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Diversity of Otolith Morphology in *Nuchequula nuchalis* (Temminck and Schlegel, 1845) Larvae and Juveniles Collected in the Tien Yen Estuary, Northern Vietnam

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ABSTRACT.– Otolith morphological changes in *Nuchequula nuchalis* larvae and juveniles are described based on 87 specimens of 6.3–37.1 mm body length (BL) collected in the Tien Yen estuary, northern Vietnam. The sagittae and asterisci were the largest and the smallest otolith, respectively. Sagittae were oval-shaped, and showed remarkable changes in morphology with growth. As the sagitta grows the margin becomes sinuated, the excisural notch forms as an acute angle, the sulcus becomes deeper, and the rostrum, antirostrum and postrostrum become clearer. The lapilli are mussel shell-shaped and their shape does not change much throughout the developmental period. In this study, the number of rings on the asterisci did not correspond to those on the sagittae and lapilli for larvae of the same size (~ 12 mm BL).

KEY WORDS: **Morphology, *Nuchequula nuchalis*, development, Tien Yen estuary, Vietnam**

INTRODUCTION

Otoliths (statoconium or otoconium) are structures located in the inner ear cavity of all teleost fish. Each side includes sagittae, lapilli and asterisci that are different in shape, size and location (Chen and Yan, 2001). The morphology of otoliths has been used in species identification, age and growth determination, larval fish ecology, fish stock identification and environmental reconstruction of the fish habitat (Mendoza, 2006). Although the use of otolith increments for aging larval and juvenile fish has become increasingly popular, characterization of the development of otolith morphology with age in different species is still poorly resolved. However, it is considered to be variable with the growth of each species (Tsukamoto and Kajihara, 1987; Umezawa et al., 1989; Morioka and Kaunda, 2005; Baker, 2006), and to exhibit a variable morphology and topography among different species (Félix et al., 2013). In addition, the spatial distribution of otoliths is different among species, and so it can be used as a valid and practical character in fish larval identification by the readily available camera-type polarization filters technique (Chen and Yan, 2001).

Nuchequula nuchalis (Fig. 1) is a common leiognathid fish that is mainly distributed along the shallow coastal and brackish waters of southern Japan to northern Vietnam in the Northwest Pacific (Woodland et al., 2001). There are eight species in the Leiognathidae family that are found in the Tien Yen River and adjacent coastal waters of northern Vietnam (Tran and Ta, 2014), but no information on *N. nuchalis* at this locality is currently available. However, during a 1 y survey, a

FIGURE 1. A juvenile (37.1 mm BL) of *N. nuchalis* collected from the Tien Yen estuary.

number of larvae and juveniles of *N. nuchalis* were collected along the shore of the Tien Yen estuary, and their otolith morphology is reported in this paper.

Baker (2006) examined the otoliths from six leiognathid fish (*Leiognathus decorus*, *L. equulus*, *L. splendens*, *Gazza minuta*, *Secutor ruconius* and *S. insidiator*), derived from the remains in the digestive tracts of predatory species from the estuaries of the Townsville region of north-eastern Queensland, Australia, where the sagittal morphology of the prey species is species-specific or genera-specific allowing their identification. However, this work did not reveal any details in the development of the sagittae because of the irregular bin size of the examined fish (Baker, 2006). Changes in sagittae morphology with fish size and development stages could be useful markers (informative characters) in larval and juvenile fish identification. However, the early development of otolith morphology in *N. nuchalis* remains largely unknown, while the species identification in the early developmental stages of fish is usually difficult. This study describes the otolith morphology (sagittae, lapilli and asterisci) throughout the development of larvae and juveniles of *N. nuchalis* collected in the Tien Yen estuary, northern Vietnam to assess if they are diagnostic morphological characters in larval and juvenile identification and to provide a preliminary observation on the increments on otolith for aging the fish.

