

DIFFERENT ELEMENT/Ca RATIOS IN THE OTOLITH OF CULTURED SEA BASS IN MALAYSIA AND RELATIONSHIP TO SALINITY

AFIZA SURIANI SARIMIN and CHE ABD RAHIM MOHAMED*

*School of Environmental and Natural Resource Sciences, Faculty of Science and Technology,
Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor Malaysia.*

*E-mail: carmohd@ukm.my

ABSTRACT

The otoliths of cultured sea bass (*Lates calcarifer*) were sampled from 24 locations in Malaysian coastal waters in order to analyse the ratio values of Sr/Ca, Mg/Ca, Ba/Ca and Zn/Ca. Then the ratio values were discriminated to reconstruct a reliable marker for the migration of wild sea bass. Cultured sea bass was collected from cages and ponds along Malaysian coastal areas from March 2007 to May 2009 with ambient water salinity from oligohaline (0.1 psu) to euhaline (30-35 psu). The study found that the ratios of Mg/Ca and Zn/Ca in cultured sea bass otoliths were not influenced by the variation in water salinity. However, contradictory pattern value ratios of Ba/Ca in sea bass otoliths were found for the thalassic series. The oligohaline waters were shown to have the highest Ba/Ca ratio with the exception of Sr/Ca ratios in saline waters. This indicates that the concentration of the Sr element is slightly dominant in the otolith whereas the content of Ba was significant with salinity values. The Partition Coefficient (DMe) value also supports the proposition that the Ba element is a good tracer for salinity fluctuations in the otolith as shown by the sea bass migration track along the salinity gradient according to thalassic series.

Key words: otolith, Ba/Ca, thalassic series, migration

INTRODUCTION

Sea bass (*Lates calcarifer*) also known as barramundi have been cultured worldwide including in French Polynesia, the United States of America and the Indo-Pacific region such as Thailand and Malaysia (Mohsin and Ambak, 1996; FAO, 2006). This euryhaline fish is cultured in a wide range of salinities in cages or ponds worldwide due to its ability to adapt to myriad conditions. Over the past ten years, it has become a species with one of the highest production volumes in the Malaysian mariculture industry (Mohsin and Ambak, 1996; DOF, 2007b). Sea bass has been cultured extensively in cages along coastal waters in freshwater, brackish water and saline waters depending on the location, topography and seawater source (Husin, 1986). Thus, the elemental chemistry of cultured sea bass otoliths was expected to reflect salinity variations of fish cage locations. Cultured sea bass is in great demand as a replacement for wild sea bass as wild fish stocks are limited, and wild sea bass landings by fishermen are low (DOF, 2007a). In the wild, this catadromous

fish migrates from freshwater to seawater to breed (Husin, 1986; Grey, 1987; Schipp, 1991; Allsop *et al.*, 2006).

Salinity acts as the proximal stimulus for sea bass breeding in their natural habitat. Previous researchers found that 30 psu is the optimal salinity that stimulates sea bass migration and spawning (Blaber, 2000). Thus, adult sea bass found in semi-tropical areas of the Indo-Pacific region will move from euhaline seas (30-35 psu) to less saline water bodies such as brackish seas to spawn (Lim *et al.*, 1986; Grey, 1987; Allsop *et al.*, 2006; Balston, 2009). Meanwhile, juveniles will stay in the estuarine area, move upriver then return to sea prior to maturity (Schipp, 1991; Milton *et al.*, 2000; Allsop *et al.*, 2006). Wild sea bass populations in Malaysia are declining and are scarcely found in the wild. In order to preserve this fish, the elemental chemistry of cultured sea bass is implicated to simulate of a wild sea bass movements.

Otoliths or earstones are an aragonitic calcium carbonate structure which grows in daily rings for the entire lifespan of the fish (Degens *et al.*, 1969; Campana and Neilson, 1985; Farrell and Campana, 1996; Miller *et al.*, 2006). Calcium is physio-

* To whom correspondence should be addressed.

logically incorporated onto otoliths while foreign elements such as Sr, Mg, Ba and Zn are incorporated onto otoliths mainly due to the abundance of the elements in ambient water; usually correlated to salinity (Kafemann *et al.*, 2000; Elsdon and Gillanders, 2003b; Elsdon and Gillanders, 2003a; Yang *et al.*, 2011). The incorporation of these elements onto otoliths is permanent and stable even if the fish is distressed or starving (Secor and Rooker, 2000). Milton and Chenery (2001) were able to track the migration pattern of sea bass in Papua New Guinea by using Sr/Ca and Ba/Ca ratios. Meanwhile, Limburg and Elfman (2010) suggested that Zn might correlate with salinity gradients. In this study, cultured sea bass collected from various locations were expected to demonstrate salinity variation fluctuations of cages grouped according to modified thalassic series by Por (1972).

