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OTOLITH MORPHOLOGY DIVERSITY OF NINE SPECIES OF GOBIES (ACTINOPTERII: GOBIIFORMES) IN THE BA LAT ESTUARY, VIET NAM

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Abstract. Otoliths are located in the inner ear of bony fishes, which represent species-specific morphology. Thus, these structures could be used as an important trait in fish identification. However, little such information is available on gobies, one of the most diverse bony fish groups, which are commonly difficult to identify using external morphology. The present study provides information on the otolith morphometry of nine species of gobies (in three families, i.e., Eleotridae, Gobiidae, and Oxudercidae) caught in the Ba Lat estuary of the Red River in 2019. Otolith morphology of species in one family resembles, but it is clearly different between species in the same genus. Otolith length and weight changed proportionally to the fish's growth, but their shapes were not much changeable in one species. These results are valuable for further investigations into a taxonomy of gobies using otoliths and confirm the species-specific characteristics of this structure in fish identification.

Keywords: sagittal morphology, fish growth, identification, gobies, northern Vietnam.

1. Introduction

Otoliths are calcified structures in the inner ear of bony fishes [1], [2], which serve as an organ of hearing and balance [3]. The otolith includes 3 pairs: sagittae, lapilli, and asterisci, which are distinguished by morphology, measurement, and location in semicircular canals. Otolith shape and morphometrics are species-specific and useful tools for fish identification, age identification, growth rate determination, migration behavior, and habitat utilization [4], [5].

Ba Lat is one of the nine estuaries of the northern delta, which is considered to be an important inundation site for biodiversity conservation in the coastal region of the Red River Delta. This area is characterized by mangroves, owning the highest biodiversity that provides daily food for local people [6]. Fishery resources play an important role in serving as different provisioning services in the Ba Lat estuary [6]-[8]. Furthermore, gobies are dominant in this area, and many species have high economic values [9]-[12]. Identification of gobies based on external morphology is usually hard due to their indistinguishable ontogeny, especially for well-preserved specimens. Thus, utilizing internal structures which are still retained after fixation, like otolith, will provide more accurate characteristics in identifying this fish group.

In Vietnam, there are a few works on otolith morphology, focusing only on early stages, e.g., sand whiting *Sillago sihama* [13] and *Nuchequula nuchalis* in the Tien Yen estuary [14], Tiger bass (*Terapon jabua*) in Kalong estuary [15]. For adult fish, Tran et al. (2021b) described the morphology of two gizzard shads, *Clupanodon thrissa* and *Nematalosa nasus,* and examined otolith microchemistry to elucidate their life history [16]. Thus, understanding the morphology of goby species in the Ba Lat estuary is necessary, contributing to providing documents for goby species' identification in Vietnam since gobies resemble external morphology. The present study aims to elucidate the diversity of otolith morphology and its change with the growth in gobies at the study site.

2. Content

2.1. Materials and methods

A total of 142 pairs of sagittae of nine goby species caught in the Ba Lat estuary in 2019 by using a trap net were used in the present study (Table 1). Fish photos were taken to support identification, and fixed directly to 80% ethanol after collection in the field and preserved in this solution in the laboratory. Furthermore, formalin-preserved specimens of these species were used to identify them into the species level. Fish was identified based on morphology according to the following documents: Nakabo (2002), Nguyen (2005), Tran et. al. (2013), and Kimura et al. (2018) [17]-[21].

After removal, sagittae were cleaned in water and then placed under a magnifying glass for observation. Its length and breadth were measured along the longest axis using a Nikon binocular magnifier, and it was weighed using an analytical balance ENTRIS224I - 1S. Sagittae's photos were taken with a Pentax camera on the magnifying glass, then edited and redrawn outlines using Photoshop CS6 software. Otolith was fixed and preserved with 70^o alcohol. Otolith morphological description referred to Secor et al. (1992) and Lin & Chang (2012) [22], [23] (Figure 1). All photos of sagittae show the fish's total length (TL) and sagittal dimensions. In addition, to understand the relationship between fish size and its otolith, the present study applied a linear regression analysis using the left sagittae of four dominant species in the study site.

