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)

MORWENNA SEE, SARA MARSHAM, CHIH WEI CHANG, VING CHING CHONG, A. SASEKUMAR, SARINDER KAUR DHILLON & KAR HOE LOH

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. melanopterus, 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. melanopterus (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

INTRODUCTION

Otoliths are calcareous structures (Popper & Lu 2000) found in the inner ear of fishes (Campana 2004). There are three pairs of otoliths; sagitae, 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 then dried overnight before being photographed using the Olympus SZX10 (Japan) Trinocular Stereomicroscope at 6.3x 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 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. macrocephalus* (\( Z = 543.0, n = 45, P > 0.05 \)) 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
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 (OAS) was calculated by dividing OL by OW. 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’ OAS and the effect of the size covariate SL.

**OTOLITH LENGTH/FISH SIZE**

The relationship between OL 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 OM and fish st. 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 OAS 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 OAS between species (ANCOVA, F = 23.74, df = 7, 465, P < 0.001). Fish size (SL) however had a significant effect on OAS 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 OAS were *C. melinopterus* (mean=1.65) and *V. engeli* (1.66); *M. cunnesius* (1.89) and *E. vaigiensis* (1.89); *M. seheli* (2.08); 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 OL (μm) and SL (cm) for the eight Malaysian Mugilidae species (Spearman’s rank correlation coefficient, r = 0.897, n = 453, P < 0.001) (Figure 3). There was a significant relationship determined by method of
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 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.
of the fish species, specifically head shape. However, this is not supported by present study; 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

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

**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

<table>
<thead>
<tr>
<th>Chelon macrolepis</th>
<th>Chelon melinopterus</th>
<th>Chelon subviridis</th>
<th>Ellochelon vaigiensis</th>
<th>Moolgarda cunnesius</th>
<th>Moolgarda seheli</th>
<th>Mugil cephalus</th>
<th>Valamugil engeli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chelon macrolepis</td>
<td>-</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>o</td>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>Chelon melinopterus</td>
<td>-</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>o</td>
</tr>
<tr>
<td>Chelon subviridis</td>
<td>-</td>
<td>-</td>
<td>*</td>
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<td>*</td>
<td>o</td>
<td>*</td>
</tr>
<tr>
<td>Ellochelon vaigiensis</td>
<td>-</td>
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<td>*</td>
<td>*</td>
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<tr>
<td>Moolgarda cunnesius</td>
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<td>o</td>
<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Moolgarda seheli</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Mugil cephalus</td>
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<td>*</td>
</tr>
<tr>
<td>Valamugil engeli</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>-</td>
</tr>
</tbody>
</table>
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

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

<table>
<thead>
<tr>
<th>Species</th>
<th>HL/SL (Mean ± S.D.)</th>
<th>Head shape ¹</th>
<th>O$_{AS}$ (Mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chelon macrolepis</td>
<td>0.26 ± 0.03</td>
<td>Depth equal or greater than width, dorsally flattened</td>
<td>2.14 ± 0.21</td>
</tr>
<tr>
<td>Chelon melinopterus</td>
<td>0.25 ± 0.04</td>
<td>Wider than deep and depressed</td>
<td>1.65 ± 0.13</td>
</tr>
<tr>
<td>Chelon subviridis</td>
<td>0.24 ± 0.02</td>
<td>Broad, but depth usually equal to or greater than width, dorsally flattened</td>
<td>2.24 ± 0.21</td>
</tr>
<tr>
<td>Ellochelon vaigiensis</td>
<td>0.29 ± 0.03</td>
<td>Broad, wider than deep, dorsally flattened</td>
<td>1.89 ± 0.29</td>
</tr>
<tr>
<td>Moolgarda cunnesius</td>
<td>0.24 ± 0.01</td>
<td>Deeper than wide, dorsally flattened</td>
<td>1.89 ± 0.26</td>
</tr>
<tr>
<td>Moolgarda seheli</td>
<td>0.26 ± 0.02</td>
<td>As wide as deep, or slightly wider, dorsally flattened</td>
<td>2.08 ± 0.15</td>
</tr>
<tr>
<td>Mugil cephalus</td>
<td>0.25 ± 0.02</td>
<td>Dorsally broad and flat, deeper than wide</td>
<td>1.87 ± 0.18</td>
</tr>
<tr>
<td>Valamugil engeli</td>
<td>0.27 ± 0.03</td>
<td>Deeper than wide, dorsally flattened</td>
<td>1.67 ± 0.12</td>
</tr>
</tbody>
</table>


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

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


Morwenna See
School of Marine Science & Technology
University of Newcastle upon Tyne

Sara Marsham
Dove Marine Laboratory
School of Marine Science & Technology
Cullercoats, North Shields, Tyne and Wear
NE30 4PZ
United Kingdom

Chih Wei Chang
National Museum of Marine Biology and Aquarium
Taiwan ROC

Chih Wei Chang
Institute of Marine Biodiversity and Evolutionary Biology
National Donghua University
Taiwan ROC

Ving Ching Chong, A. Sasekumar & Sarinder Kaur Dhillon
Institute of Biological Sciences
University of Malaya
50603 Kuala Lumpur
Malaysia

Ving Ching Chong & Kar Hoe Loh
Institute of Ocean and Earth Sciences, C308
Institute of Postgraduate Studies Building
University of Malaya, 50603 Kuala Lumpur
Malaysia

*Corresponding author; email: khloh@um.edu.my

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APPENDIX 1. Sample site: A. Langkawi; B. Kuala Selangor; C. Bachok; D. Kuantan; E. Sandakan.