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Otolith biometrics and their relationships with fish sizes of *Butis koilomatodon* living in Mekong Delta, Vietnam

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ABSTRACT

Otolith dimension and mass of *Butis koilomatodon* sampled along estuaries in Mekong Delta from January 2020 to December 2020 were used to test the similarity of left and right otolith in a pair, as well as the otolith variation between male and female monthly in four sampling locations. The left otolith was not different in size and mass from the right one. The otolith length and weight of males were greater than females but not the width. The highest otolith dimensions were recorded in the dry season, but the otolith weight was similar in both dry and wet seasons. Besides, otolith biometric and weight were also similar in four sampling sites. The otolith mass had relatively to fish body weight, demonstrating that otolith weight could be considered an indicator to predict the fish length, body height, head length, and weight.

INTRODUCTION

Fish otoliths, a crystal form of calcium carbonate **(Popper & Lu, 2000)**, are detected in the inner ear of fish **(Campana, 2004; Schulz-Mirbach *et al.*, 2010)**. Otoliths have been used for classification, age identification, growth rate, migrant behavior, and certain fish habitat **(Tsukamoto & Kajihara, 1987; Rodríguez Mendoza, 2006)**. Otolith is typical for species **(Popper *et al.*, 2005)**, and plays an important role in hearing and balancing as mechanoreceptor **(Popper *et al.*, 2005; Rodríguez Mendoza, 2006)**. In this study, both otolith morphometric and morphology of *Butis koilomatodon* were analyzed to understand deeply the role of otolith in the growth and development of this fish.

Butis koilomatodon (Bleeker, 1849) (Perciformes: Butidae) is one of three goby species of the genus *Butis* **(Froese & Pauly, 2021)**. The species *B. koilomatodon* dwells in a sand or mud–bottom and is a native of coastal regions and river mouth in the Indo-Pacific Ocean **(Dawson, 1973; Miller *et al.*, 1989; Contente *et al.*, 2016)**, including the Mekong Delta, Vietnam **(Tran *et al.*, 2013; Diep *et al.*, 2014; Tran *et al.*, 2020)**. The fish body size is moderate (3.19 cm to 5.78 cm SL) with five to seven dark bands on the lateral surface and caudal fin **(Yokoo *et al.*, 2006; Contente *et al.*, 2016)**. In Cambodian Mekong, crustaceans and small fish are main food items found in its stomach **(Rainboth, 1996)**. It can tolerate water salinity up to 37% together with the ability to adapt to the different cryptic benthic habitats **(Contente *et al.*, 2016)**. This species is also a carnivore in the Vietnamese Mekong Delta **(Dinh *et al.*, 2020b; Nguyen *et al.*, 2020)**. Its morphometrics, reproductive and population have been doccumented in this area as well **(Dinh *et al.*, 2020a; Lam & Dinh, 2020; Dinh *et al.*, 2021a)**. So far, the studies of morphology and morphometry of *B. koilomatodon* otoliths are insufficient and fragment. Consequently, both morphometry and morphology of otolith were studied in the current study. The use of the otolith dimensions is faster, less time, more simple equipment, and more manipulation than morphology **(Fossen *et al.*, 2003; Isermann *et al.*, 2003; Matic-Skoko *et al.*, 2011)**. Some study results showed a regression correlation of fish body size with otolith dimensions and weight **(See *et al.*, 2016; Maciel *et al.*, 2019; Nguyen & Dinh, 2020)**. Therefore, the study aimed to investigate the relationship between fish sizes and otolith morphometric traits of *B. koilomatodon.*

