



Research article

Otolith morphometry and its role in determining the growth of *Periophthalmus chrysospilos* distributed in some coastal provinces in the Mekong Delta

Tran Thi Huyen Lam¹, Quang Minh Dinh^{2*}, Ton Huu Duc Nguyen²

¹ Cuu Long University, Phu Quoi Commune, Long Ho District, Vinh Long Province 890000, Vietnam

² Department of Biology, School of Education, Can Tho University, Xuan Khanh ward, Ninh Kieu district, Can Tho 900000, Vietnam

Abstract

This article first provided information on otolith shapes, the variation of otolith weight by sex, fish size, season, and sampling site, and the linear relationship between otolith weight and fish size. A total of 815 *Periophthalmus chrysospilos* specimens from Duyen Hai, Tra Vinh; Tran De, Soc Trang; Dong Hai, Bac Lieu; and Dam Doi, Ca Mau in the Mekong Delta ranged from 4.20 to 12.50 cm total length and from 1.26 to 16.88 g weight were analysed. The analysis results revealed that *Ps. chrysospilos* had a square otolith, deeply notched at the anterior apex and flat at the posterior apex. The weight of the left otolith showed a statistical difference according to survey factors, including gender, fish size, season, and sampling site. The otolith weight had a proportional relationship with to the total length, body weight, and head length of mudskippers ($r^2 = 0.7\sim0.9$), which meant that fish size could be predicted from the otolith weight and about 70-90% of the otolith weight of *Ps. chrysospilos* varied corresponded the growth of the fish.

Keywords: A proportional relationship, Mekong Delta, Otolith shapes, Otolith weight, *Periophthalmus chrysospilos*

***Corresponding author:** Quang Minh Dinh, Department of Biology, School of Education, Can Tho University, Xuan Khanh ward, Ninh Kieu district, Can Tho 900000, Vietnam. Email: dmquang@ctu.edu.vn

Funding: This work was funded by Cuu Long University for research funding for the period of 2022-2023 under the project entitled: "Otolith morphometry and its role in determining the growth of *Periophthalmus chrysospilos* distributed in some coastal provinces in the Mekong Delta"

Article history:
received manuscript: 15 June 2023
revised manuscript: 7 September 2023
accepted manuscript: 11 September 2023
published online: 2 October 2023

Academic editor: Nguyen Trong Ngu



INTRODUCTION

According to Jaafar and Murdy (2017), the genus *Periophthalmus* includes 19 species of amphibian fish, commonly known as “muddy skippers”. Their regular activities are foraging, defending their territory, pursuing mates, and maintaining burrows on exposed mudflats at low tide (Polgar and Crosa, 2009; Jaafar and Murdy, 2017). The commercialization of mudskippers as aquarium fish has skyrocketed over the years, with the strong growth of global trade in aquariums (Schäfer, 2005; Monks, 2006; Dang and Nguyen, 2009). Commercial fish largely depend on wild-caught, not captive-bred fish, especially mudskippers. In coastal Asian populations, fishes of the goby family, including mudskippers, are a daily source of protein. In coastal populations of Asia, mudskippers and other gobioid fish have long been popular foods supplementing protein in the daily diet (Clayton, 1993; Nguyen, 2000).

Tran et al. (2013) recorded three *Periophthalmus* species (*Ps. chrysospilos*, *Ps. gracilis* and *Ps. variabilis*), occurring in Vietnam. Among these three species, *Ps. chrysospilos* is more common and used for food and ornamental fish based on our surveys. At the river mouth in the Mekong Delta, *Ps. chrysospilos* is an opportunistic mesopredator with a food composition of 7 species, of which mainly *Acetes* spp., *Uca* spp., *Dolichoderus* sp. The main food of this mudskipper varies according to habitat and food source, indicating their adaptation to hunting prey in coastal areas (Dinh et al., 2021a). Mudskipper *Ps. chrysospilos* spawns all year round, highly concentrated in the wet season (July to October). According to Dinh et al. (2022a), the fecundity of the batch ranges from 2,614 to 23,465 eggs/female and is proportional to the fish's total length and body weight. *Ps. chrysospilos* has a positive allometric growth, i.e., fish grows in weight faster than length because the b-value is greater than 3, and is well adapted to the habitat due to its CF value being approximately the well-being value of one (Dinh et al., 2022b).