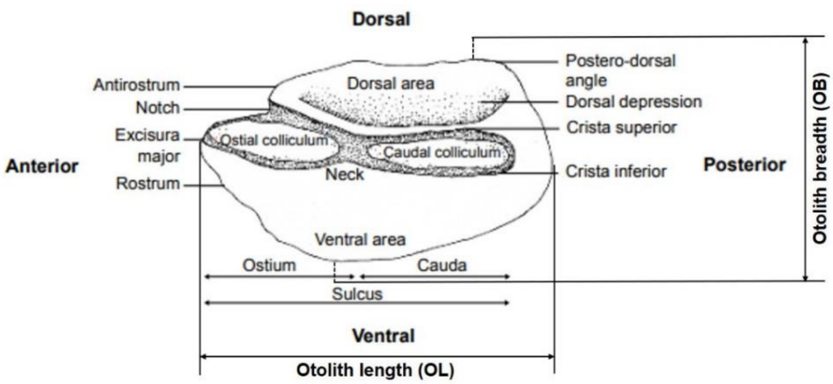

Figure 1. Sagitta structure and measurements in bony fish (Lin and Chang, 2012)

Table 1. Otolith data of gobies from the Ba Lat estuary, Vietnam was used in the present study

No.	**Family and species name**	**Fish total length (TL, mm)**	**Fish weight (W, mg)**	**No. of specimens**	**No. of otolith removed**	
					Left	***Right***
I	**Eleotridae**					
1	*Butis butis* (Hamilton, 1822)	93.8-124.5	5.90-17.34	5	5	5
2	*Butis koilomatodon* (Bleeker, 1849)	38.2-90.0	0.40-8.30	30	30	30
II	**Gobiidae**					
3	*Acentrogobius viridipunctatus* (Valenciennes, 1837)	72.4-95.9	3.31-6.76	5	5	5
4	*Aulopareia unicolor* (Valenciennes, 1837)	47.3-84.8	0.63-4.77	5	5	5
5	*Glossogobius giuris* (Hamilton, 1822)	70.3-119.2	1.56-9.61	30	30	30
6	*Glossogobius olivaceus* (Temminck & Schlegel, 1845)	54.7-103.8	1.12-8.66	30	30	29
7	*Gobiopsis macrostoma* (Steindachner, 1861)	60.8-83.5	1.36-4.34	5	5	5
III	**Oxudercidae**					
8	*Odontamblyopus rubicundus* (Keith, Hadiaty, Busson & Hubert, 2014)	159.1-204.1	3.37-4.34	2	2	2
9	*Tridentiger barbatus* (Günther, 1861)	47.2-76.9	0.92-4.39	30	30	30
	Total			142	142	141

2.2. Results and discussion

2.2.1. Otolith morphometrics diversity

The otolith morphology of nine species ranging from 76.9 to 159.1 mm TL is shown in Figure 2. In the *Butis* genus, the otolith of *B. butis* was white and transparent (Figure 2A). The sagitta was square-shaped with a convex top and slightly concave bottom, and the outer margin was serrated on both the dorsal and ventral surfaces. The sulcus was evident on the otolith surface. The rostrum has a slightly bent protrusion. The rostrum and antirostrum are clear, and the postrostrum protrudes posteriorly, evident in both the left and right otoliths. The postero-dorsal angle is curved with visible saw lines. In *Butis koilomatodon*, the sagitta is the most distinctive, with a square to oval shape (Figure 2B). The margin of the otolith tends to be clear. The sulcus is deep and obvious. The antirostrum is slightly curved, making it difficult to distinguish between the rostrum and the antirostrum. The postrostrum is pointed downwards. Compared to *B. butis,* the sagitta of *B. koilomatodon* has a similar appearance, but the number of serrated crests is larger and clearer, which is an important sign to distinguish between the two species. Compared to Eleotridae's otolith between this study and those in Taiwan [23], we can see that their sagittal all have a shaped square. However, there is a disparity in the otolith of species in Eleotridae in Taiwan, which is shaped from square to rectangular. When comparing the otolith of these two species with other works, it shows that the sagittal morphology of *B. koilomatodon* in the Mekong River region has a protruding pointed rostrum, serrated postrostrum, slightly flat upper surface, and protruding lower surface [24], which is similar to this study. In addition, the sagittal morphology of *B. but is* collected in Sumatra [25] and that in the present study are quite alike.