MATERIALS AND METHODS

Study sites and fish collection

The *B. koilomatodon* specimens were collected consecutively from January 2020 to December 2020 from four sites along the coastal and estuarine regions in Mekong Delta; namely, Duyen Hai, Tra Vinh (DH, 9°41'18.6"N 106°30'35.8"E); Tran De, Soc Trang (TB, 9°29'26.8"N 106°11'58.5"E); Hoa Binh, Bac Lieu (HB, 9°12'24.8"N 105°42'54.9"E); and Dam Doi, Ca Mau (DD, 8°58'17.5"N 105°22'51.8"E). This set of sites had the immense mudflat edged with *Avicennia marna* (Forssk.) Vierh. and *Sonneratia caseolaris* (L.) A. Engl. The type of tide is semi-diurnal with two high and low tides equally every day. The typical climate is tropical monsoon. It is almost rarely raining in the dry season; on the contrary, rainfall intensity is high in the wet season (around 400mm monthly). The yearly temperature is ~27^{o}C **(Le *et al.*, 2006)**.

Fish samples of all sizes were taken randomly by bottom gill nets every month. The size of the mesh was 1.5 cm. The classification was taken place right in the field base on morphology following the description of **Tran *et al.* (2013)**. *B. koilomatodon* specimens were stored in 5% formalin to avoid being spoiled and then shipped to the lab. The other fishes were released back to the natural habitat.

Fish analysis

In the laboratory, the fish genders were identified by observing the external shape of the genital papilla: oval shape in female and triangle shape in male **(Dinh *et al.*, 2021a)**. The fish total length (TL), body height (BH), head length (HL), and body weight (W) were recorded (nearest 0.1 cm and 0.01g of precision). The otolith was weighed using a digital balance with an accuracy of 0.1 mg. A stereo microscope linked to the camera was used to shoot otolith images. The Motic Image Plus v2.0 software was then applied to measure the length and width ((m) of the otoliths based on those images.

Data analysis

The changes between left otolith (O_L) and right otolith (O_R) in length, width, and weight were tested by T-test **(Matic-Skoko *et al.*, 2011)**. If the weight of O_L did not differ from O_R, the weight of O_R (WO) would be used to examine the relationships among WO and fish measurements by the following linear formulas: WO = a×TL + b and WO = a×W + b; WO = a×HL + b; WO = a×BH + b, where WO is the weight of otolith, TL is fish total length, W is fish body weight, HL is fish head length, BH is fish body height, a and b are the constant coefficients. T-test was also used to quantify the otolith variation in weight (WO), length (OL), and width (OW) according to genders and seasons. To test whether WO, OL, and OW varied amongst sampling sites, One-way ANOVA was performed. All analyses were performed using the SPSS .v21 software at the level significance $p < 0.05$.

RESULTS

Otolith morphology

The shape of *B. koilomatodon* otolith is shown in Figs (1, 2). The left otolith is quite symmetrical with the right one. The length is greater than the width in each one. The anterior apex is pointed, and the posterior side is serrated. The upper side is slightly flat, but the lower protrudes. The inner surface of otolith (inward fish body) is rough, while the outer surface is smooth.

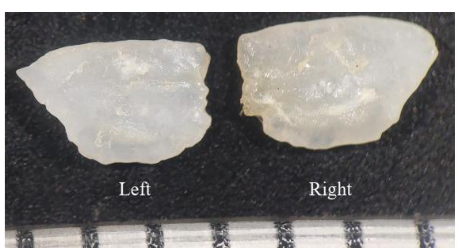

Fig. 1: Left and right otoliths of *Buits koilomatodon*.

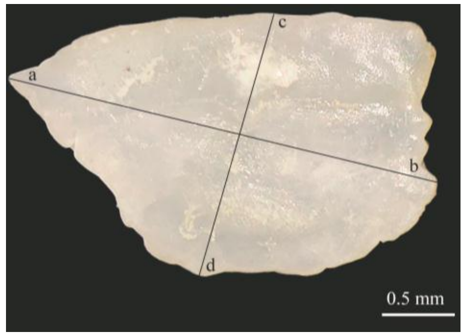

Fig. 2: Dimension measurement of left otolith of *Buits koilomatodon* (ab: otolith length, cd: otolith width, e: otolith core).

or width between left and right (t-test, t_{OL}=0.02, t_{OW}=-0.35, p>0.05, Table 2). Similarly, the **Differences in otoliths morphometries**

The total length, body weight of fish, and otolith dimensions are shown in Table (1).