Throughout the fish's life, otoliths develop continuously, and their shape expands from a simple shape in immature to a very complex lobe pattern as they grow older. The cross-section of the otolith has growth rings similar to the growth rings in plants (Arneri et al., 2002). The otoliths are a sensory organ located in the fish's head that provides information about orientation, movement, and sound vibrations. Sagitta (the largest), lapillus, and asteriscus are the three common types of ear stones found in fish. The otoliths are composed mainly of calcium carbonate crystals - usually in the form of aragonite, but sometimes vaterite, combined with a proteinous matrix. Calcium carbonate crystals (in the form of aragonite or vaterite) and a protein substrate make up otoliths. Each fish species has a unique otolith size and shape. In general, the sagitta of benthonic species (e.g., snapper, grouper, croaker) is larger than that of pelagic species (e.g., mackerels, tunas, swordfish, marlins) (Proctor et al., 2021). In all bony fishes, lapillus and asteriscus are very small (Taştan and Sönmez, 2021). Several studies have shown that the size of fish bony structures has a strong and proportional relationship with the fish length, one of these structures is the otolith (Copp and Kovac, 2003). Due to species specificity, the size of the otolith plays a vital role in species identification studies (Bani et al., 2013; Kontaş and Bostancı, 2015), categorization (Campana, 2004), and palaeontology (Campana et al., 2015). Recent studies related to otoliths include: determining the size of otoliths (width, length, weight), the otolith size relationship with

fish length and determine the growth based on this relationship (Taştan and Sönmez, 2021). This relationship helps predict fish size from the otolith length that can be detect at archaeological sites and in predator stomachs (Echeverria, 1987). From this perspective, estimating fish size from otolith biometrics and subsequent school management studies is essential. The results of this study are a valuable step toward supplying the biological and ecological data of this fish in the Mekong Delta, which is key to informing population patterns as well as policy and management decisions.

MATERIALS AND METHODS

Sample collection

Pairs of otoliths from selected 815 mudskippers *Ps. chrysospilos* (399 females and 416 males) were examined to obtain the average shape and size of the otoliths. Fish samples were collected monthly by hand on the last three days from August 2022 to March 2023 in four coastal alluvial areas, including Duyen Hai, Tra Vinh (9°40'29.5 "N 106°34'49.5 "E; TV), Tran De, Soc Trang (9°26'19.7 "N 105°10'48.1 "E; ST), Dong Hai, Bac Lieu (9°05'50.5 "N 105°29'54.7 "E; CM) and Dam Doi, Ca Mau (8°58'10.4 "N 105°22'58.9 "E; CM) in the Mekong Delta (Figure 1). The specimens of *Ps. chrysospilos* were classified according to the description of Tran et al. (2013).

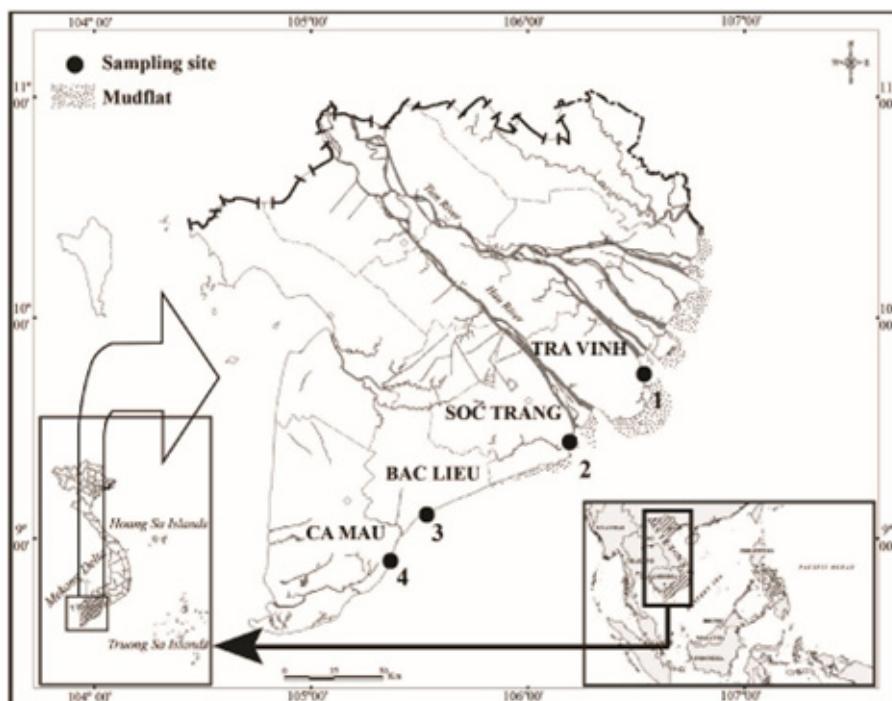


Figure 1 Map of sample collection at the Mekong Delta (1: Duyen Hai, Tra Vinh; 2: Tran De, Soc Trang; 3: Dong Hai, Bac lieu; and 4: Dam Doi, Ca Mau; Source: modified from Dinh (2018)).

