# Sr/Ca, Mg/Ca and Ba/Ca Ratios in the Otolith of Sea Bass in Peninsular Malaysia as Salinity Influence Markers

(Nisbah Sr/Ca, Mg/Ca dan Ba/Ca dalam Otolit Ikan Siakap di Semenanjung Malaysia sebagai Penunjuk Pengaruh Saliniti)

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# ABSTRACT

The otoliths of sea bass (Lates calcarifer) were sampled from 23 locations in Peninsular Malaysia in order to analyse the Sr/Ca, Mg/Ca and Ba/Ca ratios. This study found that these elements/Ca ratios in sea bass otoliths were correlated with the salinity zone (thalassic series) compared with elements/Ca ratios in ambient water. The contradictory pattern of Sr/Ca ratios and Ba/Ca ratios in otoliths was found according to salinity zone variations. Thus, oligohaline waters showed the highest Ba/Ca ratios while the highest Sr/Ca ratios were recorded for saline waters. The terrigenous enrichment of Ba in water also affects the Ba content in otoliths. The presence of Mg in otoliths acts as a rival to Ba, thus affecting Mg and Ba deposition. Meanwhile, Ba is the tracer for salinity fluctuations based on the partition coefficient (D) and it shows significant changes. However, the elements in otoliths were found to originate indirectly from the water column. The sequence of the elements/Ca ratios in otoliths and ambient water was Sr/Ca > Mg/Ca > Ba/Ca and Sr/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Ba/Ca > Ba/Ca > Mg/Ca > Ba/Ca > Ba/Ca > Ba/Ca > Ba/Ca > Ba/Ca

Keywords: Ba/Ca; Mg/Ca; Otolith; Sr/Ca; thalassic series

### ABSTRAK

Sebanyak 23 lokasi di Semenanjung Malaysia telah dipilih untuk persampelan ikan siakap (Lates calcarifer). Otolit ikan siakap dianalisis untuk mendapatkan nisbah Sr/Ca, Mg/Ca dan Ba/Ca. Kajian ini mendapati nisbah elemen/Ca dalam otolit mempunyai korelasi dengan saliniti berbanding nisbah elemen/Ca dalam air ambien. Nisbah Sr/Ca dalam otolit didapati bertentangan dengan nisbah Ba/Ca dalam otolit dan berubah mengikut saliniti. Oleh itu, nisbah Ba/Ca dalam otolit adalah tinggi dalam air oligohalin manakala nisbah Sr/Ca dalam otolit. Manakala, kehadiran Mg dalam otolit adalah pesaing kepada Ba lalu mempengaruhi pengenapan Ba pada otolit. Unsur Ba merupakan penyurih berdasarkan pekali sekatan (D) menunjukkan perubahan yang signifikan. Walau bagaimanapun, sumber elemen dalam otolit adalah Sr/Ca>Mg/Ca>Mg/Ca.

Kata kunci: Ba/Ca; Mg/Ca; otolit; Sr/Ca; siri talasik

### INTRODUCTION

In a natural habitat, salinity acts as a proximal stimulus for sea bass (*Lates calcarifer*) to spawn. According to previous research, 30 psu is the optimal salinity which stimulates sea bass to migrate (Blaber 2000; Moore 1979). Thus, adult sea bass found in semi-tropical areas of the Indo-Pacific ascend from euhaline seas (30-35 psu) to less saline water bodies such as brackish seas to spawn (Grey 1987). Meanwhile, juveniles will stay in estuarine areas and return to sea prior to maturity (Russell & Garrett 1983). As a result of its ability to adapt, this protandrous hermaphrodite is the most abundant fish reared by mariculturists in cages and ponds nationwide. The farming of sea bass has also become the most popular fin fish produced in Malaysia due to its texture and taste (DOF 2007). Native sea bass is a popular fish not only in Malaysia, but also in South East Asia. The elemental chemistry of sea bass otoliths was used as a tool to indicate changes in salinity according to the thalassic series for the entire lifespan of the fish.