The left and right otoliths of 1,007 *B. koilomatodon* individuals (271 females and 736 males) were measured and weighed. The comparison results of OL, OW, and WO between left and right otoliths are presented in Table (2). There was no statistically significant difference in otolith length left otolith's weight was not different from the right (t_{WO}=0.97, p>0.05). Since the left otolith's measurement and weight were similar to the right, the right otolith's values would be used for the following analysis.

Table (3) exhibits the biometric data taken from the right otolith of 1007 *B. koilomatodon* males and females in two seasons at four sampling sites. The otolith length of females was larger than males (t-test, t_{length}=1.38, p<0.01, Table 3), but their otolith widths were not significantly different (t_{widh}=1.58, p=0.09). Besides, the mass of otoliths in males was heavier than in females (t_{weight}=-4.37 p=0.04). In the dry season, otolith morphometries were greater than those in the wet season (t_{length}=5.37, t_{width}=5.62, p<0.01 for all cases) as shown in Table (3). However, the otolith weight in both dry and wet seasons was similar (t_{weight}=4.06, p=0.43). Similarly, the otolith biometric and mass differences were not found at the four sampling sites (One-way ANOVA, F_{lenght}=0.42, F_{width}=0.60, F_{weight}=0.96, all p>0.01) as presented in Table (3).

Table 1: Range of the measurements observed in body size and otolith of *Butis koilomatodon* in sampling sites.

Site	No		Total length (cm)		Weight (g)		Left OL (μm)		Right OL (μm)		Left OW (μm)		Right OW (μm)		Left WO (mg)		Right WO (mg)	
	Female	Male	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
DH	44	214	4.41	10.40	1.00	16.70	1620.78	3919.19	1931.75	3919.19	1466.13	3111.02	1684.05	3111.02	0.55	6.15	0.88	6.15
TD	70	132	4.20	10.91	1.04	18.37	1564.11	4202.73	1752.15	3967.90	1395.33	3234.84	1527.49	3252.82	0.60	6.95	0.80	7.17
HB	79	211	4.80	10.48	2.07	15.03	2147.12	4028.54	2180.00	3980.03	1761.39	3111.02	1838.56	3111.02	1.37	5.90	1.12	6.19
DD	78	179	4.33	10.29	1.87	14.95	1905.30	3990.46	1985.91	3990.46	1591.92	3153.77	1648.57	3153.77	1.11	6.37	1.20	6.37

(OL: otolith length; OW: otolith width; WO: weight of otolith; DH: Duyen Hai, Tra Vinh; TD: Tran De, Soc Trang; HB: Hoa Binh, Bac Lieu; DD: Dam Doi, Ca Mau)

Table 2: The length, width, and weight of the left and right otoliths.

Comparison	No. of fish	Mean	Standard error of mean	t-value	df	*P*-value
Left otolith length	1007	3122.83	10.7	0.02	1006	0.99
Right otolith length	1007	3122.74	10.30			
Left otolith width	1007	2478.35	8.47	-0.35	1006	0.73
Right otolith width	1007	2479.93	8.20			
Left otolith weight	1007	3.12	0.03	0.97	1006	0.33
Right otolith weight	1007	3.11	0.03			

Table 2: The variation of length, width, and weight of right otoliths according to genders, seasons, and sampling sites.