The samples of *Ps. chrysospilos* were anesthetized with Tricaine methanesulfonate (10 g/L), preserved from spoil by 5% formal solution, and then brought the samples to the laboratory. The mud skippers *Ps. chrysospilos* were measured to the nearest 0.1 cm total length (TL), head length (HL), body depth (BD) to the nearest 0.1 cm, and body weight (W) to the nearest 0.1 g. Gender was determined by observing the shape of the genital spines. The genital papillae are narrow, wider at the bottom, and narrow towards the apex in males. On the contrary, the genital papillae are wide, and the base and tip of the papilla are of equal width in females. The quantity and handling of fish samples used in this experiment were evaluated and approved by The Council for Science and Education, School of Education, Can Tho University (Animal Welfare Assessment number: BQ2020-03/KSP).

Otolith morphometry

The head of the fish was cut off from the body, then cut lengthwise, and using forceps to pick the otoliths. Rinse the otoliths with distilled water, and let it dry naturally at room temperature for 15-20 minutes. The otoliths were weighed by an analytical balance with an error of 0.1 mg. The images of otoliths were magnified 100 times and taken with a microscope connected to the camera. The otolith length and width were measured on images taken with Motic Image Plus software v.2.0. The longest axis was the otolith length, the width was the line perpendicular to the length of the otolith was the longest axis, and the width was (Paladin et al., 2023). The otolith morphology was described using the terminology proposed by Tusset et al. (2008).

Statistical Analysis

Because of the regression relationship, the size of the otolith was used to predict the fish's total length and body weight, (Francis and Campana, 2004; Ilkyaz et al., 2011). However, according to Jawad et al. (2011), the otolith weight predicted growth better than length because otolith length did not increase further when total fish length was maximal. Therefore, in the scope of this study, the weight of otolith was used to analyze the regression analysis. The weight difference between left otolith (O_L) and right otolith (O_R) was tested by t-test (Matic-Skoko et al., 2011). If the difference was insignificant, the left otolith weight (WOL) was then used to analyze the regression relationship between the otolith weight and fish weight and size. The coefficient of determination (r^2) was used to evaluate the quality of the linear regression.

The t-test was also used to examine for variations in otolith weight by sex, fish size, and season. Meanwhile, One-way ANOVA was used to test the otolith weight difference between the four sampling points. SPSS v.21 software was applied to analyze the data with a statistical significance level of $p < 0.05$.

RESULTS

In total, 815 of *Ps. chrysospilos* individuals from four sampling sites in the Mekong Delta were analyzed. The total length range was from 4.20 to 12.50 cm, divided into five length classes (Figure 2), and the weight range was from 1.26 to 16.88 g. The fish specimens included 399 females (48.96%), and 416 males (51.04%). Regarding fish size, there were 224 immature fishes (27.48%) and 591 mature fishes (72.52%). The total length ranges of immature and mature fishes ranged from 4.20 to 8.50 cm and 6.30 to 12.50 cm, respectively. The weight of immature fishes ranged from 1.26 to 6.96 g, and the weight of mature fishes fluctuated from 1.86 to 16.88 g.

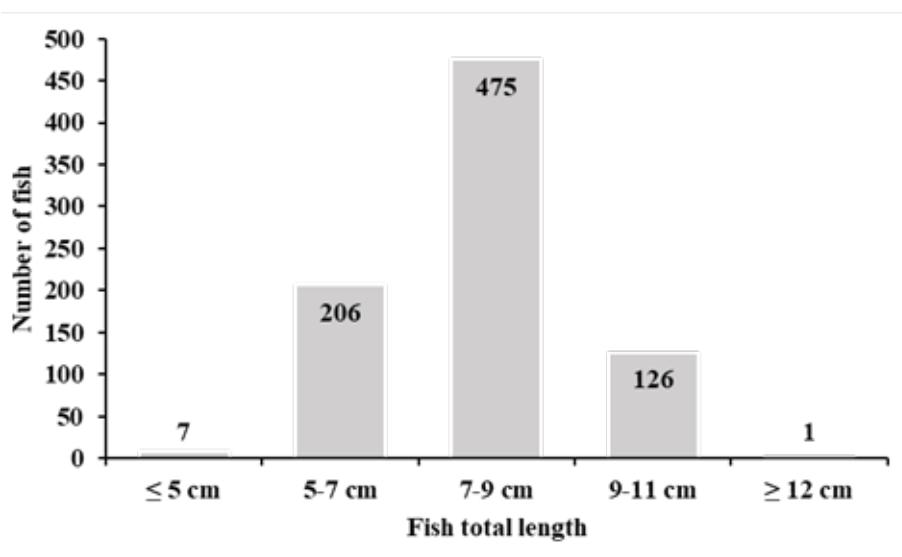


Figure 2 Five length classes of the total sample of *Ps. chrysospilos* (The number in each column is the number of fish).