Previous studies focussed on the chemical analysis of otolith daily rings to determine migratory patterns of fish (Milton *et al.*, 2000; Secor and Rooker, 2000; Arai *et al.*, 2007; Honda *et al.*, 2012); to determine fish age, reproduction and mortality (Dwyer *et al.*, 2003); and also to investigate the impact of oil spills on fish growth (Gallego *et al.*, 1995; Kendall *et al.*, 2009). Not much is known about the relations between the trace elements in otolith with salinity variation (Leakey *et al.*, 2009; Sarimin and Mohamed, 2012). But this study aimed to determine which elements are suitable as salinity indicators and to reconstruct a model to track wild sea bass movements over their life cycle.

The tracking of sea bass migration patterns began in 1986 by Davis (1986) using fishing mortality in Australia. Over the years, several techniques have been developed to track fish migration especially for diadromous fish such as conventional tagging which depends on the capture-recapture methodology (Morton *et al.*, 1993) and advanced tagging such as satellite telemetry (Elsdon and Gillanders, 2003a). However, these techniques might not be useful for benthic fish species (Elsdon and Gillanders, 2003a). The elemental chemistry of otoliths is an alternative method to replace these earlier methods as the current method is suitable for all fish types including benthic fish.

MATERIALS AND METHODS

Sampling locations, storage and sample analysis

A total of 24 sampling stations were chosen in order to obtain salinity variations in sea bass cages. Overall, 122 sea bass specimens were collected, covering 11 Malaysian states from 24 March 2007 to 18 May 2009 (Fig. 1, Table 1). The selected fish cages receive seawater influences from the Straits of Malacca, Straits of Johor and the South China Sea (Fig. 1) as well as freshwater influences from adjacent rivers. The Straits of Malacca and the Straits of Johor separate Peninsular Malaysia with Sumatera, Indonesia and Singapore respectively while the South China Sea separates Peninsular Malaysia with East Malaysia (Sabah and Sarawak).

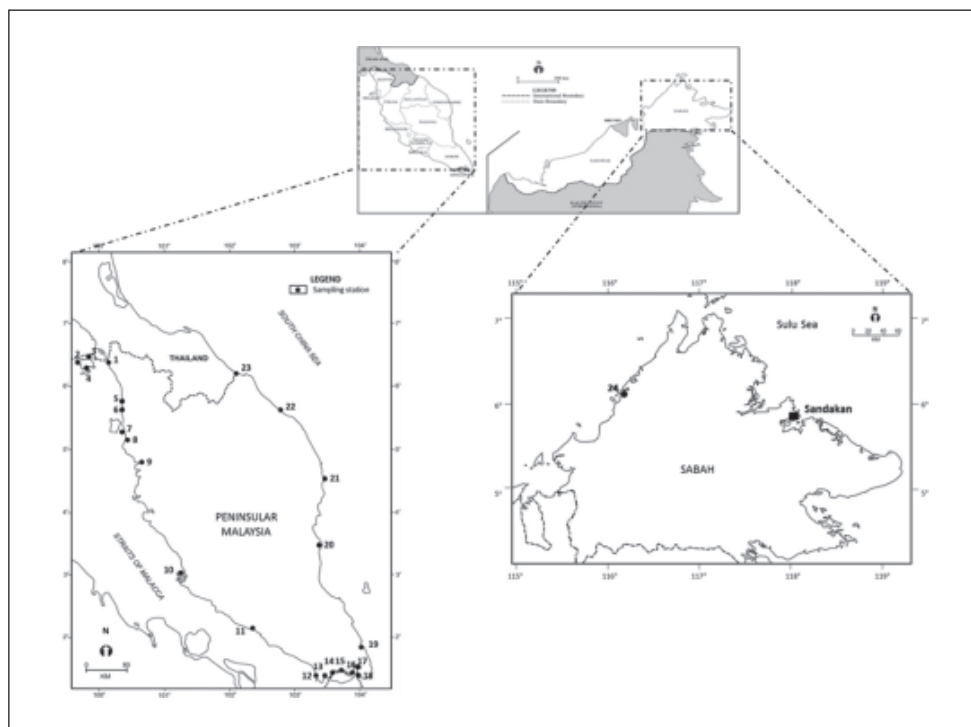


Fig. 1. Sampling stations (St 1 – St 24) of cultured sea bass in Malaysia.