Otoliths or earstones are the structures that are found to record changes of salinity in its daily rings throughout the lifespan of a fish (Campana & Neilson 1985; Degens et al. 1969; Farrell & Campana 1996; Miller et al. 2006). Each teleost inner ear system consists of three types of otoliths in pairs; sagitta, asteriscus and lapillus located in the hindbrain (Platt & Popper 1981; Popper & Coombs 1980). Calcium is physiologically incorporated onto otoliths proportionally with fish growth (Popper et al. 2005). While foreign elements such as Sr and Ba are incorporated mainly due to the natural abundance of these elements in water bodies inhabited by the fish (Farrell & Campana 1996). Usually, the concentration of these elements is synchronized with water salinity (Elsdon & Gillanders 2003a, 2003b; Gillanders 2005; Kafemann et al. 2000). The Sr and Ba concentrations in otoliths have been successfully used to detect changes of salinity experienced by fish (Milton & Chenery 2001; Secor & Rooker 2000). Previous studies focused on the chemical analysis of otoliths to determine migratory patterns of fish (Arai & Miyazaki 2001; Milton & Chenery 2001; Secor & Rooker 2000), fish age, growth patterns, reproduction and mortality (Arai et al. 2002; Dwyer et al. 2003; Grandcourt et al. 2006) as well as to investigate the impact of oil spills on fish growth (Morales-Nin et al. 2007). Most researchers analyse otolith microchemistry, but only Leakey et al. (2009) analysed otolith macrochemistry. However, the history of otolith research for sea bass in the South-east Asia region is scarce aside from work by Milton and Chenery (2001) in Papua New Guinea. Other than that, no research was found regarding the otolith chemistry of sea bass in this region. In Malaysia, wild sea bass is rarely found. Thus not much is known about the adult of sea bass before and after they migrate to brackish water to breed as they are rarely captured by local fishermen in the open sea. Most adult wild sea bass are captured during their migration to estuarine areas. Otolith macrochemistry has great potential to be developed as a reflector of the variations in salinity inhabited throughout the entire lifespan of a fish. The needs of sea bass' otolith research in Malaysia triggered this preliminary research on this hermaphroditic anadromous fish. This research will be the first attempt to determine otolith chemistry affected by different series of salinity. The normal classification of water bodies according to salinity is freshwater, brackish water and seawater. Therefore, the modified thallasic series from Por (1972) was used to discriminate between the salinity of sampling stations' ambient water. In this series, brackish water was also divided into two series; mesohaline and polyhaline in order to discriminate between the variations among the sampling stations.

In this study, the otoliths of cultured sea bass were analysed to determine the relationship between salinity zone effects on otoliths. These salinity zones were categorized according to the thalassic salinity series. This study thus aimed to determine the Sr/Ca, Mg/Ca and Ba/Ca ratios in otoliths, as well as the ratios (Sr/Ca, Mg/Ca and Ba/Ca) in the salinity series, thus determine which element/ Ca ratio is a better indicator of salinity fluctuations.

### MATERIALS AND METHODS

# SAMPLING LOCATIONS, STORAGE AND SAMPLE ANALYSES

From March 2007 to July 2008, 115 sea bass specimens were collected from cages and ponds located around Peninsular Malaysia (Figure 1, Table 1). The selected fish cages and ponds are currently influenced by seawater from the Straits of Malacca, the Straits of Johor and the South China Sea (Figure 1) and also by freshwater from adjacent rivers. The Straits of Malacca and Straits of Johor separate Peninsular Malaysia from Sumatra, Indonesia and Singapore, respectively, while the South China Sea separates Peninsular Malaysia from East Malaysia (Sabah and Sarawak). The sampling locations were chosen in order to obtain the differentiation of salinity series.

Water salinity was recorded using a YSI 30 salinometer at the time the specimens were collected. Each sampling site was characterized according to the thalassic series (Table 1). The sampled specimens were kept frozen for further analysis in the laboratory.

The sampling stations' salinity levels were divided according to Por (1972). Brackish waters were divided into two zones; mesohaline and polyhaline. Brackish water sampling stations experience the mixing of both fresh and seawater but there was no abrupt changes were found. The sampled fish did not experience severe changes of salinity. Fish survival rates were found to be high; if abrupt variations of salinity were to occur, it could be fatal to the fish. There are changes in salinity as the sampling stations are influenced by both fresh and seawater. However, the changes are not varied such that it might change the series i.e. oligohaline to mesohaline and vice versa.