The otolith of *Acentrogobius viridipunctatus* is a flat oval shape, with the dorsal margin and ventral margin quite clearly defined. The sulcus is truly narrow and not evident in the samples obtained (Figure 2C). The rostrum and antirostrum are curved with the antirostrum tending upwards while the rostrum is downward. They are distinctly separated. The postrostrum is bowed, not protruding, and hard to identify. The sagitta of *Aulopareia unicolor* is oval, and its surface is smooth (Figure 2D). The outer margin of the sagittae appears deeper and more pronounced. The distance between the rostrum and antirostrum is shortened. The postrostrum tends to be inward, bent at the bottom. In the size range obtained by *Gobiopsis macrostoma*, the sagittae showed little variation, which is species-specific (Figure 2E). It has oval shapes and a transparent color. The dorsal and ventral are smooth, with few or no serrations. The sulcus is shallow and tricky to define. The rostrum has a clear boundary that distinguishes it from the antirostrum. The antirostrum does not protrude and it is difficult to identify. The postero-dorsal angle is arched, and straight with the postrostrum. The present study provides the first data on the otoliths of these three species in the world.

In the *Glossogobius* genus, the otolith of *G. giuris* has a relatively stable oval shape within the size range (Figure 2F). The dorsal and ventral surfaces are slightly raised. The serrated folds on the dorsal and ventral are small and not numerous. The sulcus in the middle can be identified. The rostrum protrudes anterior to form a triangle, distinguished by the antirostrum at the point of intersection. The postrostrum is curved and straight with a postero-dorsal angle. There is a clear resemblance between the shape of *G. giuris*' otolith in this work and that of Phan et al. (2021) [26]. The otolith shapes of *G. giuris* and *G. olivaceus* are clearly different (Figure 2F, G). Sagittae of *G. olivaceus* also are oval (Figure 2G). The ventral has more serrations than the dorsal. The serrations are clear and deep. The sulcus is deep and easy to identify. The rostrum extends to the anterior and is differentiated from the antirostrum. The postrostrum is curved and does not convex outward. Therefore, to differentiate the two species in this genus, we can use surface smoothness and the number of serrated folds on the otolith margin. *G. olivaceus*' sagittal morphology in the present study is different from Taiwan's (Figure 2G in the present study; Plates 57, 125 in Lin and Chang, 2012 [16]). The sagitta in the description by Lin and Chang (2012) had a smoother surface and crests than that in the present study, but the otolith sizes are different (5.14 OL mm for Taiwan and 3.8 OL mm in the current study). There is a possibility that the otolith surface changes during growth. Thus, it should be better to use an otolith of the same fish size for identification.