Category		No. of fish	Otolith length (μm)	Otolith width (μm)	Otolith weight (mg)
			Mean±SE	Mean±SE	Mean±SE
Genders	Female	271	3147.71±21.88	2501.20±17.03	2.91±0.05
	Male	736	3113.54±11.56	2472.10±9.29	3.19±0.03
t-test	*T*		1.38	1.58	-4.37
	P		0.02	0.09	0.04
Seasons	Dry	407	3184.17±11.94	2531.24±9.69	3.26±0.05
	Wet	600	3081.07±15.05	2445.13±11.88	3.02±0.04
t-test	*T*		5.37	5.62	4.06
	p		<0.01	<0.01	0.43
Sampling sites	Duyen Hai, Tra Vinh	258	3142.20±21.55	2497.66±16.87	3.09±0.06
	Tran De, Soc Trang	202	3119.79±25.91	2480.28±20.58	3.06±0.06
	Hoa Binh, Bac Lieu	290	3114.75±16.69	2470.02±13.73	3.19±0.05
	Dam Doi, Ca Mau	257	3114.54±19.88	2473.04±15.64	3.09±0.61
One-way ANOVA	*F*		0.42	0.60	0.96
	p		0.74	0.62	0.41

Fish size and otolith biometric relationships

Figs. (3, 4, 5& 6) illustrate the relationship between otolith mass and fish size (total length, body height and head length, respectively). Linear regression analysis of the otolith weight with total length, body height, head length and fish body weight indicated statistically significant and average correlations between them (r^2_{TL}= 0.50, r^2_{BH}=0.44, r^2_{HL}=0.46 and r^2_{W}= 0.57) (Figs. 3, 4, 5& 6). The mass of fish (r^2=0.57) showed a closer relationship with otolith weight (Fig. 6) compared to fish dimensions such as total length (Fig. 3), body height (Fig. 4) and head length (Fig. 5). WO=0.62TL-1.37 was the regression equation between otolith weight and the fish total length, whereas WO=0.30W+1.38 was the linear formula between otolith weight and fish body weight. WO=2.26BH-0.05 was the regression equation between otolith weight and the fish body height, and WO=2.23HL-1.02 was the linear formula between otolith weight and fish head length. Thus, the otolith increased mass as the fish raised in morphometries such as total length, body height, head length and body weight.

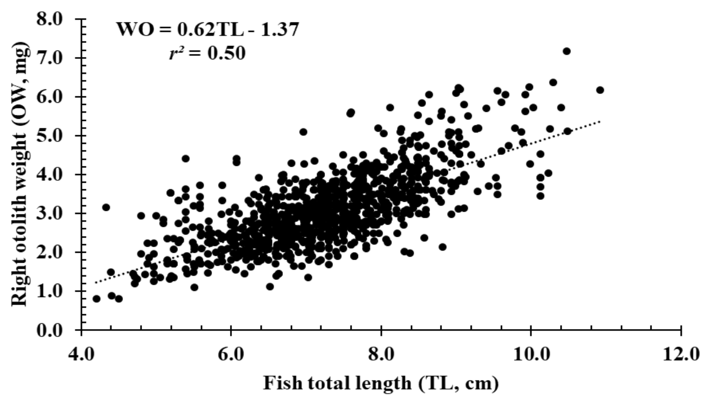

Fig. 3 The linear regressions between right otolith weight and total length (TL) of *B. koilomatodon*

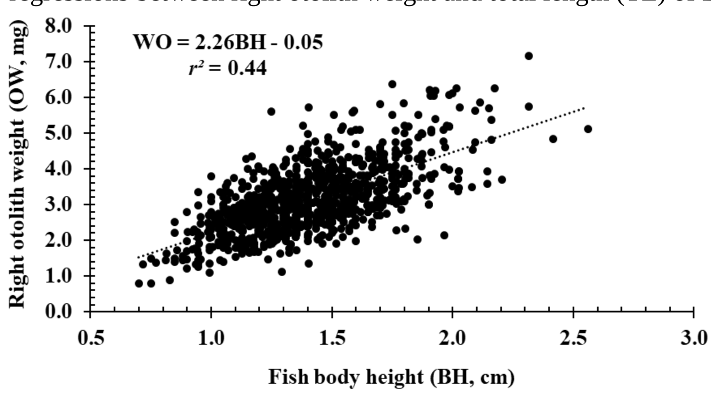

Fig. 4: The linear regressions between right otolith weight and body height (BH) of *B. koilomatodon.*