The age of the collected fish ranged from 12 to 16 months and the fish varied by size, according to the mariculturist's rearing time. Sampled specimens were collected based on fish availability. The specimens were then measured and weighed before otolith extraction (Table 1). Once extracted, the otoliths were rinsed with deionised water and were stored dry. All the glassware was soaked in 5% of HNO<sub>3</sub> overnight prior to analysis. Only right sagitta otoliths were used for elemental analysis as both right and left otoliths are similar in weight, length and width (paired T-TEST, p>0.05) (Figure 2). The otoliths were weighed using an electronic balance with an accuracy of  $\pm 0.01$  mg. The otoliths were digested in 10 mL of HNO<sub>2</sub> in beakers. The samples were then dried to dryness on a hotplate and 0.5M HNO3 was added before being analysed with a Perkin Elmer 9000 inductively coupled plasma mass spectrometer (ICP-MS) to determine Sr and Ba while Ca was determined using a Perkin Elmer AA 800 flame atomic absorption spectrometer (AAS).

Ambient water samples were collected from the cages and pond area in Peninsular Malaysia. Surface water samples were collected in 12 L of High-density polyethylene (HDPE) containers filtered using a 0.45  $\mu$ m membrane filter and acidified with HNO<sub>3</sub> to pH < 3 using a hand-held pH meter. Three replicates of 250 mL water samples were used to extract Ca, Mg and Ba using the APDC-MIBK extraction method (Kumar & Riyazuddin 2009). Purified water samples were then diluted before being detected with Flame AAS (Mg and Ca) while Graphite Furnace AAS for Ba. For the Sr analysis, water samples were diluted (1:10) and then detected directly with ICP-MS.

Replicates of Certified Reference Material No. 22 (Otolith) were also prepared using the same procedure as otolith samples where the measurement results were in the range of certified values. For seawater, multi-element standards were spiked into artificial seawater and purified



FIGURE 1. Sea bass sampling locations around Peninsular Malaysia

using the same procedure as actual seawater samples before being detected with AAS. Standards were also spiked into artificial seawater for ICP-MS detection. Both of these procedures were done in order to obtain the calibration curve and method validation.

# STATISTICAL ANALYSIS AND CALCULATION

One way ANOVA was used to determine the significance of elemental/Ca ratio with salinity series. The relationship between the salinity of ambient water with elemental/ Ca ratios in otoliths and ambient water were quantified using Pearson's partial correlations analysis computed by SPSS 18.0 version software. The Partition coefficients of elements were calculated using this formula as:

$$D_{Element} = (A) / (B)$$
(1)

where the (A) is the ratio of Element/Ca in otoliths and the (B) is the Element/Ca in the water column.

# RESULTS AND DISCUSSION

### RELATIONSHIP OF ELEMENT/CA RATIOS IN OTOLITHS WITH SALINITY

The ambient salinity value of water at the sampling stations was determined from oligohaline to euhaline series modified from the classification of Por (1972). Sampling stations were classified as oligohaline (0-5 psu) followed by mesohaline (6-18 psu), polyhaline (19-30 psu) which dominated the sampling stations and euhaline (31-35 psu) (Table 1). The varied salinity of ambient waters of the fish cages and ponds indicate that adult sea bass successfully tolerate fluctuations of salinity (Table 1). The relationship