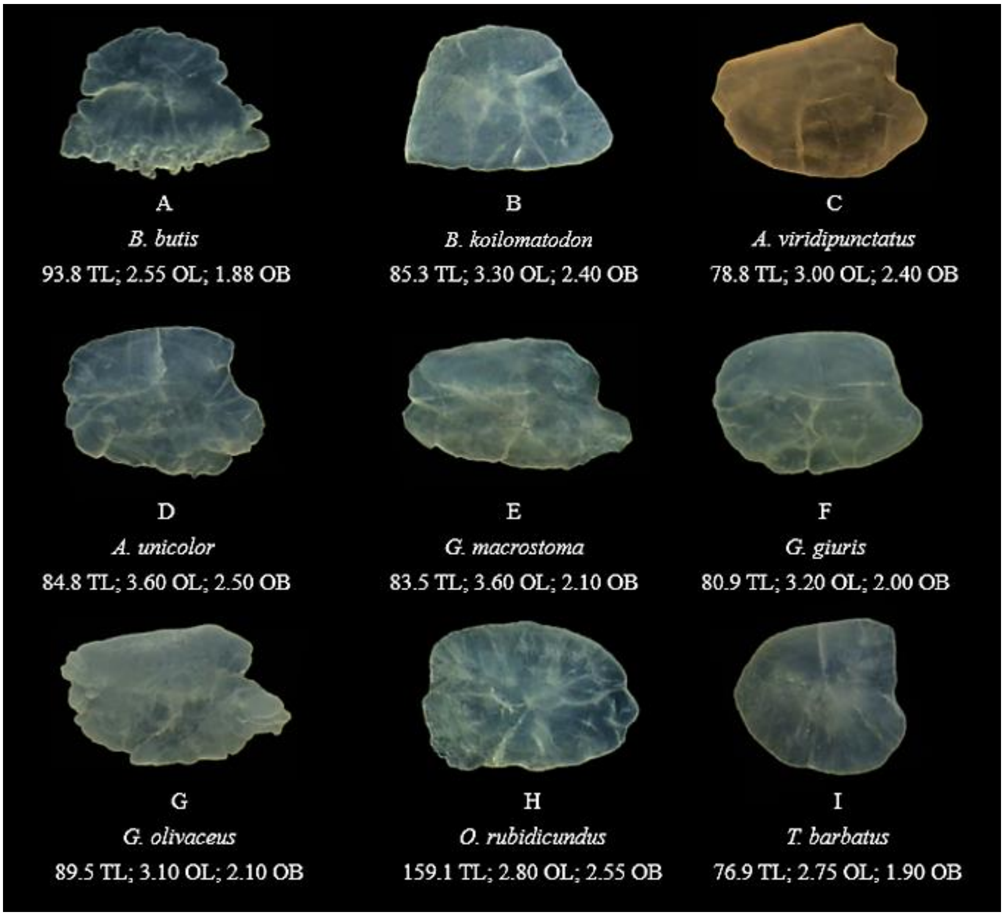

Figure 2. Otolith morphology of nine gobies in the Ba Lat estuary, northern Vietnam, TL. Total length of fish (mm); OL. Otolith length (mm); OB. Otolith breadth (mm)

This study described the otolith morphology of two species of Oxuderxidae. The otolith of *Odontamblyopus rubicundus* is a nearly round oval shape, transparent, the color becoming more opaque towards the center (Figure 2H). The surface is smooth, not rough, and not prominent. The dorsal and ventral margins are soft without any breaks. The rostrum and antirostrum extend to form a V-shaped fork with a bottom towards the center. The postrostrum is slightly convex, in line with the sulcus. In the obtained sagittae of *Tridentiger barbatus*, the otolith exhibits an oval to elliptical shape with little serration on the outer margin (Figure 2I). The deep sulcus can be identified. The rostrum is relatively convex to distinguish it from the antirostrum. The postrostrum is pointed posteriorly. Currently, otolith data of two species in the family Oxuderxidae has no atlas or descriptions for comparison.

2.2.2. Otolith sizes and weight

In nine species, *Acentrogobius viridipunctatus* has the greatest average otolith length (OL = 3.75 mm) and the maximum average mass (OW = 0.0080 g). The maximum average otolith breadth species is *Odontamblyopus rubicundus* (OB = 2.75 mm). *Tridentiger barbatus* has the minimum average otolith length, breadth, and weight (OL = 2.37 mm; OB = 1.59 mm; OW = 0.0027 g). The ratio OL/OB displays the distinction in morphology in each species, with the highest in *Glossogobius olivaceus* (1.54), and the lowest in *Odontamblyopus rubicundus* (1.0) (Table 2).

Table 2. The sagittal dimension of nine gobies from the Ba Lat estuary

No.	Species name	Sagittal length (OL, mm)		Sagittal breadth (OB, mm)		Sagittal weight (OW, g)		OL /OB (Ave.)
		Min-Max	Ave.	Min-Max	Ave.	Min-Max	Ave.	
1	*Butis butis*	2.5-3.4	2.78	1.8-2.3	2.09	0.0022-0.0044	0.0033	1.33
2	*Butis koilomatodon*	1.6-3.3	2.47	1.1-2.4	1.82	0.0006-0.0068	0.0030	1.36
3	*Acentrogobius viridipunctatus*	3.0-4.7	3.75	2.4-3.0	2.66	0.0058-0.0097	0.0080	1.41
4	*Aulopareia unicolor*	2.3-3.6	3.09	1.9-2.5	2.26	0.0024-0.0123	0.0072	1.37
5	*Glossogobius giuris*	2.7-3.7	3.19	1.8-2.3	2.11	0.0032-0.0081	0.0058	1.51
6	*Glossogobius olivaceus*	2.1-3.8	2.94	1.5-2.2	1.91	0.0016-0.0082	0.0047	1.54
7	*Gobiopsis macrostoma*	2.8-3.6	3.1	1.6-2.1	1.82	0.0028-0.0054	0.0039	1.70
8	*Odontamblyopus rubicundus*	2.5-3.1	2.75	2.6-2.9	2.75	0.0069-0.007	0.0069	1.00
9	*Tridentiger barbatus*	1.9-2.8	2.37	1.3-1.9	1.59	0.0013-0.0041	0.0027	1.49