Otolith shape

The otolith was squared-shaped, with notched edges with apparent changes in shape and size through the development stages (Figure 3). The shift in otolith shape was expressed through the change in otolith surface, otolith margin, bend angle and depth of sulcus on otolith surface, and otolith radius according to the increase in fish size corresponding to different stages of development.

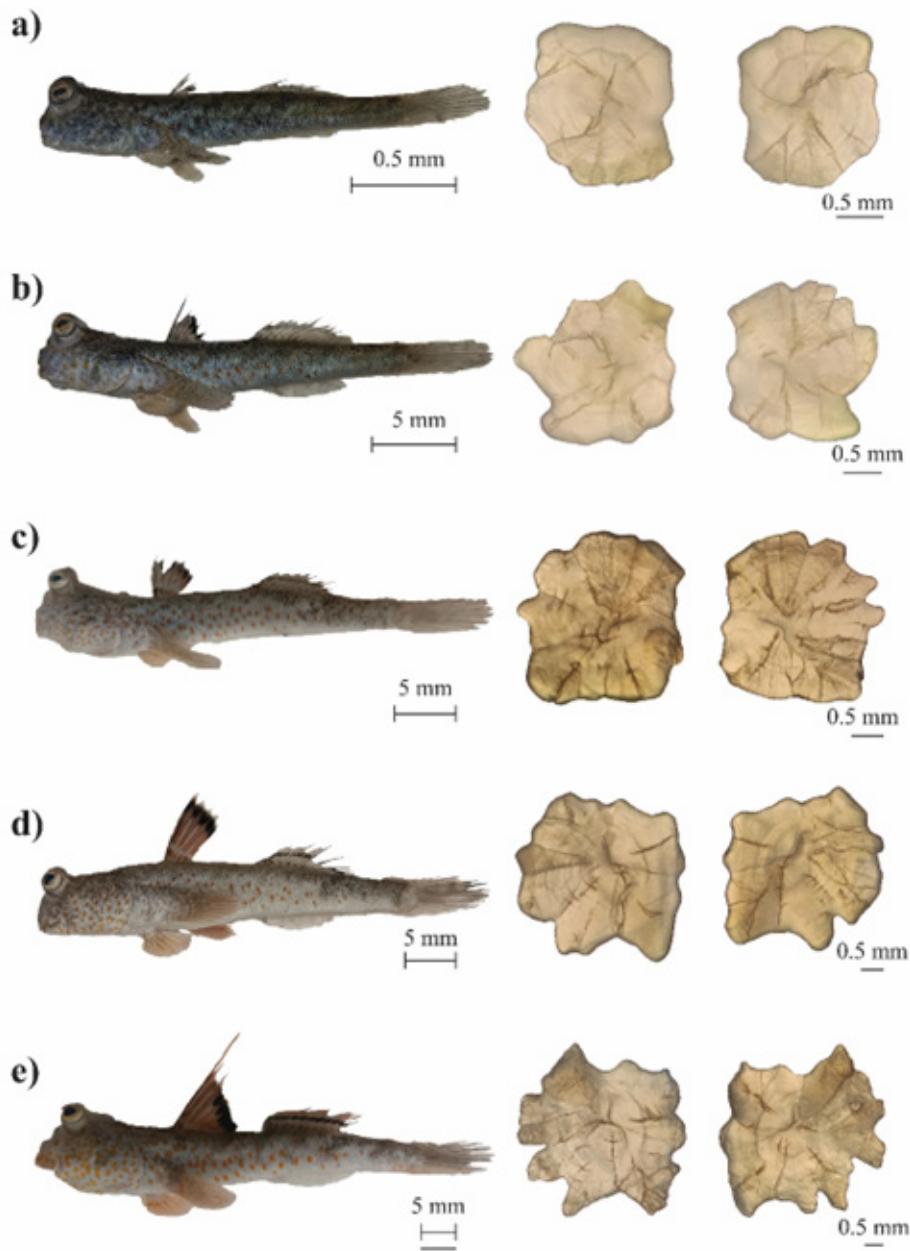


Figure 3 Morphological changes of otolith of *Ps. chrysospilos* in this study (a) ≤ 5 cm TL, b) 5-7 cm TL, c) 7-9 cm TL, d) 9-11 cm TL, e) ≥ 12 cm TL).