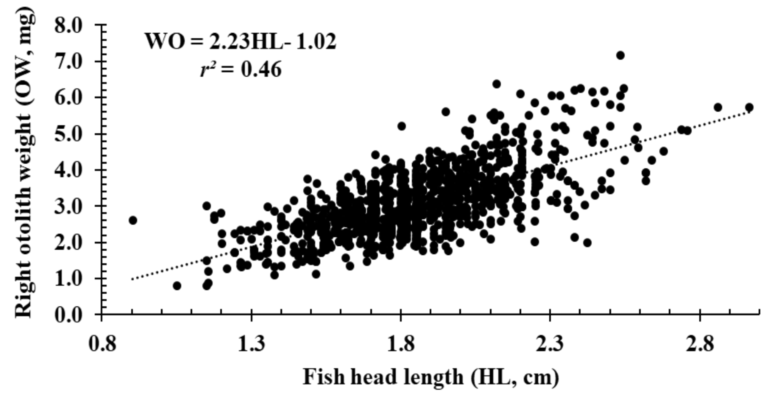

Fig. 5: The linear regressions between right otolith weight and head length (HL) of *B. koilomatodon*.

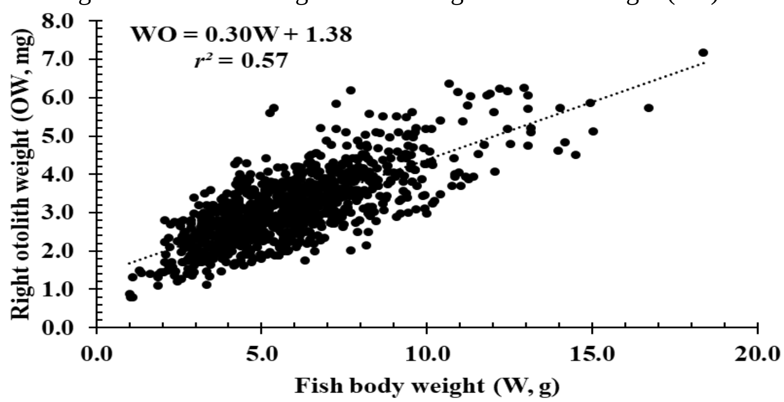

Fig. 6: The linear regressions between right otolith weight and fish body weight of *B. koilomatodon*.

DISCUSSION

Otolith dimension and mass investigation aimed to understand whether there were significant differences within the pair of all *B. koilomatodon* specimens. The present results showed the left otolith resembled the right one closely. Similar findings were also found in *Parapocryptes serperaster* in Soc Trang, Mekong Delta **(Dinh *et al.*, 2015)**; *Glossogobius sparsipapillus* collected along the Mekong Delta coastline **(Nguyen & Dinh, 2020)**; *Periophthalmodon septemradiatus* **(Dinh *et al.*, 2021b)**. The otolith size and weight of *G. sparsipapillus* were similar between two left and right sides, males and females but varied amongst the sampling locations. The other fish, tiger bass (*Terapon jabua*) in Kalong estuary, Vietnam, also had no differences in otolith pair size **(Tran *et al.*, 2017)**. The study outcomes of **Bani *et al.* (2013)** on the otolith morphology of three different gobies: the Caspian goby *Neogobius caspius* (Eichwald 1831), the deepwater goby *Ponticola bathybius* (Kessler 1877) and the bighead goby *Ponticola gorlap* (Iljin, 1949) in Talebabad, Anzali coast, Guilan, Iran also validated the size, shape and mass of the left sagittal otoliths were symmetrical to the right one in the three species. However, some of fishes such as *Pagrus auratus* and *Platycephalus* in south- eastern Australia **(Hamer and Jenkins, 2007)**;

Chlorurus sordidus and *Hipposcarus harid* in the Red Sea coast of Egypt **(Mehanna *et al.*, 2016)** showed the size differences between left and right otoliths.