2.2.3. Otolith morphological changes with fish growth

The change of sagittal morphology with fish growth is depicted in Figures 3, 4. The present study expresses morphological changes of otoliths with the growth of only four species (*B. koilomatodon*, *T. barbatus*, *G. olivaceus*, and *G. giuris*), which have numerous specimens with a wide range in size. It shows that all four species do not change in shape when they grow, but the otolith dimension increases with fish growth (Figures 3, 4, Table 3). These figures confirm that the relationship between fish size and otolith dimensions is positive, especially being high R-squared value in *Butis koilomatodon* ($R^2 > 0.6$ in all cases). Thus, the otolith size and mass could be used to determine the fish growth, which is consistent with previous works (e.g., Phan et al., 2021; Ta et al., 2015; Tran et al., 2015 [13], [14], [26]).

Figure 3. The growth of the left otolith in B. koilomatodon* and *T. Barbatus in the study site

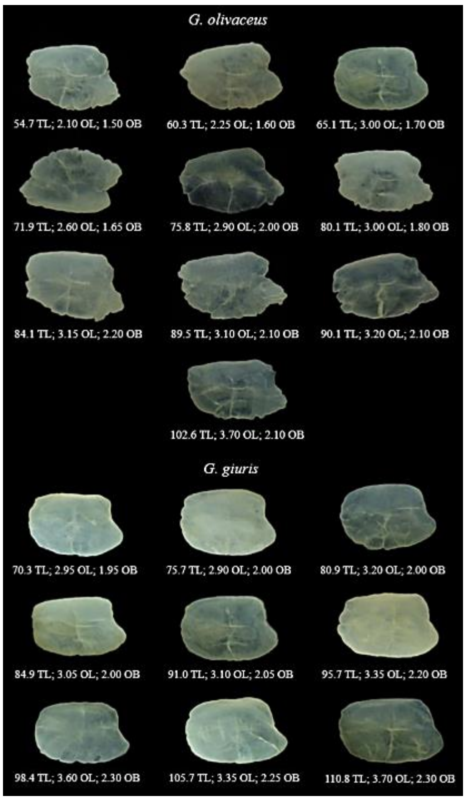

Figure 4. The growth of the left otolith in two species of the genus Glossogobius in the study site

Table 3. The relationship between fish and otolith in four goby species in the study site

Species		***B. koilomatodon*** (n = 30)	***T. barbatus*** (n = 29)	***G. olivaceus*** (n = 29)	***G. giuris*** (n = 29)
Left sagittal weight - Fish total length	y	$y = 0.0003e^{0.035x}$	$y = 0.0002e^{0.0396x}$	$y = 0.0006e^{0.0252x}$	$y = 0.0017e^{0.0135x}$
	R^2	0.8516	0.6597	0.6291	0.683
Left sagittal length - Fish total length	y	$y = 0.9866e^{0.0137x}$	$y = 1.2387e^{0.0108x}$	$y = 1.3074e^{0.0103x}$	$y = 2.003e^{0.0051x}$
	R^2	0.9301	0.8283	0.7582	0.719
Left sagittal breadth - Fish total length	y	$y = 0.7632e^{0.013x}$	$y = 0.908e^{0.0093x}$	$y = 1.0742e^{0.0073x}$	$y = 1.4657e^{0.004x}$
	R^2	0.8590	0.6051	0.6500	0.5606
Left sagittal weight - Fish weight	y	$y = 0.0015e^{0.1883x}$	$y = 0.0016e^{0.2997x}$	$y = 0.0026e^{0.1545x}$	$y = 0.0045e^{0.0499x}$
	R^2	0.6061	0.3528	0.4946	0.4392
Left sagittal length - Fish weight	y	$y = 1.9102e^{0.0774x}$	$y = 2.0133e^{0.0827x}$	$y = 2.3501e^{0.063x}$	$y = 2.8996e^{0.0194x}$
	R^2	0.7248	0.6038	0.6000	0.5104
Left sagittal breadth - Fish weight	y	$y = 1.4281e^{0.0725x}$	$y = 1.3975e^{0.0639x}$	$y = 1.6404e^{0.0434x}$	$y = 1.9551e^{0.0151x}$
	R^2	0.6568	0.3563	0.4800	0.3991