Table 1. Sampling stations of cultured sea bass in Malaysian cages and ponds with the analyzed fish weight, total length and otolith weight (\pm sd)

Thalassic Series	St	Location	Sample size (n)	Date of sampling	Salinity (psu)	Body weight (kg)	Total length (cm)	Otolith weight (g)
Oligohaline (0-5 psu) n=15	6	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
	20	Tanjung Agas, Pekan	6	22-Mar-08	0.1	1.30 \pm 0.27	47.80 \pm 3.55	0.130 \pm 0.020
	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 (6-18 psu) n=13	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
	5	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
	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
Polyhaline (19 - 29 psu) n=51	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	6	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
	17	Kg. Teluk Sengat, Kota Tinggi	6	20-Mar-08	22.9	0.62 \pm 0.11	35.48 \pm 1.76	0.066 \pm 0.006
	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
	9	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
	7	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
	24	Kg. Sg. Mengkabong, Tuaran	7	18-May-09	27.3	0.38 \pm 0.05	29.66 \pm 1.56	0.051 \pm 0.003
	Euhaline (30-35 psu) n=32	12	Pulau Kukup, Pontian	4	16-Nov-07	29.9	0.40 \pm 0.10	32.51 \pm 1.92
2		Kubang Badak, Langkawi Island	6	17-Jan-08	30	0.47 \pm 0.52	31.85 \pm 1.79	0.043 \pm 0.007
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
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
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
3		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	7	21-Apr-07	33.1	0.84 \pm 0.20	39.79 \pm 2.68	0.076 \pm 0.009

The unmarked locations were cages, whereas those marked with asterisks were fish ponds

The salinity of ambient waters at the sampling stations was recorded using the calibrated YSI 30 salinometer, where fish samples were collected and divided according to the thalassic series (Table 1). The specimens sampled were kept frozen for further analysis in the laboratory.

Briefly, the specimens were measured and weighed before being dissected for otolith extraction (Table 1). The extraction of otoliths was done by dissecting the fish head vertically parallel with the fish preopercle until the otolith is exposed. The sagitta otolith was removed from the head using clean forceps and was rinsed with deionised distilled water and dried at room temperature. All the glassware was soaked in 5% of HNO₃ 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). The otolith was weighed with an electronic balance with an accuracy of ± 0.01 mg (Table 1).

The weighed otolith was digested with 10mL of HNO₃ 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 the elements of Sr, Mg, Ba and Zn while Ca was determined using a Perkin Elmer AA 800 Atomic Absorption Spectrometer (AAS).

Ambient waters inhabited by cultured sea bass were sampled to obtain background information on the environment such as salinity and trace element values. Surface water samples were collected using 12 L of high-density polyethylene (HDPE) containers and acidified with concentrated HNO₃ to pH <3. In the laboratory, water samples were filtered using a 0.45 µm WHATMAN membrane filter to remove suspended solids. Three replicates of 250 mL filtered water samples were used to extract trace elements such as Mg, Ba and Cd using the metal complexation of ammonium pyrrolidinedithiocarbamate (APDC) and were continuously extracted using the methyl isobutyl ketone (MIBK) method

(Brooks *et al.*, 1967; Jan and Young, 1978; Hirata *et al.*, 2003). The extracted trace elements were diluted then detected. The Perkin Elmer AA800 Flame Atomic Absorption Spectrometer (AAS) was used to detect Ca and Mg while, Graphite-Furnace Atomic Absorption Spectrometer (GF-AAS) was used to detect Ba and Cd. As for Sr and Zn, the water samples were diluted (1:10) and then detected directly with ICP-MS. All the glasswares used were soaked in 5% of HNO₃ overnight prior to analysis.