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Thalassic Series	St	Location	и	Date of sampling	Salinity (psu)	Body weight (kg)	Total length (cm)	Otolith weight (g)
Oligohaline	9	Sg. Kuala Muda, Kuala Muda	5	1-Sep-07	0.1	$0.86 \pm 0.30$	$38.06 \pm 3.66$	$0.070 \pm 0.013$
(0-5 psu)	20	Tanjung Agas, Pekan	9	22-Mar-08	0.1	$1.30 \pm 0.27$	$47.80 \pm 3.55$	$0.130 \pm 0.020$
n=15	11	Sg. Muar, Muar	4	17-Nov-07	5.3	$0.41 \pm 0.23$	$31.68 \pm 1.14$	$0.047 \pm 0.041$
Mesohaline	8	Sg. Udang, Nibong Tebal *	5	4-Jul-08	11.2	$0.71 \pm 0.38$	$36.36 \pm 1.15$	$0.060 \pm 0.010$
(6-18 psu)	S	Sg. Jagung, Sungai Petani	4	27-Apr-08	16.8	$0.86 \pm 0.09$	$42.33 \pm 1.70$	$0.056 \pm 0.009$
n=13	1	Kg. Bukit Tok Poh, Kuala Perlis *	4	5-Jul-08	18.4	$0.56 \pm 0.10$	$35.00 \pm 1.92$	$0.059 \pm 0.005$
	16	Sg. Penderam, Pasir Gudang	4	14-Nov-07	19.4	$0.51 \pm 0.63$	$33.78 \pm 1.38$	$0.056 \pm 0.009$
	19	Kg. Sedili Kechil, Kota Tinggi	9	22-Mar-08	19.8	$0.556 \pm 0.09$	$36.15 \pm 2.14$	$0.070 \pm 0.006$
	15	Kg. Teluk Jawa, Pasir Gudang	3	15-Nov-07	21.3	$0.98 \pm 0.13$	$41.70 \pm 0.60$	$0.094 \pm 0.012$
Polyhaline	17	Kg. Teluk Sengat, Kota Tinggi	9	20-Mar-08	22.9	$0.62 \pm 0.11$	$35.48 \pm 1.76$	$0.066 \pm 0.006$
(19 - 29 psu)	14	Sg. Danga, Johor Bahru	5	20-Mar-08	23.4	$0.49 \pm 0.57$	$33.67 \pm 2.00$	$0.051 \pm 0.009$
n=51	6	Larut Matang, Taiping	8	3-Jul-08	23.6	$0.81 \pm 0.26$	$39.65 \pm 3.23$	$0.089 \pm 0.017$
	13	Kg. Pendas Laut, Gelang Patah	5	15-Nov-07	25.6	$0.53 \pm 0.10$	$34.77 \pm 2.46$	$0.072 \pm 0.009$
	18	Kg. Linting, Penggerang	5	21-Mar-08	27.4	$0.37 \pm 0.08$	$31.24 \pm 2.56$	$0.053 \pm 0.006$
	Г	Pulau Aman, Nibong Tebal	5	28-Apr-08	29	$0.53 \pm 0.32$	$34.98 \pm 0.65$	$0.068 \pm 0.002$
	23	Pantai Sri Tujuh, Tumpat	5	24-Mar-07	29	$0.63 \pm 0.39$	$35.95 \pm 0.98$	$0.059 \pm 0.005$
	12	Pulau Kukup, Pontian	4	16-Nov-07	29.9	$0.40 \pm 0.10$	$32.51 \pm 1.92$	$0.118 \pm 0.010$
	0	Kubang Badak, Langkawi Island	9	17-Jan-08	30	$0.47 \pm 0.52$	$31.85 \pm 1.79$	$0.043 \pm 0.007$
Euhaline	10	Pulau Ketam, Port Klang	4	4-Mar-08	31.2	$1.44 \pm 0.14$	$45.00 \pm 1.96$	$0.115 \pm 0.025$
(30-35 psu)	21	Sg. Paka, Paka	5	24-Apr-07	31.4	$0.49 \pm 0.10$	$32.54 \pm 1.86$	$0.061 \pm 0.008$
n=32	4	Pulau Dayang Bunting, Langkawi Island	5	17-Jan-08	31.5	$0.11 \pm 0.13$	$43.58 \pm 2.55$	$0.118 \pm 0.010$
	б	Sungai Kilim, Langkawi Island	4	17-Jan-08	31.7	$0.44 \pm 0.12$	$34.33 \pm 2.42$	$0.061 \pm 0.011$
	22	Kg. Gong Batu, Setiu	Г	21-Apr-07	33.1	$0.84 \pm 0.20$	$39.79 \pm 2.68$	$0.076 \pm 0.009$

TABLE 1. Sea bass sampling stations around Peninsular Malaysia with the analysed fish weight, total length and otolith weight ( $\pm$  SE) *n*, number, \*fish pond



FIGURE 2. A pair of panhandle sagitta otoliths extracted from sea bass

between divalent elements/Ca ratios in this study showed the connection between these elements and the salinity of ambient water (Figure 4). Generally, the uptake of Ca was physiologically incorporated by the fish to maintain fish orientation, hearing and balance in proportion with increasing weight (Campana 1999). Foreign elements such as Sr, Mg and Ba are the same divalent elements as Ca. Furthermore, Sr (atomic radius = 0.135 Å) Mg (0.065 Å) and Ba (0.113 Å) present in otoliths were the cause of the similarity between these elements' atomic radii with Ca atomic radius, 0.099 Å (Gauldie et al. 1995). These foreign elements were considered a mistake deposition onto otoliths (Afiza Suriani et al. 2009a). Figure 3 shows the distribution of elemental/Ca ratios based on the sampling stations in Peninsular Malaysia according to the salinity series inhabited by sea bass throughout its entire lifespan.