3. Conclusions

The present study first described otoliths of nine goby fishes collected in the Ba Lat estuary. Of which, otoliths of four species have already provided in other areas (i.e., *Butis butis*, *B. koilomatodon*, *Glossogobius olivaceus* and *G. giuris*), and those are new data for five species (i.e., *Acentrogobius viridipunctatus*, *Aulopareia unicolor*, *Gobiopsis macrostoma*, *Odontamblyopus rubicundus*, and *Tridentiger barbatus*). Differences in the otolith morphology of species in one genus (*Butis* and *Glossogobius*) obtained from the present study further confirm that otolith morphology is species-specific, which is valuable for fish identification. Also, this study presents data on the otolith size and weight of these nine species. In addition, the otolith measurements increase with the fish growth but the shape is not so much changed, implying that the fish length and weight could be indicated by otolith size and mass. The current findings are helpful to further work on using otoliths as an identification trait in bony fishes.

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REFERENCES

[1] Popper AN & Lu Z, (2000). Structure–function relationships in fish otolith organs. *Fisheries research*, 46(1-3), 15-25.

[2] Campana SE, (2004). *Photographic atlas of fish otoliths of the Northwest Atlantic Ocean*. Canadian: NRC Research Press, p. 284.

[3] Popper AN, Ramcharitar J & Campana SE, (2005). Why otoliths? Insights from inner ear physiology and fisheries biology. *Marine and Freshwater Research*, 56(5), 497-504.

[4] Ghanbarifardi M, Gut C, Gholami Z, Esmaeili HR, Gierl C & Reichenbacher B, (2020). Possible link between the structure of otoliths and amphibious mode of life of three mudskipper species (Teleostei: Gobioidei) from the Persian Gulf. *Zoology in the Middle East*, 66(4), 311-320.

[5] Purrafee Dizaj L, Esmaeili HR & Teimori A, (2020). Comparative otolith morphology of clupeids from the Iranian brackish and marine resources (Teleostei: Clupeiformes). *Acta Zoologica*, 103(1), 29-47.

[6] Pham TTN, Tran DH & Nguyen VQ, (2021). An initial overview of ecosystem services from mangrove forests in Vietnam. *Journal of Science Natural Science.* DOI: 10.18173/2354-1059.2021-0065.

[7] Nguyen DT & Hoang TTN, (2013). Species diversity of fishes in Ba Lat estuary and Xuan Thuy National Park, Nam Dinh province. *Proceedings of the 5th National Scientific Conference on Ecology and Biological Resources*. Natural Science and Technic Publishing Houses, 678-681.

[8] Pham VL, Dang TTH, Nguyen TTD, Nguyen XH & Tran DH, (2023a). Values and conservation of goby (Actinopteri: Gobiiformes) in Xuan Thuy National Park. *TNU Journal of Science and Technology*, 228(05), 363-371.

[9] Tran DH, Nguyen LHT & Nguyen TN, (2020). First data of goby fish in Tien Hai Wetland Nature Reserve, Thai Binh Province. *HNUE Journal of Science*, 65(10), 143-153. DOI: 10.18173/2354-1059.2020-0058.

[10] Tran DH, Nguyen HH & Ha ML (2021a). Length-weight relationship and condition factor of the mudskipper (*Periophthalmus modestus*) in the Red River delta. *Regional Studies in Marine Science*, 46.

