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The Use of Otolith Morphometrics in Determining the Size and Species Identification of Eight Mullets (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 (µm), width (µm) 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 (µm), lebar (µm) 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 utricule, 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 Stereomicrscope at 6.3× magnification with an attached Olympus DP25 camera (Japan) equipped with the imaging system cell^a. An image was taken of the interior and exterior of both the left and right otoliths. Using the same imaging software, measurements (µm) 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

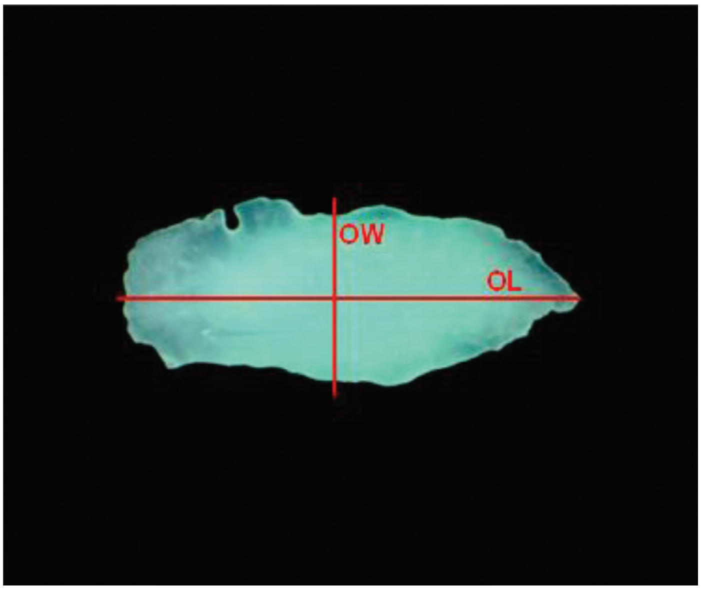

FIGURE 1. Positioning of otolith morphometrics measured (in µm) from *Chelon subviridis*, O_L (otolith length) the longest distance between the most anterior and posterior points, O_W (otolith width) the longest distance between the ventral and dorsal edges

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 ± S.D. = 1.65 ± 0.13), while *C. subviridis* had the highest (2.24 ± 0.21). *E. vaigiensis* had the highest standard deviation and therefore the highest variation between otolith measurements (1.89 ± 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 (µm) 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

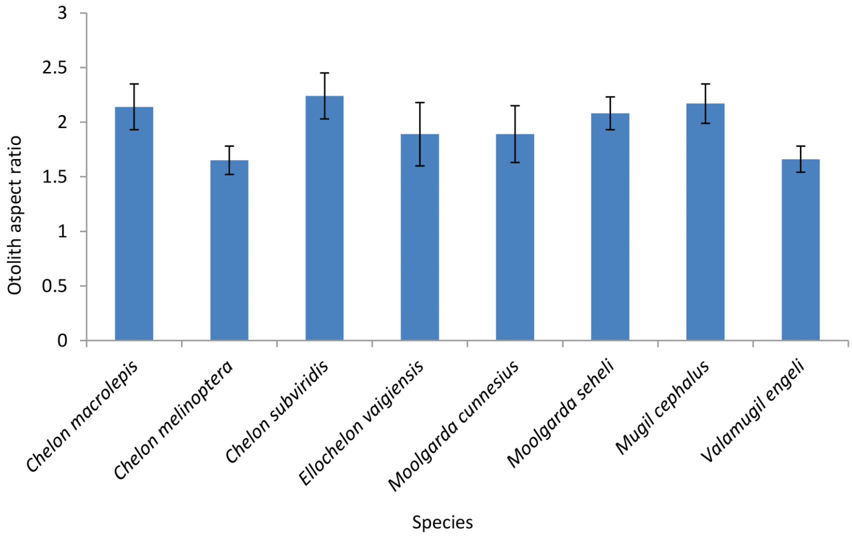

FIGURE 2. Mean left otolith aspect ratio (the length of the longest distance between the most anterior and posterior points divided by the length of the longest distance between the ventral and dorsal edges) for eight different Mugilidae species, presented with standard deviation

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 ± S.D.
Chelon macrolepis	C D	2.14 ± 0.21
Chelon melinopterus	A	1.65 ± 0.13
Chelon subviridis	D	2.24 ± 0.21
Ellochelon vaigiensis	B	1.89 ± 0.29
Moolgarda cunnesius	B	1.89 ± 0.26
Moolgarda seheli	C	2.08 ± 0.15
Mugil cephalus	C D	2.17 ± 0.18
Valamugil engeli	A	1.66 ± 0.12

least squares regression between otolith length (µm) (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 ± 0.9593 otolith length (µm), 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^2 (g) which accounted for 44.8% (R^2) of the variation in the Y variable.