The fluctuations of Sr/Ca ratios were not so gradual according to increasing salinity even though Sr was abundant in higher salinity water, especially in euhaline waters (Campana & Tzeng 2000; Farrell & Campana 1996). The one way ANOVA test supports the patterns with insignificant differences between Sr/Ca ratios in otoliths with the all thalassic series (p > 0.05). It is evident that Sr/Ca ratios do not significantly differ with fluctuations of salinity (Farrell & Campana 1996). Thus, high Sr/Ca ratios indicate that Sr<sup>2+</sup> has the priority in being incorporated onto the otolith matrix even though it is in oligohaline waters because Sr deposition onto otoliths are less strictly controlled than essential Ca (Figure 3a). Plus, saline waters contain more Sr than Ba as the highest ratio is Sr/Ca ratios, followed by Ba/Ca and Mg/Ca (Figure 4).

Meanwhile, Ba/Ca ratios showed the contradictory pattern with Sr/Ca ratios. The content of Ba was abundant in less saline waters because of the terrestrial input although one way ANOVA exhibited a significant difference (p< 0.05). Consequently, variation pattern of Ba/Ca suggest that Ba uptake was diversified by salinity changes. The strong relationship between Ba/Ca and Sr/Ca



FIGURE 3. Element/Ca ratio values in otoliths according to the thallasic salinity series

ratios was determined by the preference of these elements to be incorporated onto otoliths (Figure 5(a) & 5(c)). This indicates that Ba/Ca ratios in otoliths act as the tracer of fluctuating salinity especially in oligohaline waters (Figure 3(b)). Furthermore, the otolith prefers Ba than Mg because of its similarity to Ca atomic radius (Milton & Chenery 2001). Similar sequences of elements/Ca ratios between giant mudskippers and sea bass otoliths support this theory (Afiza Suriani et al. 2009a).

The Mg/Ca ratios showed a insignificant difference in one way ANOVA analysis (p > 0.05) and showed no exact trend of Mg/Ca ratios. Additionally, it is suggested that Mg exists in ambient water from oligonaline to euhaline



FIGURE 4. Element/Ca ratio values in water according to the thallasic salinity series

(Figures 3(b) & 4(b)). Hence, all Sr/Ca, Mg/Ca and Ba/Ca ratios in the otoliths were found to be from the same source which is ambient water as all the elements were correlated to each other with an insignificant difference (p< 0.05) as shown in Figure 5.

### RELATIONSHIP OF ELEMENT/CA RATIOS IN AMBIENT WATER ACCORDING TO SALINITY SERIES

Generally, Sr, Mg and Ba in seawater are strongly related with salinity (Elsdon & Gillanders 2003b). These elements act as salinity markers in order to discriminate between fluctuations of salinity. However, no synchronizing or contradictory patterns were shown in this study (Figure 4). The sequence of elements/Ca ratio (Sr/Ca>Ba/Ca>Mg/Ca) differs from the sequences discovered in previous studies (Mg/Ca>Sr/Ca>Ba/Ca) (Hamer et al. 2006; Summerhayes



FIGURE 5. Significant correlations between Mg/Ca, Sr/Ca and Ba ratio values in otoliths

& Thorpe 1996). The high elements/Ca ratio in mesohaline waters suggests that all these elements were abundant in mesohaline waters which are the intermediate range of the thalassic series (Figure 4). The enrichment of these elements might be due to terrestrial input from fish ponds (Table 1). Terrestrial input was suspected to be the enrichment factor of Sr, Mg and Ba from the shallow soil-made ponds, especially Ba which originates from terrigenous soil (de Vries et al. 2005). Furthermore, pond water circulation is closed-system and not free-flowing, unlike fish cages. The limitations of water flow and enclosed circulation may cause enrichment of these divalent elements. The enrichment of Sr, Mg and Ba came from the soil which is rich with excess fish food thus leaching from soil to ambient water affects otolith composition. Meanwhile, Ba, Sr and Mg exists in low concentrations that might be diluted by spatial elements such as rainwater input during sampling at oligohaline stations (Figure 4).

The ratios of Mg/Ca and Sr/Ca in ambient water showed a significant difference (one way ANOVA; p<0.05). This shows that Mg and Sr from adjacent seawater affects the sampling areas' ambient water (Gauldie et al. 1995; Tzeng & Tsai 1994). At the same time, the lowest ratio of Ba/Ca ratios in euhaline water support the claim that Ba originates from freshwater (de Vries et al. 2005). The strong

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and similar correlation (r > 0.6; p < 0.05) between all the elements/Ca ratios verifies that these elements originate from non-anthropogenic inputs into seawater (Figure 6).