[11] Ha ML, Nguyen HH, Ta TT, Nguyen XH & Tran DH, (2022). Spatio-temporal occurrence of different early life stages of *Periophthalmus modestus* in a tropical estuary. *Animal Biology*, 72(2). DOI: 10.1163/15707563-bja10074.

[12] Pham VL, Chu HN, Dang TTH, Nguyen HH & Tran DH, (2023b). Additional data on species diversity of gobies (Actinopteri: Gobiiformes) in Xuan Thuy National Park, Nam Dinh province. *Hue University Journal of Science: Natural Science,* 132(1b), 49-58.

[13] Tran DH, Nguyen TT & Nguyen TTD, (2015). Morphological variations of otolith in larvae and juveniles of sand whiting *Sillago sihama* (Forssakal, 1775) collected in the Tien Yen estuary, Quang Ninh. *Proceedings of the 5th National Scientific Conference on Ecology and Biological Resources*. Natural Science and Technic Publishing Houses, 1378-1383.

[14] Ta TT, Tran DH, Nguyen TTD & Tran TT, (2015). Diversity of Otolith Morphology in *Nuchequula nuchalis* (Temminck and Schelegel, 1845) Larvae and Juveniles Colleted in the Tien Yen Estuary, Northern Vietnam. *Tropical Natural History*, 15(1), 69-79.

[15] Tran DH, Nguyen PH & Ha ML, (2017). Study on otolith structure of larvae and juveniles of Tiger bass (*Terapon jarbua*) in Kalong estuary, Viet Nam. *Proceedings of the 7th National Scientific Conference on Ecology and Biological Resources.* Natural Science and Technic Publishing Houses, 687-693.

[16] Tran DH, Mari K & Pham MH, (2021b). Migration patterns of two gizzard shads, *Clupanodon thrissa* (L., 1758) and *Nematalosa nasus* (Bloch, 1795) (Clupeiformes: Clupeidae), from Vietnam as revealed by otolith microchemistry analyses. *Acta Zoologica Bulgarica, 73(3), 409-416.*

[17] Nakabo T, (2002). *Fishes of Japan with pictorial keys to the species*, English edition II, Tokai Univerdity Press.

[18] Nguyen VH, (2005). *Freshwater fish in Vietnam* (Vol. 2). Agriculture Publishing House, Hanoi, p. 759

[19] Nguyen VH, (2005). *Freshwater fish in Vietnam* (Vol. 3). Agriculture Publishing House, Hanoi, p. 755

[20] Tran DH, Shibukawa K, Nguyen TP, Ha PPH, Tran VL, Mai VH & Utsugi K, (2013). *Fishes of the Mekong Delta, Vietnam*. Can Tho University Publishing House.

[21] Kimura S, Imamura H, Nguyen VQ & Pham TD, (2018). *Fishes of Ha Long Bay, the World Natural Heritage Site in Northern Vietnam.* Fisheries Research Laboratory, Mie University, Shima, Japan, p. 314

[22] Secor DH, Dean JM & Laban EH, (1992). Otolith removal and preparation for microstructural examination. In: Stevenson DK. and Campana SE. (Eds), *Otolith microstructure examination and analysis,* 19-57. Canadian Special Publication of Fisheries and Aquatic Sciences.

[23] Lin CH & Chang CW, (2012). *Otolith atlas of Taiwan fishes.* National Museum of Marine Biology & Aquarium.

[24] Lam TTH, Nguyen THH, Dinh MQ & Nguyen D, (2021). Otolith biometrics and their relationships with fish sizes of *Butis koilomatodon* living in Mekong Delta, Vietnam. *Egyptian Journal of Aquatic Biology & Fisheries*, 25 (3), 803-814.

[25] Schwarzhans W, Scofield RP, Tennyson AJ, Worthy JP & Worthy TH, (2011). Fish remains, mostly otoliths, from the non-marine early Miocene of Otago, New Zealand. *Acta Palaeontologica Polonica,* 57, 319-350.

[26] Phan HG, Dinh MQ, Truong TN, Tran SN & Nguyen DHT, (2021). Using the otolith mass in growth determining of *Glossogobius giuris* in the Mekong Delta. *Egyptian Journal of Aquatic Biology & Fisheries*, 25 (6), 193-203.