Replicates of Certified Reference Material No. 22 (Otolith, NIES, Japan) were also prepared using the same procedure as otolith samples for an analytical procedure validation (Table 2). For a seawater standard, a multi-element standard was spiked into artificial seawater and purified using the same procedure with 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 a calibration curve (r>0.90) and method validation.

Statistical analysis and calculation

One way ANOVA was used to determine the significance of elements/Ca ratio with the salinity series. The relationship between the salinity of ambient water with elements/Ca ratios in otoliths and ambient water were quantified using Pearson's partial correlations analysis. The Post hoc Tukey's test was performed to obtain differences between salinity classes. All the statistical analyses were computed using the SPSS 16.0 version software. Partition coefficients (D_{Me}) of elements were calculated using equation 1 where ME is the average concentration for the elements (Bath *et al.*, 2000; Lin *et al.*, 2007). D_{Me} were calculated to discriminate the uptake value of incorporated waterborne elements incorporated during sea bass lifetime. The greater value of D_{Me} (D_{Me}>1) showed the efficient uptake level meanwhile lower value of D_{Me} (D_{Me}<1) estimated the inefficient uptake level.

$$D_{Me} = \frac{(\text{Me/Ca}) \text{ otolith}}{(\text{Me/Ca}) \text{ ambient water}} \quad (1)$$

Table 2. The analyzed value of triplicates of CRM N0.22 – Fish Otolith (NIES) and the recovery (%) based on certificate and reference values

Element	Analysed Value	Certificate/Reference Value*	Recovery Value%
Ca	37.49 ± 3.75%	38.8 ± 0.5%	96.62 ± 9.67
Sr	2.19 ± 1.3 mg g ⁻¹	2.36 ± 0.05 mg g ⁻¹	92.75 ± 5.49
Mg	18.40 ± 0.06 µg g ⁻¹	21 ± 1 µg g ⁻¹	87.63 ± 0.28
Ba	2.76 ± 0.24 µg g ⁻¹	2.89 ± 0.08 µg g ⁻¹	95.51 ± 8.25
Zn	0.71 ± 0.06 µg g ⁻¹	0.74 µg g ⁻¹ *	96.15 ± 8.36

*reference value

RESULTS AND DISCUSSION

Relationship of elements/Ca ratios in otoliths and water with salinity

The salinity of ambient water at sampling stations ranged from the oligohaline to euhaline series (Por, 1972). Sampling stations classified as polyhaline (19-30 psu) dominated the sampling locations follows by euhaline (31-35 psu), oligohaline (0.1-5 psu), mesohaline (6-18 psu) and lastly polyhaline (0.1-5 psu) stations as shown in Table 1. The variety in salinity of ambient water around the fish cages indicate that adult sea bass can adapt to a wide range of salinity fluctuations (Table 1). The relationship of divalent elements/Ca ratios in otoliths in this study revealed the connection between Sr, Mg and Ba with salinity and these ratios in ambient water (Fig. 2). The incorporation of divalent elements onto otoliths was considered inevitable (Campana, 1999). The elements mimic calcium as they have similar atomic radius and are therefore incorporated onto the otolith. At the same time, the estimation of otolith uptake from the background environment was estimated using partition coefficient (Bath *et al.*, 2000; Milton *et*

al., 2008) and most favourable element that was chosen to be incorporate onto otolith composition from the ambient water (Fig. 3).

No exact trends were found for Mg/Ca ratios in otoliths and water with variations in salinity (Fig. 2b ANOVA $P > 0.05$). The ratio values of Mg/Ca in otoliths and water salinity however showed contradictory patterns with the highest in the oligohaline and euhaline series respectively. The high magnitude of Mg incorporation onto otolith formation was found in euhaline waters only. Thus, the Mg/Ca ratio in otolith is not applicable to determine water chemistry or salinity variations especially in low degrees of salinity (Sarimin *et al.*, 2009). Similar patterns were observed for Zn/Ca ratios in the otolith and water (Fig. 2d) suggesting that ratio values of Zn/Ca in the water column were not affecting the concentration of elements in the otolith. The enrichment of zinc in the water column and fish otoliths might be caused by anthropogenic activities close to sea bass rearing areas (Sarimin and Mohamed, 2012). This study revealed that the Zn/Ca ratio could not be used as a marker to trace sea bass movements but might be useful to salmonid species only (Limburg and Elfman, 2010). The