At sample sizes less than 5 cm TL and 5-7 cm TL (Figures 3a and 3b): Otolith was thin and clear, smooth surface; the margin of otolith on both ventral and dorsal surface was smooth at size ≤ 5 cm TL (Figure 3a) but began to notch when the fish reached to 5-7 cm TL in size (Figure 3b), shallow sulcus. The posterior apex is quite flattened; the anterior apex is notched. At this size, the rings on the otolith were visible (Figures 4a and 4b).

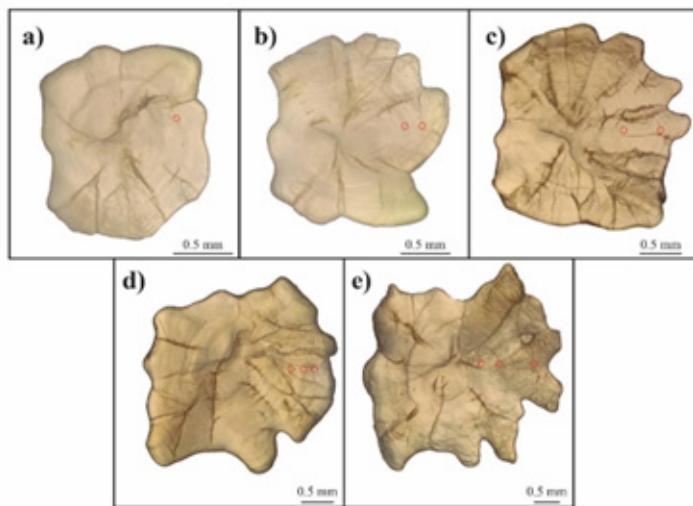


Figure 4 The rings of otolith of *Ps. chrysospilos* in this study (a) ≤ 5 cm TL, b) 5-7 cm TL, c) 7-9 cm TL, d) 9-11 cm TL, e) ≥ 12 cm TL.

At the size of 7-9 cm TL, 9-11 cm TL, and ≥ 12 cm TL (Figure 3c, 3d, and 3e): The otolith has a more complex shape: the surface is more rough, and the outer margin is thickened. The posterior apex is relatively flat at 7-9 cm TL but later shallowly notched at larger sizes. The larger the fish, the deeper and more pronounced the anterior apex is divided into distinct lobes. The bottom is slightly concave in the center; the upper surface is slightly convex. In these size ranges, the number of otolith rings increases with fish size (Figures 4c, 4d, and 4e).

Differences in otolith morphometries

Regarding weight, the left otolith weighed 1.20-3.70 mg (2.39 ± 0.01 SE), while the weight of the right one was 1.10-3.80 mg (2.33 ± 0.02 SE). Their mean values were not much different, so the left otolith weight was used to analyze for the next analysis.

The variation of WO_L according to gender, fish size, sampling season, and sites was expressed in Table 1. The weight of the female otolith was slightly heavier than that of the male, corresponding to the TL and W of the female (8.22 cm and 5.79 g) was also more significant than that of the male (4.47 cm and 4.24 g) (t-test, $t=8.55$, $p<0.01$) (Table 1).

Table 1 The variation of left otolith weight according to fish size, sampling season and sites.

Caterory		No. of fish	Fish total length (cm)	Fish weight (g)	Left otolith weight (mg)
Fish size	Immature	224	6.58	2.90	2.00±0.02 ^a
	Mature	591	8.31	5.80	2.53±0.01 ^b
t-test	<i>t</i>				-21.09
	<i>p</i>				<0.01
Season	Dry	308	8.09	5.52	2.45±0.02 ^b
	Wet	507	7.68	4.68	2.35±0.02 ^a
t-test	<i>t</i>				3.14
	<i>p</i>				<0.01
Sites	Duyen Hai, Tra Vinh	177	8.11	5.43	2.47±0.03 ^b
	Tran De, Soc Trang	264	7.52	4.47	2.28±0.02 ^a
	Dong Hai, Bac Lieu	169	8.01	5.32	2.44±0.04 ^b
	Dam Doi, Ca Mau	205	7.86	5.04	2.40±0.03 ^b
One-way ANOVA	<i>F</i>				9.04
	<i>p</i>				<0.01
Total		815	7.83	5.00	2.39±0.01

Because the mature fish was larger than the immature (shown by two values of TL and W), the otolith of the mature fish was also heavier ($t=-21.09, p<0.01$) (Table 1). In the dry season, mudskippers increased in size and weight better than in the wet season, so their otolith was also heavier ($t=3.14, p<0.01$). There was a difference in the otolith weight at four sampling points, the lowest value recorded at Tran De, Soc Trang (2.28±0.02), the remaining three sampling sites, the otolith weight was not statistically different (One-way ANOVA, $F=9.04, p<0.01$).