### ELEMENT/CA RATIOS IN OTOLITH VERSUS WATER AND SALINITY

Most previous research designed experimental tanks under controlled environments to minimize the effect of environmental variables onto fish and otolith incorporation (Farrell & Campana 1996; Lin et al. 2007). Changes in Sr/Ca and Ba/Ca ratios were examined in otolith rings using equipment such as laser ablation ICP-MS (Milton & Chenery 2001). The controlled environment of the experimental design enabled the reduction of spatial, temporal and environmental variables in order to obtain more significant data. Most of the indoor experimental designs were found to be correlated with element contents in ambient water. This relationship was often triggered by salinity variations (Lin et al. 2007). In spite of that, this study showed a clearer pattern between elements/Ca in otoliths with salinity than elements/Ca in water (Figures 3 & 4). However, some studies also found a correlation between elements/Ca ratios and salinity (Lin et al. 2007; Milton & Chenery 2001). Similarly, Leakey et al. (2009) also found no relationship between elements/Ca in otoliths and elements/Ca in water. Ba/Ca ratios show a significant difference compared to Mg/Ca in otoliths providing information on salinity changes affect otolith chemical composition (Figure 3(b) & 3(c)). Sr/Ca value in otoliths also show significant concentrations compared to Sr/Ca ratios in water as Sr and Ba concentrations in water were similar (Figures 3(a) & 4(a)). Sr/Ca ratios in otoliths were the highest compared to Mg/Ca and Ba/Ca ratios (Figure 3). The entire lifespan of sea bass in the study areas can be reflected by implicating the fractionation of Sr, Mg and Ba to Ca in otoliths. It is assumed that otoliths have the potential to be an indicator for the entire lifespan of a fish. On the other hand, the interpretation of Sr/Ca and Ba/Ca to determine the influence of salinity can only be achieved under natural environments with insignificant contamination of Ba and Sr from anthropogenic sources (Payan et al. 1997). This study suggests that Sr/Ca and Ba/



FIGURE 6. Significant correlations between Sr/Ca, Mg/Ca and Ba/Ca ratio values in ambient water

Ca ratios in whole otolith elemental analysis can determine the influence of salinity of ambient water inhabited by the fish for their entire lifespan. Hence, it showes that Sr/ Ca and Ba/Ca ratios can still be used to indicate salinity changes in background water considering that otoliths were fully analysed and not just by their rings (Afiza Suriani et al. 2009a, 2009b).

# PARTITION COEFFICIENT

The major uptake of otoliths is usually from water while other factors like temperature and diet have an insignificant effect on sea bass (Milton & Chenery 2001). In this study, the estimation of otolith uptake from the background environment was considered from the correlation coefficient calculation for the entire lifespan of the fish (Figure 7). It roughly indicated that the most favourable element that was chosen to be incorporated onto otolith composition was from ambient water. The uptake of Ba was greater than Sr and Mg might indicated that Ba can be a tracer for the detection of salinity changes especially in polyhaline water, even though it occurred in low concentrations in both otolith and ambient water (Figure 7(c)). The preferences of these elements were usually affected by the abundance of these elements in the ambient water (Payan et al. 1997).

However, the elemental composition in ambient water was significantly influenced by the intrusion of seawater which naturally leads to water salinity changes (Milton & Chenery 2001). In this study, the higher Ba/Ca ratio in ambient water especially in polyhaline waters is believed to be from terrestrial leaching of Ba into the water (Figure 4(c)). This might affect the uptake of the Ba onto otoliths due to enrichment. The similar coefficient value of Mg and Sr suggests that both of these elements are competing with each other to be incorporated onto non-lattice sites of otoliths (Figure 7(b) & 7(c)). In fact, Sr uptake is considered more preferable as shown by the greater Sr content in ambient water, the trend of Sr/Ca ratios in otoliths and the similarity of the atomic radius (Figures 5(a)& 3(a)). Sr and Ba are known as the biological tracers and Mg is considered a supporting element to validate seawater input in ambient water (Afiza Suriani et al. 2009a, 2009b; Milton & Chenery 2001).