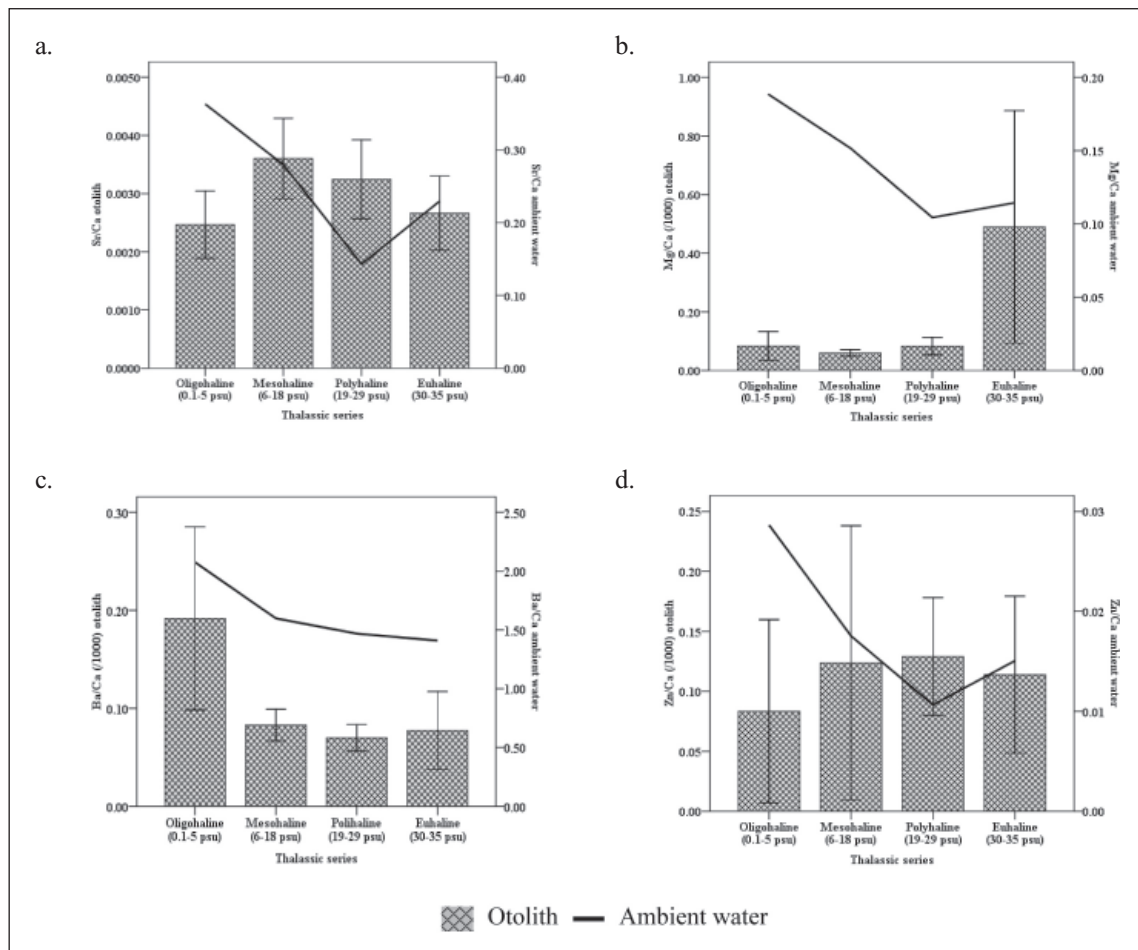


Fig. 2. Distribution element/Ca ratios according to thalassic series (mean \pm 95% confidence interval).

