  $O_M$  compared to  $O_L$ , but the correlation seems similarly positive. There was a statistically significant positive relationship between  $O_M$  (g) and SL (cm) of the eight Malaysian Mugilidae species (Spearman's rank correlation coefficient,  $r_s = 0.795$ ,  $n = 453$ ,  $P < 0.001$ ). There was a significant relationship between otolith mass (g) (X variable) and standard length (cm) (Y variable) (Regression ANOVA: Linear:  $F = 354.67$ ,  $df = 1,451$ ,  $P < 0.001$ ; Quadratic:  $F = 6.78$ ,  $df = 1,451$ ,  $P < 0.05$ ). The equation for the line of fit through the points was: Standard length (cm) =  $103.5 + 0.3095$  otolith mass (g) +  $0.000777$  otolith mass<sup>2</sup> (g) which accounted for 44.8% ( $R^2$ ) of the variation in the Y variable.

#### OTOLITH ASPECT RATIO

The otolith aspect ratio ( $O_{AS}$ ) separates eight Malaysian mugilid species into four homogenous groups. The group with the smallest  $O_{AS}$  was a distinct group (A) that comprised *Chelon melinopterus* and *Valamugil engeli*. The group with the largest  $O_{AS}$  (D) comprising *Chelon subviridis*, *C. macrolepis* and *Mugil cephalus* is not a distinct group, since their  $O_{AS}$  values overlapped with another group (C). This probably resulted from the larger variations due to allometric growth or the effect of fish size. The results also show no particular pattern in term of  $O_{AS}$  similarity among congeneric species, e.g. *Chelon* and *Moolgarda* species were separated in different groups (Table 1). During the study, it became apparent that some species such as *C. melinopterus* and *V. engeli* were the easiest to identify due to their deeper body and head shape compared to the rest of the species (Carpenter & Niem 1999). Some studies have previously suggested that otolith size and shape could be constricted by head size (Bani et al. 2013; Kumar et al. 2012), so it could be possible that the morphology of otoliths is affected by the morphology



TABLE 3. A comparison of morphological characteristics for eight Mugilidae species

Species	HL/ SL (Mean $\pm$ S.D.)	Head shape <sup>1</sup>	O <sub>AS</sub> (Mean $\pm$ S.D.)
<i>Chelon macrolepis</i>	0.26 $\pm$ 0.03	Depth equal or greater than width, dorsally flattened	2.14 $\pm$ 0.21
<i>Chelon melinopterus</i>	0.25 $\pm$ 0.04	Wider than deep and depressed	1.65 $\pm$ 0.13
<i>Chelon subviridis</i>	0.24 $\pm$ 0.02	Broad, but depth usually equal to or greater than width, dorsally flattened	2.24 $\pm$ 0.21
<i>Ellochelon vaigiensis</i>	0.29 $\pm$ 0.03	Broad, wider than deep, dorsally flattened	1.89 $\pm$ 0.29
<i>Moolgarda cunnesius</i>	0.24 $\pm$ 0.01	Deeper than wide, dorsally flattened	1.89 $\pm$ 0.26
<i>Moolgarda seheli</i>	0.26 $\pm$ 0.02	As wide as deep, or slightly wider, dorsally flattened	2.08 $\pm$ 0.15
<i>Mugil cephalus</i>	0.25 $\pm$ 0.02	Dorsally broad and flat, deeper than wide	2.17 $\pm$ 0.18
<i>Valamugil engeli</i>	0.27 $\pm$ 0.03	Deeper than wide, dorsally flattened	1.66 $\pm$ 0.12

<sup>1</sup>Head shape descriptors taken from Carpenter, K.E. and Niem, V.H. (1999) *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 4. Bony fishes part 2 (Mugilidae to Carangidae)*. Rome

TABLE 4. A comparison of behavioural characteristics for eight Mugilidae species, adapted from (Carpenter &amp; Niem 1999)

Species	Habitat	Diet
<i>Chelon macrolepis</i>	Shallow coastal waters, estuaries and backwaters	Small algae, diatoms, foraminifera, benthic polychaetes, crustacea, molluscs, organic matter and detritus
<i>Chelon melinopterus</i>	Shallow coastal waters, enters lagoons, estuaries and rivers to feed	Plant detritus, microalgae, minute bottom-living organisms, organic matter
<i>Chelon subviridis</i>	Shallow coastal waters, enters lagoons, estuaries and fresh waters to feed	Microalgae, diatoms, detrital material and filamentous algae
<i>Ellochelon vaigiensis</i>	Shallow coastal areas, protected sandy shores in lagoons, coastal creeks, estuaries and reef flats	Small algae, diatoms, benthic polychaetes, molluscs, crustacea, living and detrital organic matter
<i>Moolgarda cunnesius</i>	Shallow coastal waters, benthic substrates	<i>Lack of data due to previous taxonomic confusion</i>
<i>Moolgarda seheli</i>	Shallow coastal waters, enters lagoons, estuaries, brackish tidal creeks, fresh water rivers	Microalgae, diatoms, detrital material, foraminifera and filamentous algae
<i>Mugil cephalus</i>	Inshore marine waters, estuaries, lagoons and rivers	Fine particulate matter, detritus, microscopic animals and algae
<i>Valamugil engeli</i>	Coastal waters, shallow lagoons, protected inlets, sandy to muddy areas of reef flats	Lack of data due to previous taxonomic confusion