FIGURE 7. Partition coefficient of elements based on the salinity series

### CONCLUSION

This research suggested that otolith macrochemistry can be used to determine salinity variations of sea bass otoliths in Peninsular Malaysia. The Sr/Ca and Ba/Ca ratio values were significantly related to the salinity of ambient water for the entire lifespan of sea bass in the study area. It was shown in this study that Sr/Ca ratios reflect saline water input while Ba/Ca ratios reflect changes in freshwater input throughout the thalassic salinity series. Although the method used was unconventional compared with previous studies, it proves that otoliths can be a tool in inspecting the influence of salinity for the entire lifespan of a fish. Sr and especially Ba appeared to show significant changes via salinity fluctuations. The traces of Ba in detecting salinity fluctuations is useful for identifying freshwater that is mixed and diluted within saline waters inhabited by the fish. Thus, the interpretation of Sr/Ca and Ba/Ca ratios in otolith macrochemistry could also be applicable as an indicator for migration of native sea bass in general. However, further studies need to be done to diversify the use of otoliths in solving many fisheries problems that might lead to decreasing fish populations.

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#### REFERENCES

- Afiza Suriani, S., Mazlan, A.G. & Mohamed, C.A.R. 2009a. Variation of Ca, Sr, Ba and Mg in the otolith of giant mudskipper in west coast of Peninsular Malaysia. *Pakistan Biological Journal of Science* 12(3): 231-238.
- Afiza Suriani, S., Noorliza, Z. & Mohamed, C.A.R. 2009b. Kepekatan unsur makro dan surih dalam otolit, isi ikan siakap dan air laut di Sedili Kechil. In *Seminar Penyelidikan Pantai Timur: Mersing Warisan Terpelihara*. Mersing, 28-29 March, Malaysia.
- Arai, T., Ikemoto, T., Kunito, T., Tanabe, S. & Miyazaki, N. 2002. Otolith microchemistry of the conger eel, *Conger myriaster*. *Journal of the Marine Biological Association of the United Kingdom* 82: 303-305.
- Arai, T. & Miyazaki, N. 2001. Use of otolith microchemistry to estimate the miratory history of the Russian sturgeon, *Acipenser guldenstadti. Journal of the Marine Biological Association of the United Kingdom* 81: 709-710.
- Blaber, S.J.M. 2000. Tropical Estuarine Fishes. Ecology, Exploitation and Conservation. Fish Aqua. Res. Ser., 7. Blackwell Science. pp. 129-140.
- Campana, S.E. & Tzeng, W.N. 2000. Section 4: Otolith composition. *Fisheries Research* 46: 287-288.
- Campana, S.E. 1999. Chemistry and compositions of fish otoliths: Pathways, mechanism and applications. *Marine Ecology Progress Series* 188: 263-297.

- Campana, S.E. & Neilson, J.D. 1985. Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Science* 42: 1014-1032.
- Degens, E.T., Deuser, W.G. & Haedrich, R.L. 1969. Molecular structure and composition of fish otoliths. *International Journal of Life Oceans and Coastal Waters* 2: 105-113.
- de Vries, M.C., Gillanders, M.B. & Elsdon, T.S. 2005. Facilitation of barium uptake into otoliths: Influence of strontium concentration and salinity. *Geochimica Cosmochimica Acta* 69: 4061-4072.
- DOF (Department of Fisheries). 2007. Fisheries Annual Statistical Vol 1., Putrajaya, p. 185.
- Dwyer, K.S., Stephen, J.W. & Campana, S.E. 2003. Age determination, validation and growth of Grand Bank yellowtail flounder (*Limanda ferriginea*). *ICES Journal of Marine Science* 60: 1123-1138.
- Elsdon, T.S. & Gillanders, B.M. 2003a. Reconstructing migratory patterns of fish based on environmental influences on otolith chemistry. *Fish Biology and Fisheries* 13: 219-235.
- Elsdon, T.S. & Gillanders, B.M. 2003b. Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*. *Marine Ecology Progress Series* 260: 263-272.
- Farrell, J. & Campana, S.E. 1996. Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus. Comparative Biochemistry and Physiology* 115A(2): 103-109.
- Gauldie, R.W., West, I.F. & Coote, G.E. 1995. Evaluating otolith age estimates for *Holosthertus atlanticus* by comparing patterns of checks, cycle in microincrement width, and cycles in strontium and calcium composition. *Bulletin of Marine Science* 56: 76-102.
- Gillanders, B.M. 2005. Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats. *Estuarine Coastal Shelf Science* 64: 47-57.
- Grandcourt, E.M., Al Abdessalaam, T.Z. & Francis, F. 2006. Age, growth, mortality and reproduction of the blackspot snapper, *Lutjanus fulviflamma* (Forsskäl, 1775), in the southern *Arabian Gulf Fisheries Research* 78: 203-210.
- Grey, D.L. 1987. An overview of *Lates calcarifer* in Australia and Asia. In *Management of Wild and Cultured Sea Bass Barramundi* Lates calcarifer, edited by Copland, J.W. ACIAR *Proceedings* 20: 15-21.
- Hamer, P.A., Jenkins, G.P. & Coutin, P. 2006. Barium variation in *Pagrus auratus* (Sparidae) otoliths: A potential indicator of migration between an embayment and ocean waters in south-eastern Australia. *Estuarine Coastal and Shelf Science* 68: 686-702.
- Kafemann, R., Adlerstein, S.M. & Neukamm, R. 2000. Variation in otolith strontium and calcium artios as an indicator of lifehistories strategies of freshwater species within a brackish water system. *Fisheries Research* 46: 313-325.
- Leakey, C.D.B., Attrill, M.J. & Fitzsimons, M.F. 2009. Multielement otolith chemistry of juvenile sole (*Solea* solea), whiting (*Merlangius merlangus*) and European seabass (*Dicentrarchus labrax*) in the Thames estuary and adjacent coastal regions. *Journal of Sea Research* 61(4): 268-274.
- Lin, S.H., Chang, C.W., Iizuka, Y. & Tzeng, W.N. 2007. Salinities, not diet, affects strontium/calcium ratios in otoliths of *Anguilla japonica*. *Journal of Experimental Marine Biology* and Ecology 341: 254-263.
- Kumar, A.R. & Riyazuddin, P. 2009. Comparative study of analytical methods for the determination of chromium in