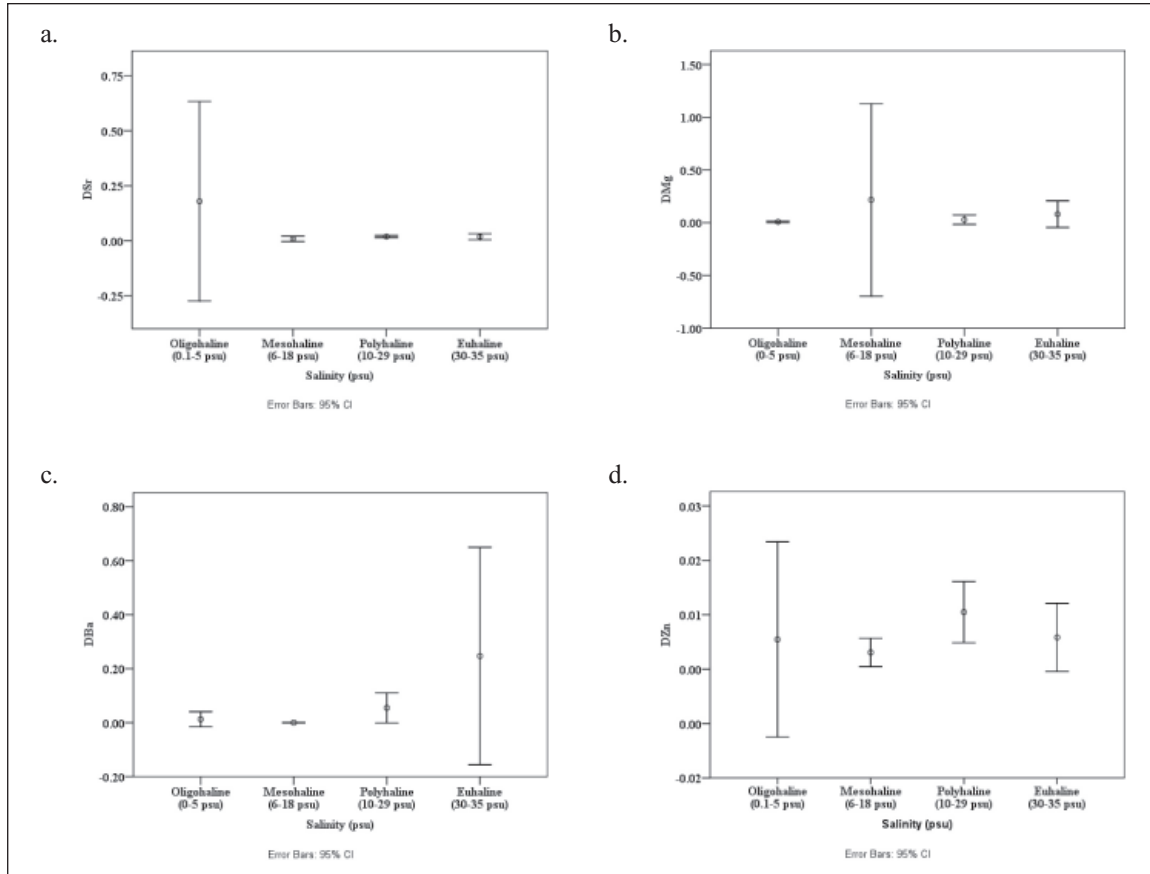


Fig. 3. Partition coefficient of Sr, Mg, Ba and Zn according to thalassic series (Mean \pm confidence interval).

Table 3. Distribution partition coefficient range, mean and the significance of the mean (ANOVA $p=0.05$)

D	Range	Mean \pm S.E.	ANOVA
Sr	0.004 – 0.38	0.04 \pm 0.02	$p<0.05$
Mg	0.0002 – 0.64	0.06 \pm 0.03	$p>0.05$
Ba	0.00001 – 0.65	0.08 \pm 0.04	$p>0.05$
Zn	0.000002 – 0.04	0.008 \pm 0.002	$p>0.05$

partition coefficient, D_{Mg} and D_{Ba} supports that the uptake of Mg and Zn was not according to salinity variations as no trends were observed (Fig. 3b & 3d, Table 3).

Fluctuations in Sr/Ca ratios were not gradual with increasing salinity values even though strontium was abundant at high degrees in saline water, especially in euhaline waters (Figure 2a; Tukey's Test $P>0.05$) (Farrell and Campana, 1996; Campana and Tzeng, 2000). However, the one way ANOVA test proved that the pattern was significant with classification of salinity variations ($p<0.05$). The ratio value of Sr/Ca in fish otoliths changed significantly with the oligohaline, mesohaline and polyhaline series (Tukey's Test, $P<0.05$) but was

insignificant in less saline waters (<29 psu). High value ratios of Sr/Ca show that Sr^{2+} has the potential to be incorporated onto otoliths even though it is in oligohaline waters (Fig. 2a). Nonetheless, no distinct patterns were observed between the Sr/Ca ratios in ambient waters with either salinity variations or Sr/Ca ratios in otoliths (Fig. 2a). Ambient water chemistry found that Sr was the highest followed by Mg, Ba and Zn.