The relationship between otolith weight and fish size

The relationship between left otolith weight and fish morphometries (W: body weight; TL: total length; HL: head length; BH: body height; ED: eye diameter; MD: mouth distance) according to high values of the coefficient of determination was presented in Table 2.

Table 2 Parameters of the linear regression between left otolith weight (WO) and fish morphometrics.

Relationship	a	b	r ²
WO vs. TL	0.31	-0.06	0.87
WO vs. W	0.16	1.61	0.79
WO vs. HL	1.16	0.46	0.72
WO vs. BH	1.47	0.93	0.65
WO vs. ED	5.09	0.48	0.56
WO vs. MD	1.49	0.68	0.53

(W: body weight; TL: total length; HL: head length; BH: body height; ED: eye diameter; MD: mouth distance; a: intercept value; b: regression slope; r²: coefficient of determination)

The data are reasonably fitted with simple linear regression. Overall, otolith weight was more linear with total length, body weight, and head length ($r^2>0.7$) than body height, eye diameter, and mouth distance ($r^2=0.53-0.65$), suggesting that otolith growth was proportionate to the growth of fish in the study. All linear relationships were statistically significant ($p<0.05$ in all cases). A high r^2 value suggested that otolith weight provided a useful prediction of fish size.

The results of further analysis of the linear correlation between the otolith weight and the TL, W, and HL of mudskippers at four sampling sites are shown in [Figure 5a](#), [5b](#) and [5c](#), respectively. At a glance at [Figures 5](#), it can be seen that the otolith weight had the closest linear relationship with W, followed by TL and, finally, HL.