OTOLITH ASPECT RATIO

The otolith aspect ration (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

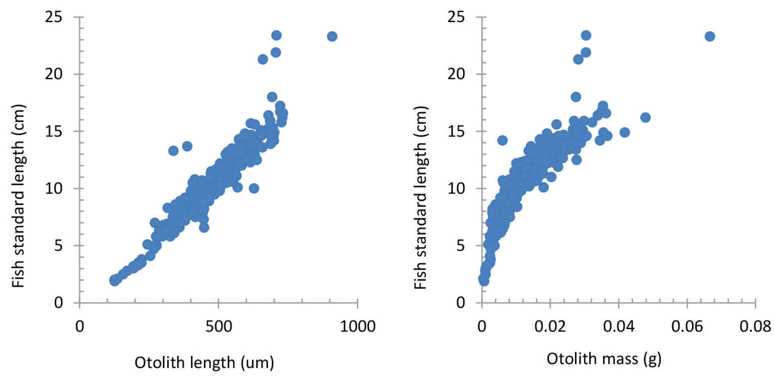

FIGURE 3. The relationship between Malaysian Mugilidae species standard length (cm) and two sagittal otolith measurements, otolith length (µm) and otolith mass (g)

of the fish species, specifically head shape. However, this is not supported by present student; the highest relative head length (HL/SL) was *E. vaigiensis* with (0.29) with a O_{AS} 1.89, whereas, *C. subviridis* (0.24) had the highest O_{AS} of (2.24) (Table 3).

The head shape of *C. melinopterus* is described to be notably different from the majority of other species, corresponding with findings suggesting this species had the most significantly different O_{AS} value (Table 1). *C. melinopterus* had the smallest mean O_{AS} value (Figure 2, Table 1), meaning that O_L could be smaller and/or O_W could be bigger. If head morphology does relate to otolith morphology, it would be acceptable to presume a deeper head shape would result in a wider otolith, as reflected in the O_{AS} (Figure 2, Table 1). However, the head of *E. vaigiensis* is described similarly, as well as *M. seheli*'s and while *E. vaigiensis* was found to have a relatively high number of significant differences, *M. seheli*'s differences were to a lesser degree (Table 1), despite the similarity in head shape (Table 3).

The results however suggest there may be some aspect of species specific otolith morphology, possibly relating to head morphology, but due to the large amount of morphological similarity between species, otolith morphometrics would only be useful as a taxonomic aid rather than as a distinguishing feature.

In the case of these Mugilidae, it appears that otolith morphology is linked with the fish's morphological traits, but there is too much variation in the patterns (Table 3) to allow for fish morphology to be the sole factor affecting otolith shape. This can be attributed to other variables influencing otolith morphology. While there is some evidence otolith shape can be coded for genetically (Ali et al. 2013), the majority of studies believe that it is environmental factors that play a greater role in determining otolith morphology (Lombarte et al. 2010; Stransky et al. 2008). These environmental factors are temperature, depth, food and mineral availability, as well as habitat type (Aguirre & Lombarte 1999; Arellano et al. 1995; Lombarte 1992; Lombarte & Lleonart 1993; Paxton 2000; Reichenbacher et al. 2007; Volpedo et al. 2003).

Table 4 examines data for two of the previously mentioned factors that can affect otolith morphology. Habitat and diet were the only factors with available data that was both detailed and showed differences between species. Many aspects of Mugilidae life histories have not been studied for the majority of species, while some sectors, spawning grounds for example, were assumed to be the same for all species (at sea). Between habitat and diet, however, there seems to be little variation between species, the biggest difference appears to be salinity limits, with some species distribution limited to more saline

TABLE 2. *Post-hoc* Duncan test to display where differences in the O_{AS} values are between the eight Malaysian Mugilidae species (level of significance: * $P < 0.05$). '0' comparisons had no significant difference

	Chelon macrolepis	*Chelon melinopterus*	*Chelon subviridis*	*Ellochelon vaigiensis*	*Moolgarda cunnesius*	*Moolgarda seheli*	*Mugil cephalus*	*Valamugil engeli*
Chelon macrolepis	-	*	o	*	*	o	o	*
Chelon melinopterus		-	*	*	*	*	*	o
Chelon subviridis			-	*	*	*	o	*
Ellochelon vaigiensis				-	o	*	*	*
Moolgarda cunnesius					-	*	*	*
Moolgarda seheli						-	o	*
Mugil cephalus							-	*
Valamugil engeli								-

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

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

[1]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 & Niem 1999)

Species	Habitat	Diet
Chelon macrolepis	Shallow coastal waters, estuaries and backwaters	Small algae, diatoms, foraminifera, benthic polychaetes, crustacea, molluscs, organic matter and detritus
Chelon melinopterus	Shallow coastal waters, enters lagoons, estuaries and rivers to feed	Plant detritus, microalgae, minute bottom-living organisms, organic matter
Chelon subviridis	Shallow coastal waters, enters lagoons, estuaries and fresh waters to feed	Microalgae, diatoms, detrital material and filamentous algae
Ellochelon vaigiensis	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
Moolgarda cunnesius	Shallow coastal waters, benthic substrates	*Lack of data due to previous taxonomic confusion*
Moolgarda seheli	Shallow coastal waters, enters lagoons, estuaries, brackish tidal creeks, fresh water rivers	Microalgae, diatoms, detrital material, foraminifera and filamentous algae
Mugil cephalus	Inshore marine waters, estuaries, lagoons and rivers	Fine particulate matter, detritus, microscopic animals and algae
Valamugil engeli	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 $CaCO_3$ 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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APPENDIX 1. Sample site: A. Langkawi; B. Kuala Selangor; C. Bachok; D. Kuantan; E.Sandakan.