habitats, while others extend into fresh water (Table 4). A more specific study would be beneficial to understand the influence of the effect that salinity of the Mugilidae's habitat has on otolith morphology.

Another possible reason for the lack of significant difference between species could be the methods of preservation. Due to the length of data collection, some of the fish samples were fixed using 10% formalin and then stored in 70% ethanol solution for sample preservation and long term storage. However, it was observed that older fish specimens had otoliths that were notably more fragile than the more recent samples, with some otoliths being fragmented even before removal. While there has been little research into the effect of preservation on otoliths, an indication of its effect can be seen in the literature. Ethanol has been shown to reduce the density of otoliths and formalin storage is advised against for age and growth studies involving otoliths due to the changes in pH potentially causing swelling or degradation in the CaCO<sub>3</sub>

structure. (Degens et al. 1969; Kristoffersen & Salvanes 1998; Suthers et al. 1992).

#### OTOLITH LENGTH AND MASS/FISH SIZE

Otolith length was shown to have a stronger correlation with fish standard length than otolith mass (Figure 2). This relationship has previously been demonstrated in earlier studies (Introduction section). However, for this information to be effectively utilised, especially relating to fisheries stock assessments, the relationship has to be linked to the age of the fish.

The current method of age estimation in Mugilidae is largely accomplished by analysing growth increments of scales, as the otolith growth increments are easily misread in Mugilidae species (Hotos 2003). However, Mugilidae scales have also been shown to be problematic to interpret as well (Matić-Skoko et al. 2012) and it would, therefore, be helpful to understand if there is a correlation between

otolith length and fish age, making age estimation easier and quicker. In order to achieve this, further studies would be needed to assess how otolith growth relates to fish growth.

It should, however, be noted that the fish specimens collected in this study did not fully represent the size ranges of the species sampled, with many of the sample groups not reaching the maximum, or even common length, as reported in the FAO Species Identification Guide by Carpenter and Niem (1999). The effects of preservation (Discussion section). should also be mentioned again, as a potential variable, possibly reducing the validity of results.

#### CONCLUSION

While the sagittal otolith morphology of the of the eight Mugilidae species in the study is most likely to be species specific, the morphological and behavioural similarity between species means that, rather than being a distinguishing taxonomic feature, otoliths could be used as an aid to species identification, when including a range of other morphological traits.

A strong positive relationship was found between the fish standard length and otolith length, and further investigation could be conducted to assess if this could potentially be used for age estimation of Mugilidae species. The data from this study can give a suggestion of otolith morphometrics in Malaysian Mugilidae species, for an aid in identification or possibly to predict age. More research is needed to allow for a more accurate indication of both taxonomy and age.