Meanwhile, the ratio value of Ba/Ca in fish otoliths and the water column were revealed to be contradictory with salinity patterns (Fig. 2c, ANOVA $p<0.05$), where this ratio in the fish otolith significantly changes with the mesohaline, polyhaline and euhaline series (Tukey's Test $P<0.05$). Moderate inverse correlations were found between Ba/Ca ratio and the thalassic series ($r=-0.48$, $p<0.05$). Consequently, variation patterns of Ba/Ca in otoliths suggest that barium uptake from the water was consistent with salinity. Previous researchers have also demonstrated that the incorporation of barium onto otoliths was related to magnesium (Kitano *et al.*, 1979; Pingitore, 1986; de Vries *et al.*, 2005) but this result was not obtained in this study. A strong relationship between Ba/Ca and Sr/Ca ratios was revealed by the preference of elements to

be incorporated onto otoliths (Fig. 3a & 3c). The uptake of Sr and Ba was incoherent with the thalassic series with strontium displaying a decreasing pattern towards saline waters despite the barium trend (Fig. 3a & 3c). This indicates that Ba/Ca ratios in the otolith will act as a tracer of fluctuations in salinity values especially in the oligohaline water series (Bath *et al.*, 2000; Milton *et al.*, 2000; de Vries *et al.*, 2005)(Fig. 3b). Barium content was also abundant in less saline waters because it is related to terrestrial non-anthropogenic and spatial anthropogenic inputs (Choudhury and Cary, 2001). Furthermore, sea bass otoliths are preferred to barium than magnesium because of its similarity to calcium atomic radius (Milton and Chenery, 2001). The domination of D_{Ba} and D_{Sr} in euhaline and oligohaline water series suggest that these elements were efficiently deposited onto otoliths (Fig. 4). The optimal uptake was estimated even though the content of barium and strontium was high in oligohaline and euhaline respectively. However, that the uptake was not consistent and dominated by magnesium in mesohaline suggests that barium content is affected by magnesium uptake.

On the other hand, interpretations of the ability of Sr/Ca and Ba/Ca to determine the influence of salinity can only be achieved under natural environments with insignificant contamination of barium and strontium from anthropogenic sources (Campana, 1999). This study suggests that ratio values of Sr/Ca and Ba/Ca in whole otoliths can discriminate the influence of salinity in ambient water inhabited by the fish during their entire lifespan (Sarimin and Mohamed, 2012). The Sr/Ca ratio in water trends was not as significant as Ba/Ca ratios. In this study, the Ba/Ca ratio in otoliths was found to be a better indicator in cultured sea bass otoliths and best demonstrated on wild sea bass movements and supported by the highest D_{Ba} mean value.

Simulation of wild sea bass movement

Wild sea bass is often found in small numbers in several estuarine and littoral areas off Malaysian coastal waters during the breeding season. This protandrous hermaphrodite fish takes years to complete its migration cycle from freshwater to seawater (Grey, 1987; Schipp, 1991). Conventional and updated tagging techniques are time consuming and involve high costs (Elsdon and Gillanders, 2003a). Due to a decrease in population, it is important to track sea bass movement in order to obtain useful data about the nursery grounds of this fish and its natal estuaries. The simulation of wild sea bass demonstrated by Ba/Ca ratios in cultured otoliths was reconstruct by eliminating elements that show weak correlations with ambient water chemistry and salinity series (Fig. 5). Thus, this simulation estimated that Ba/Ca ratios in otoliths of oligohaline and euhaline waters would be greater than 0.006 and 0.002 respectively. This means that

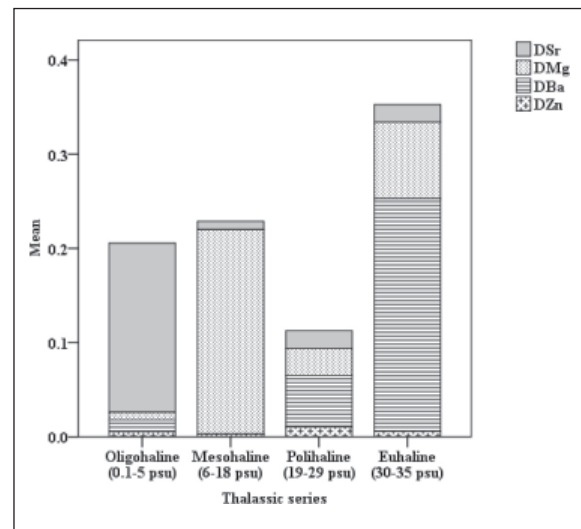


Fig. 4. The partition coefficient (D) of Sr, Mg, Ba and Zn according to thalassic series.