Because of correlating together, the otolith morphometry has usually been used for predictions about total length, body weight, and age of fish **(Francis & Campana, 2004; Ilkyaz *et al.*, 2011)**. In some cases, nevertheless, the otolith weight could be better than the length as an indicator of fish growth because the otolith length stops increasing when total fish length reaches up to the maximum **(Jawad *et al.*, 2011)**. Consequently, the otolith mass was chosen for regression analysis. In the present research, otolith's weight had the average correlations (r^2 around 0.5) with fish total length, body height, head length and body weight. This clued two critical implications: the otolith mass had a regression relationship with the fish body size and weight and could be used to predict morphometries and weight of fish. This regression relationship was also found in *Parapocryptes serperaster* in the Mekong Delta (r^2>0.6) **(Dinh *et al.*, 2015)**; *Glosogobius sparsipapillus* in Mekong Delta (r^2>0.6) **(Nguyen & Dinh, 2020)**; tiger bass (*Terapon jabua*) in Kalong estuary, Viet Nam (r^2>0.7) **(Tran *et al.*, 2017)**. The findings of **Santana *et al.* (2018)** about *Prochilodus lineatus* also indicated the fish's measurements could be predicted from otolith size. Moreover, **Bani *et al.* (2013)** showed the linear relationship between somatic mass and otolith weight in the three goby species; namely, *P. bathybius* (r^2=0.3), *P. gorlap* (r^2= 0.5), and *N. caspius* (r^2 = 0·3). This clued that there was about 50% otolith weight of *P. gorlap* changed corresponded to somatic mass, while there was 30% in two remain gobies. *Rastrelliger* kanagurta, collected from the Oman Sea, exhibited the strong relationship between total length of fish and otolith dimension **(Jawad *et al.*, 2011)**.

Kontaş and Bostancı (2015) stated that the changes in the relationship between the otolith and fish size could correspond to the growth somatically between genders. In some fish species, the male could be larger than the female because it is responsible for constructing and defending the nest like *Gobiusculus lavescens* **(Houde, 2001)**. Thus, in the current research, the otolith weight of males was greater than females. Likewise, there was an asymmetry between otolith pairs of males and females of *Trachinus draco* from North Tunisia **(Fatnassi *et al.*, 2017)**. The opposite results were found in *Glosogobius sparsipapillus* in Mekong Delta **(Nguyen and Dinh, 2020)** and *Periophthalmodon septemradiatus* **(Dinh *et al.*, 2021b)**. Both of those fishes did not vary on the otolith size according to gender. Moreover, the differences in food availability and environmental conditions amongst sampling locations in dry and wet seasons could explain the variation in biometry and weight of *B. koilomatodon* otolith. These characteristics helped explain why the greater size of the otoliths was found in the dry season compared to the wet season. There was no variations about mass or biometric of otolith amongst four sampling sites in the present study. Contrary, **Nguyen and Dinh (2020)** reported that the otolith of *G. sparsipapillus* in the Mekong Delta exhibited a variation in size and weight and corresponded to different sampling sites. In the study of **Dinh *et al.* (2021b)**, the otolith dimension reached the highest value in the upstream of Bassac River in Binh Duc, Long

Xuyen, An Giang compared to Long Phu and Ke Sach, Soc Trang; Cai Rang and Thot Not, Can Tho.

CONCLUSION

This study showed the critical role of otolith morphometry and weight in finding out the total length, body height, head length and body weight of *B. koilomatodon*. The biometrics of the left otolith was similar to the right one in each pair. The genders and seasons, but not sampling sites, showed a marked impact on the otolith size and mass. Otolith morphometrics of *B. koilomatodon* could support taxonomy rather than as a main distinguishing feature though further research is recommended.

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