groundwater samples containing iron. *Microchemical Journal* 93(2): 236-241.

- Miller, B.M., Clough, A.M., Batson, J.H. & Vachet, R.W. 2006. Transition metal binding to cod otolith proteins. *Journal of Experimental Marine Biology and Ecology* 329: 135-143.
- Milton, D.A. & Chenery, S.R. 2001. Sources and uptakes of trace metals in otoliths of juvenile barramundi (*Lates calcarifer*). *Journal of Experimental Marine Biology and Ecology* 264: 47-65.
- Moore, R. 1979. Natural sex invasion in the giant sea perch (*Lates calcarifer*). Australian Journal of Marine and Freshwater Research 30: 803-813.
- Morales-Nin, B., Geffen, A.J., Cardona, F., Kruber, C. & Sabarido-Rey, F. 2007. The effect of prestige oils ingestion on the growth and chemical composition of turbot otoliths. *Marine Pollution Bulletin* 54: 1732-1741.
- Payan, P., Kossmann, H., Watrin, A., Mayergostan, N. & Boeuf, G. 1997. Ionic composition of endolymph in teleosts – origin and importance of endolymph alkalinity. *Journal of Experimental Marine Biology* 200: 1905-1912.
- Platt, C. & Popper, A.N. 1981. Fine structure and function of the ear. In *Hearing and Sound Communication in Fishes*, edited by Tavaloga, W.N., Popper, A.N. & Fay, R.R. New York. pp. 1-36.
- Popper, A.N., Ramcharitar, J. & Campana, S.E. 2005. Why otoliths? Insights from the inner ear physiology and fisheries biology. *Marine Freshwater Research* 56: 497-504.
- Popper, A.N. & Coombs, S. 1980. Auditory mechanisms in teleost fishes. *American Scientist* 68: 429-440.

- Por, F.D. 1972. Hydrobiological notes on the high-salinity waters of the Sinai Peninsula. *Marine Biology* 14(2): 111-119.
- Russel, D.J. & Garrett, R.N. 1983. Use by juvenile barramundi, Lates calcarifer (Bloch) and other fishes of temporary supralittoral habitats in a tropical estuary in northern Australia. Australian Journal of Marine Freshwater Research 34: 805-811.
- Secor, D.H. & Rooker, J.R. 2000. Is otolith strontium a useful scalar of life cycles in estuarine fishes? *Fisheries Research* 46: 359-371.
- Summerhayes, C.P. & Thorpe, S.A. 1996. *Oceanography: An Illustrated Guide*. London: Manson Publishing Ltd.
- Tzeng, W.N. & Tsai, Y.C. 1994. Changes in otolith microchemistry of the Japanese eel, *Anguilla japonica*, during its migration from the ocean to the river of Taiwan. *Journal of Fish Biology* 45: 1671-1683.

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