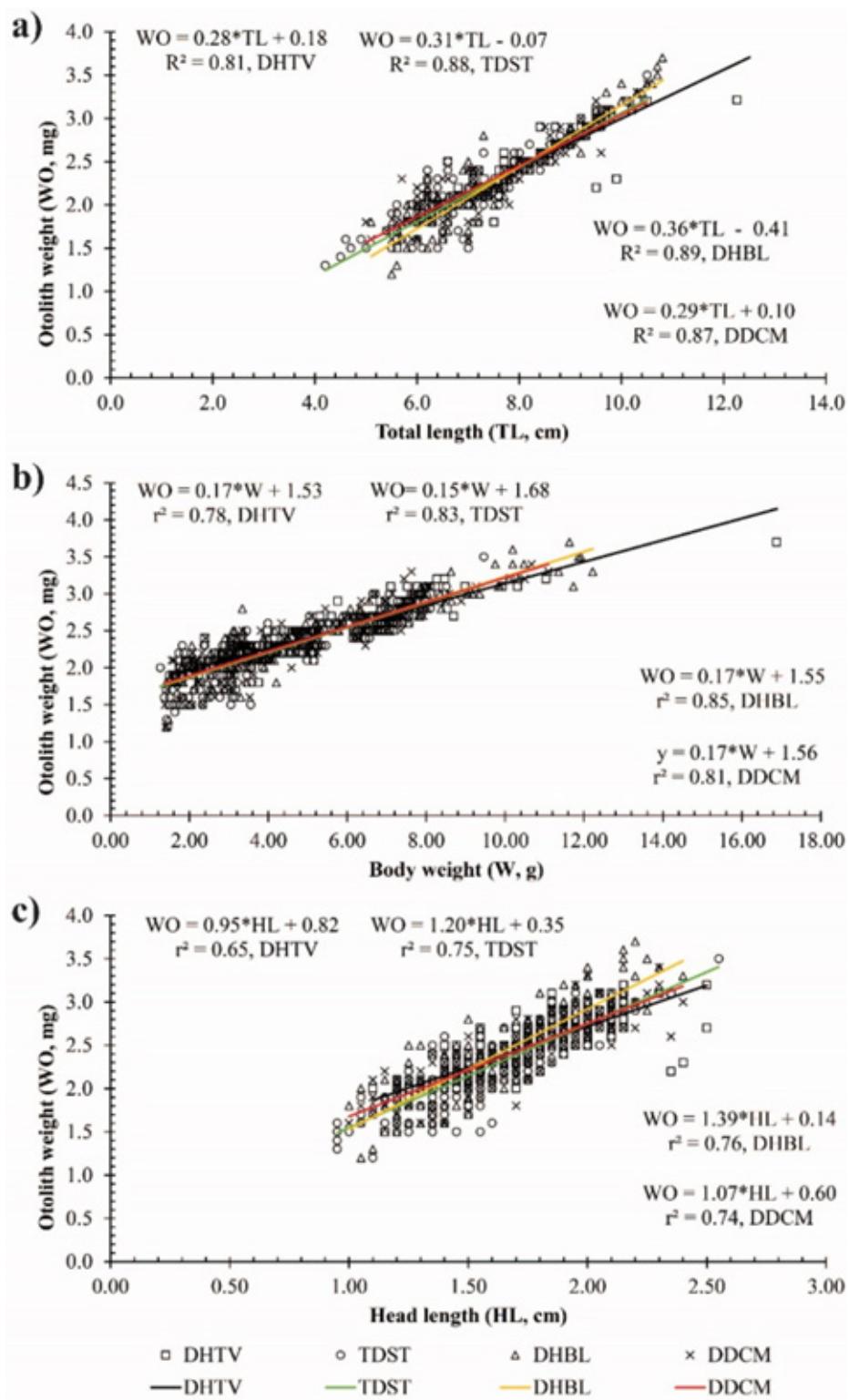


Figure 5 The relationships between otolith weight and fish total length (a), body weight (b), and head length (c) at four sampling sites (DHTV: Duyen Hai, Tra Vinh; TDST: Tran De, Soc Trang; DHBL: Dong Hai, Bac Lieu; DDCM: Dam Doi, Ca Mau).

The proportional correlation of otolith weight with TL, W, and HL in DHBL and TDST was stronger than in DDCM and DHTV, as shown by decreasing the r^2 value from 0.89 to 0.65. This meant that about 70%-90% of otolith weight increased in proportion to the growth of the fish. The graphs in Figure 4 again showed that the otolith weight was useful in predicting the TL, W, and HL of mudskippers.

DISCUSSION

The shape of otolith is very diverse with considerable variation intra and inter-species. However, this property is entirely subjective because there is no standard for classifying shapes, and the classification depends heavily on the researcher. The otolith's shape does not always match one of the identified typical shapes or any variation in otolith shape in a sample of a particular species (Tuset et al., 2008). In this study, the otolith of *Ps. chrysospilos* was square when immature and more clearly lobed in mature, the posterior apex was relatively flat, and the anterior apex was deeply notched. In other fishes, the otolith shape varied, such as triangular in *Scomberoides lysan*; ovate in *Caranx crysos*, *Lethrinus lentjan*, *Dicentrarchus punctatus*; rhomboidal in *Sardinella aurita*, *Barbonymus schwanenfeldii*; or pyriform in *Oreochromis niloticus* and *Tilapia zillii* (Alwany and Hassan, 2008). In the case, of *Pagellus bogaraveo* collected in the Adriatic Sea, the otolith was elongated to oval with irregular margins (Paladin et al., 2023).

Otolith measurements are vital for fish species and population identification studies because of their species specificity (Campana and Casselman, 1993). Statistically, the left otolith weight was heavier than the right in this study. Similar results were found in *Pagrus auratus* and *Platycephalus* from Australia (Hamer and Jenkins, 2007); *Chlorurus sordidus* and *Hipposcarus harid* originated from the Red Sea coast of Egypt (Mehanna et al., 2016). Inversely, the otolith weight of *Butis koilomatodon* from the Mekong Delta were similar between the left and right (Lam et al., 2021). *Glossogobius sparsipapillus* (Nguyen and Dinh, 2020), *Parapocryptes serperaster* (Dinh et al., 2015) and *Periophthalmodon septemradiatus* (Dinh et al., 2021b), all collected in the Mekong Delta, also exhibited similarity in the size of the pair of the otoliths. Bani et al. (2013) studied the otolith morphometry of three fish species: *Neogobius caspius* (Eichwald, 1831), *Ponticola bathybius* (Kessler, 1877), and *Ponticola gorlap* (Iljin, 1949) collected from Talebabad, Anzali coast, Guilan, Iran; the results showed that the left and right otolith were symmetrical in all three.