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#### REFERENCES

- Aguirre, H. & Lombarte, A., 1999. Ecomorphological comparisons of sagittae in *Mullus barbatus* and *M. surmuletus*. *Journal of Fish Biology* 55(1): 105-114.
- Ali, A., Khaled, S. & Bettina, R. 2013. Inter-population differences in otolith morphology are genetically encoded in the killifish *Aphanius fasciatus* (Cyprinodontiformes). *Scientia Marina* 77(2): 269-279.
- Andrus, C.F.T., Crowe, D.E., Sandweiss, D.H., Reitz, E.J. & Romanek, C.S. 2002. Otolith  $\delta^{18}O$  record of mid-holocene sea surface temperatures in Peru. *Science* 295(5559): 1508-1511.
- Arellano, R.V., Hamerlynck, O., Vincx, M., Mees, J., Hostens, K. & Gijssels, W. 1995. Changes in the ratio of the sulcus acusticus area to the sagitta area of *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae). *Marine Biology* 122(3): 355-360.
- Bani, A., Poursaeid, S. & Tuset, V.M. 2013. Comparative morphology of the sagittal otolith in three species of south Caspian gobies. *Journal of Fisheries Biology* 82(4): 1321-1332.
- Cailliet, G.M., Andrews, A.H., Burton, E.J., Watters, D.L., Kline, D.E. & Ferry-Graham, L.A. 2001. Age determination and validation studies of marine fishes: Do deep-dwellers live longer? *Experimental Gerontology* 36(4-6): 739-764.
- Campana, S. 2004. *Photographic Atlas of Fish Otoliths of the Northwest Atlantic Ocean*. Ottawa: National Research Council Canada.
- Campana, S. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* 59(2): 197-242.
- Carpenter, K.E. & Niem, V.H. 1999. *FAO Species Identification Guide for Fishery Purposes. The Living Marine Resources of the Western Central Pacific*. Volume 4. Bony fishes part 2 (Mugilidae to Carangidae). Rome: FAO. pp. 2069-2790.
- Degens, E.T., Deuser, W.G. & Haedrich, R.L. 1969. Molecular structure and composition of fish otoliths. *Marine Biology* 2(2): 105-113.
- de Vries, D.A., Grimes, C.B. & Prager, M.H. 2002. Using otolith shape analysis to distinguish eastern Gulf of Mexico and Atlantic Ocean stocks of king mackerel. *Fisheries Research* 57(1): 51-62.
- Dub, J.D., Redman, R.A., Wahl, D.H. & Czesny, S.J. 2013. Utilizing random forest analysis with otolith mass and total fish length to obtain rapid and objective estimates of fish age. *Canadian Journal of Fisheries and Aquatic Sciences* 70(9): 1396-1401.
- Fossen, I., Albert, O.T. & Nilssen, E.M. 2003. Improving the precision of ageing assessments for long rough dab by using digitised pictures and otolith measurements. *Fisheries Research* 60(1): 53-64.
- Harvey, J.T., Loughlin, T.R., Perez, M.A. & Oxman, D.S. 2000. *Relationship between Fish Size and Otolith Length for 63 Species of Fishes from the Eastern North Pacific Ocean*. Seattle: NOAA Technical Report NMFS. p. 150.
- Hotos, G.N. 2003. A study on the scales and age estimation of the grey golden mullet, *Liza aurata* (Risso, 1810), in the lagoon of Messolonghi (W. Greece). *Journal of Applied Ichthyology* 19(4): 220-228.
- Isermann, D.A., Meerbeek, J.R., Scholten, G.D. & Willis, D.W. 2003. Evaluation of three different structures used for walleye age estimation with emphasis on removal and processing times. *North American Journal of Fisheries Management* 23(2): 625-631.
- Kristoffersen, J.B. & Salvanes, A.G.V. 1998. Effects of formaldehyde and ethanol preservation on body and otoliths of *Maurollicus muelleri* and *Benthosema glaciale*. *Sarsia* 83(2): 94-102.
- Kumar, P., Chakraborty, S.K. & Jaiswar, A.K. 2012. Comparative otolith morphology of sciaenids occurring along the north-west coast of India. *Indian Journal of Fisheries* 59(4): 19-27.
- Lin, C.H., Li, K.T. & Chang, C.W. 2013. Identification of *Pomadourys* species (Pisces, Haemulidae) from an archaeological midden site in Nankuanli East (Taiwan), based on otolith morphology. *The Raffles Bulletin of Zoology* 61(1): 293-302.
- Lombarte, A., Palmer, M., Matallanas, J., Gómez-Zurita, J. & Morales-Nin, B. 2010. Ecomorphological trends and phylogenetic inertia of otolith sagittae in Nototheniidae. *Environmental Biology of Fishes* 89(3-4): 607-618.