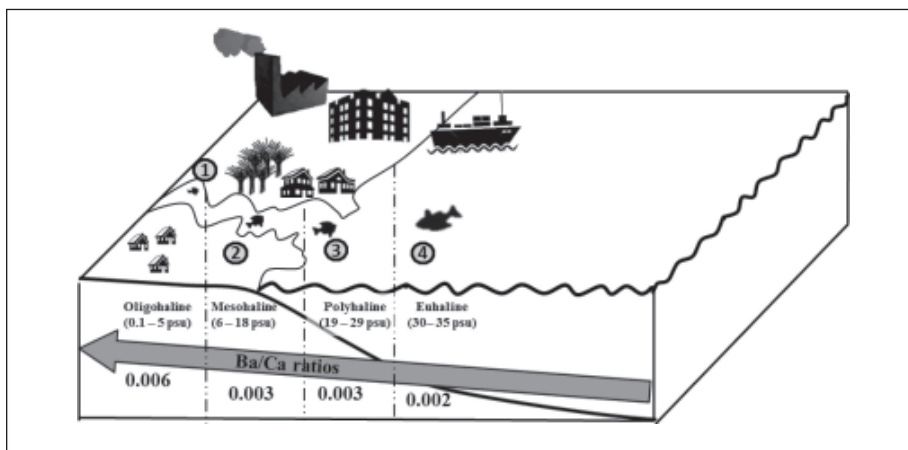


Fig. 5. Simulation movement of wild sea bass based on the Ba/Ca ratios in Malaysian waters.

the Ba/Ca ratios in freshwater is three times higher than seawater. However, the discrimination of mesohaline and polyhaline waters were found to be similar and this suggests that Ba/Ca in the otoliths of sea bass would be greater than 0.003, where the juvenile sea bass is the riverward movement as shown by increasing Ba/Ca ratios. Meanwhile, mature male fish that move towards the sea show decreasing Ba/Ca ratios. The adult fish that inhabit coastal zones such as littoral, mangrove areas and estuaries show Ba/Ca ratios ranging from low to moderate. Thus, the application of the Ba/Ca ratio to wild sea bass that are captured in future might reveal the nursery grounds and natal streams of the fish over their life cycle.

CONCLUSIONS

This research concluded that only Sr and Ba are significantly related to the salinity of ambient waters for the entire lifespan of sea bass in the study area compared to Mg and Zn. Sr/Ca ratios reflect saline water input while Ba/Ca ratios reflect changes in freshwater input throughout the thalassic salinity series. Ba/Ca ratios in whole otolith microchemistry could be used as a tool in inspecting the influence of salinity for the entire lifespan of cultured sea bass. Divalent elements which are Sr and especially Ba appear to show significant changes via salinity fluctuations and Ba/Ca ratio in ambient water. However, only Ba was considered as a tracer of freshwater input and Ba content in water. Furthermore, D_{Ba} also supports the proposition that Ba acts as a freshwater tracer and is effectively deposited onto otoliths in saline waters even though the Ba/Ca ratio in water is limited. The interpretation of Ba/Ca ratios in cultured sea bass in various salinity gradients might be applicable as an indicator for the migration patterns of wild sea bass in general. However, further study needs to be done to diversify the use of otoliths in solving myriad fisheries problems that might lead to the decreasing fish populations.

ACKNOWLEDGEMENTS

The authors would like express their highest gratitude to the National Oceanography Directorate for grant NOD/R&D/01, Ministry of Science, Technology and Innovation, Malaysia (MOSTI), for the scholarship and to UKM for the use of its facilities. Heartiest appreciation also goes to all the fish culturists and the Department of Fisheries (DOF) lab assistants, and the lab members of LAOI for their help during sampling and laboratory work.

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