The weight of otolith can help to predict fish length or weight. However, estimating fish weight directly from otolith weight was sometimes inaccurate as it can vary with spawning conditions (Alwany and Hassan, 2008). According to Kontaş and Bostancı (2015), the relationship between otolith weight and fish size may change with growth between the sexes. In the study scope, the mudskipper female was more significant than the male, so the otolith weight of the female was also heavier. Pauly (2019) claimed that females generally got bigger and achieved larger sizes than males, although females had a higher reproductive output. The asymmetry of left-right otoliths was also found in some fish species, such as *Thunnus thynnus* collected in the

Mediterranean Sea (Megalofonou, 2006), *Pagrus auratus* and *Platycephalus* from south-eastern Australia (Hamer and Jenkins, 2007), three fish species of *Neogobius caspius*, *Ponticola bathybius* and *Ponticola gorlap* in Iran (Bani et al., 2013), and *Chlorurus sordidus* and *Hipposcarus harid* in Egypt (Mehanna et al., 2016). However, previous studies showed no difference about the size of otoliths between male and female fish. For example, the otoliths size of *Periophthalmodon septemradiatus* (Dinh et al., 2021b) and *Glosogobius sparsipapillus* in the Mekong Delta (Nguyen & Dinh, 2020) did not differ between male and female fish. Another example is several marine fish species in Brazil: *Ctenosciaena gracilicirrhus*, *Macrodon ancylodon*, *Menticirrhus americanus*, *Haemulon steindachneri*, *Pellona harroweri*, and *Polydactylus virginicus* were similar in morphometry between left and right otoliths (Oliveira et al., 2019). In the current study, the otolith weight at Tran De, Soc Trang was statistically lower than that of the other three sampling sites, possibly due to variations in food sources and habitat conditions. (Dinh et al., 2020). Similarly, the otolith of *G. sparsipapillus* in the Mekong Delta differs in size and weight at each sampling site (Nguyen and Dinh, 2020); among the samples of *Periophthalmodon septemradiatus* collected in An Giang, Soc Trang, and Can Tho, the highest otolith size was recorded in Long Xuyen, An Giang (Dinh et al., 2021b).

The otolith length was proportional to the fish's length until the fish reached its maximum size (Blacker, 1974), so the otolith weight was better than the length in predicting growth (Jawad et al., 2011). Hence, this research used the otolith weight to analyze the relationship with fish morphometry. The results showed that the otolith weight strongly correlated (r^2 about 0.7~0.9) with TL, W, and HL of *Ps. chrysospilos*. This result meant that otolith gain was proportional to fish size (TL and HL) and body weight, and the otolith weight could be used to predict fish morphometry and weight. A close relationship between otolith size and fish size has been recorded in many marine and freshwater fish species, including studies of Alwany and Hassan (2008) on 28 species from the Suez Canal and Gulf of Suez, especially the four species (*Tilapia zillii*, *Lethrinus lentjan*, *Liza carinata* and *Mugil cephalus*) ($r^2 > 0.8$); Paladin et al. (2023) on blackspot seabream *Pagellus bogaraveo* (Brunnich, 1768) in the Eastern Adriatic Sea ($r^2 > 0.85$); (Dinh et al., 2021b) on *Periophthalmodon septemradiatus* ($r^2 \geq 0.7$).

CONCLUSIONS

The otolith of *Ps. chrysospilos* was squared; the posterior apex was rather flat, and the anterior apex was distinctly notched. The left otolith was asymmetrical to the right one, and the otolith weight changed by sex, fish size, season, and sampling site. This outcomes reported the first figures on the proportional relationship between otolith weight and fish size in mudskippers *Ps. chrysospilos* illustrated by a linear model (r^2 about 0.7~0.9). This relationship helped predict fish size at different stages at different sampling sites from the weight of otoliths.

ACKNOWLEDGEMENTS

This was the result of the project “Otolith morphometry and its role in determining the growth of *Periophthalmus chrysospilos* distributed in some coastal provinces in the Mekong Delta,” funded by Cuu Long University for research funding for the period of 2022-2023.

AUTHOR CONTRIBUTIONS

Tran Thi Huyen Lam; Investigation, methodology, formal analysis, manuscript preparation

Quang Minh Dinh; Investigation, methodology, formal analysis, manuscript preparation

Ton Huu Duc Nguyen; Conceptualization and design the experiment, investigation, supervision, editing and finalization

CONFLICT OF INTEREST

We have no conflict of interest.

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How to cite this article;

Tran Thi Huyen Lam, Quang Minh Dinh, Ton Huu Duc Nguyen. Morphological and structural otolith of *Periophthalmus chrysospilos* distributed in some coastal provinces in the Mekong Delta. *Veterinary Integrative Sciences*. 2024; 22(1): 299 - 313