- Lombarte, A. & Lleonart, J. 1993. Otolith size changes related with body growth, habitat depth and temperature. *Environmental Biology of Fishes* 37(3): 297-306.
- Lombarte, A. 1992. Changes in otolith area: Sensory area ratio with body size and depth. *Environmental Biology of Fishes* 33(4): 405-410.
- Lord, C., Morat, F., Lecomte-Finiger, R. & Keith, P. 2011. Otolith shape analysis for three Sicyopterus (Teleostei: Gobioidae: Sicydiinae) species from New Caledonia and Vanuatu. *Environmental Biology of Fishes* 93(2): 209-222.
- Matić-Skoko, S., Ferri, J., Kraljević, M. & Pallaoro, A. 2012. Age estimation and specific growth pattern of boxlip mullet, *Oedalechilus labeo* (Cuvier, 1829) (Osteichthyes, Mugilidae), in the eastern Adriatic Sea. *Journal of Applied Ichthyology* 28(2): 182-188.
- Matić-Skoko, S., Ferri, J., Škeljo, F., Bartulović, V., Glavić, K. & Glamuzina, B. 2011. Age, growth and validation of otolith morphometrics as predictors of age in the forkbeard, Phycis phycis (Gadidae). *Fisheries Research* 112(1-2): 52-58.
- Paxton, J.R. 2000. Fish otoliths: Do sizes correlate with taxonomic group, habitat and/or luminescence? *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 355(1401): 1299-303.
- Popper, A.N. & Lu, Z. 2000. Structure-function relationships in fish otolith organs. *Fisheries Research* 46(1-3): 15-25.
- Popper, A.N., Ramcharitar, J. & Campana, S.E. 2005. Why otoliths? Insights from inner ear physiology and fisheries biology. *Marine and Freshwater Research* 56(5): 497-504.
- Radhakrishnan, K.V., Liu, M., He, W., Murphy, B. & Xie, S. 2010. Otolith retrieval from faeces and reconstruction of prey-fish size for Great Cormorant (*Phalacrocorax carbo*) wintering at the East Dongting Lake National Nature Reserve, China. *Environmental Biology of Fishes* 89(3-4): 505-512.
- Reichenbacher, B., Sienknecht, U., Küchenhoff, H. & Fenske, N. 2007. Combined otolith morphology and morphometry for assessing taxonomy and diversity in fossil and extant killifish (*Aphanius, Prolebias*). *Journal of Morphology* 268(10): 898-915.
- Sadighzadeh, Z., Valinassab, T., Vosugi, G., Motallebi, A.A., Fatemi, M.R., Lombarte, A. & Tuset, V.M. 2014. Using otolith shape for stock identification of John's snapper, *Lutjanus johnii* (Pisces: Lutjanidae), from the Persian Gulf and the Oman Sea. *Fisheries Research* 155: 59-63.
- Steward, C.A., De Maria, K.D. & Shenker, J.M. 2009. Using otolith morphometrics to quickly and inexpensively predict age in the gray angelfish (*Pomacanthus arcuatus*). *Fisheries Research* 99(2): 123-129.
- Stransky, C., Baumann, H., Fevolden, S.E., Harbitz, A., Høie, H., Nedreaas, K.H., Salberg, A.B. & Skarstein, T.H. 2008. Separation of Norwegian coastal cod and Northeast Arctic cod by outer otolith shape analysis. *Fisheries Research* 90(1-3): 26-35.
- Suthers, I.M., Fraser, A. & Frank, K.T. 1992. Comparison of lipid, otolith and morphometric condition indices of pelagic juvenile cod *Gadus morhua* from the Canadian Atlantic. *Marine Ecology Progress Series* 84: 31-40.
- Sweeney, J.M. & Harvey, J.T. 2011. Diet estimation in California sea lions, *Zalophus californianus*. *Marine Mammal Science* 27(4): 279-301.
- Tournois, J., Ferraton, F., Velez, L., McKenzie, D.J., Aliaume, C., Mercier, L. & Darnaude, A.M. 2013. Temporal stability of otolith elemental fingerprints discriminates among lagoon nursery habitats. *Estuarine, Coastal and Shelf Science* 131(0): 182-193.
- Turan, C. 2006. The use of otolith shape and chemistry to determine stock structure of Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner). *Journal of Fish Biology* 69: 165-180.
- Tuset, V.M., Rosin, P.L. & Lombarte, A. 2006. Sagittal otolith shape used in the identification of fishes of the genus *Serranus*. *Fisheries Research* 81(2-3): 316-325.
- Veinott, G., Westley, P.A.H., Warner, L. & Purchase, C.F. 2012. Assigning origins in a potentially mixed-stock recreational sea trout (*Salmo trutta*) fishery. *Ecology of Freshwater Fish* 21(4): 541-551.
- Volpedo, A. & Echeverri, D.D. 2003. Ecomorphological patterns of the sagitta in fish on the continental shelf off Argentine. *Fisheries Research* 60(2-3): 551-560.
- Wells, R.J.D., Kohin, S., Teo, S.L.H., Snodgrass, O.E. & Uosaki, K. 2013. Age and growth of North Pacific albacore (*Thunnus alalunga*): Implications for stock assessment. *Fisheries Research* 147(0): 55-62.
- Wenzel, F.W., Polloni, P.T., Craddock, J.E., Gannon, D.P., Nicolas, J.R., Read, A.J. & Rosel, P.E. 2013. Food habits of Sowerby's beaked whales (*Mesoplodon bidens*) taken in the pelagic drift gillnet fishery of the western North Atlantic. *Fishery Bulletin* 111(4): 381-389.
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APPENDIX 1. Sample site: A. Langkawi; B. Kuala Selangor; C. Bachok; D. Kuantan; E. Sandakan.