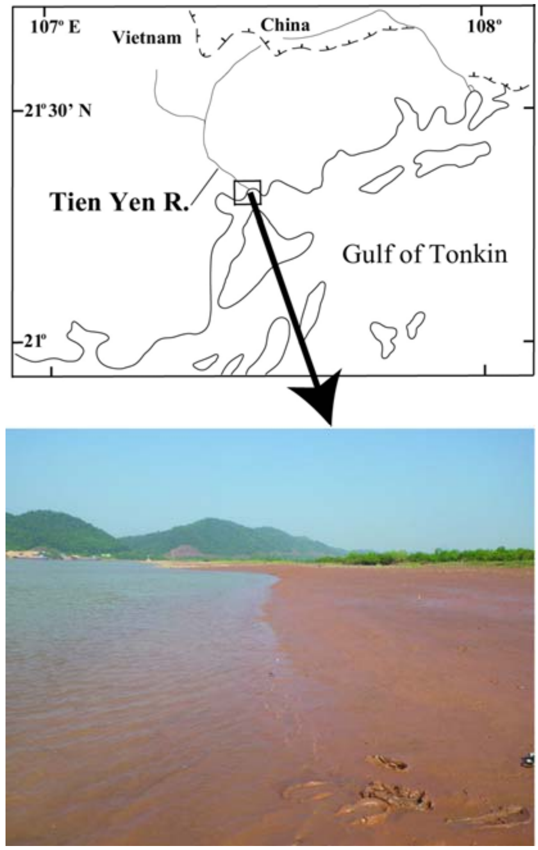

FIGURE 2. Location of the Tien Yen estuary in northern Vietnam, showing one of the dominant collection sites (below).

MATERIALS AND METHODS

Description of the otolith morphology of *N. nuchalis* was based on 87 specimens captured by a small seine net (1 x 4 m, mesh-size 1 mm) in the Tien Yen estuary from March 2013 to February 2014 (Fig. 2). Specimens were immediately fixed in 4–5% (w/v) buffered formalin solution in the field, sorted within 2–3 h, and then transferred to 70% (v/v) ethanol. One day later the fish

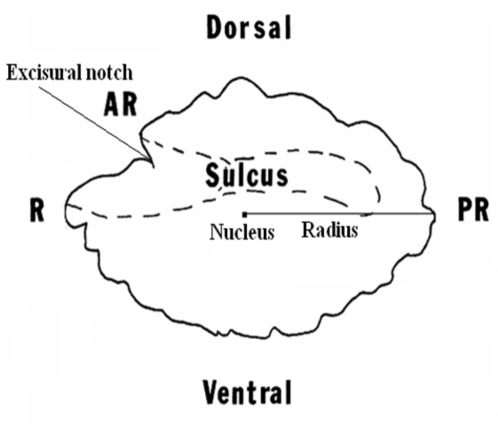

FIGURE 3. Medial view of the right sagitta of a typical teleost. AR. antirostrum, PR. postrostrum, R. rostrum (Secor et al., 1992).

were changed to fresh 70% (v/v) ethanol solution for preservation. Fish were divided into the different developmental stages (Kendall et al., 1984), and the body length (BL) was measured to the nearest 0.1 mm using an ocular micrometer attached to a binocular microscope. In this report the BL rather than the total length (TL) of the fish was used since the BL is the standard length for postflexion larvae and juveniles.

The bin size of specimens was selected based on the ontogenetic changes of the otolith morphology. Based on the range of size of specimens collected, a quick examination of the fish was performed to ascertain the changes in the otolith size of fish from the smallest to largest samples, and from this the otolith bin size was determined. Specimens were mainly in the size range of 8.0–12.0 mm BL, with a few smaller specimens and, rarely, larger specimens being found during the study period (Table 1). Thirty specimens in the two first bins and 15 in the third bin were randomly selected to remove the otoliths, and all specimens collected in the remaining bins were used to remove the otoliths (Table 1).

The right-side otolith or that from both sides was removed from the 87 specimens as previously reported (Tsukamoto and Kajihara, 1987; Mendoza, 2006). In brief, fish were immersed in a few drops of water on a normal-glass petri dish and the otoliths were located under 10–40 x magnification with cross-polarized light, which causes the otolith to become birefringent. The otoliths were then teased out from the head using small dissecting needles and any adhering tissue was gently scraped away whilst keeping the otoliths separate from all the other tissues of the dissected fish. They were then transferred to clean slides, photographed and fixed in epoxy resin. Because of the limited size of the specimens and that some otoliths were damaged on removal, from the 87 specimens of *N. nuchalis*, only 107 sagittae, 88 lapilli and six asterisci were obtained (Table 1).

Morphological observation of the fixed otoliths and measurement of the sagittae radii were performed with a binocular microscope under reflected light at 40–100 x magnification. Of 107 sagittae, 55 were used to measure the radius, measured from the nucleus to the margin along the longest axis (Fig. 3). The Excel software was used to perform the linear regression of the sagittal radius (y; μm) on the BL (x; mm) of *N. nuchalis*. Photographs were taken with a Pentax digital camera on the light microscope, and images were processed with Adobe Photo Shop CS5. The morphological description of otolith components was performed as reported in Secor et al. (1992).

The identification of *N. nuchalis* larvae and juveniles (Fig. 1) was based on Haque and Ozawa (1995), Kinoshita (1989), Leis and Trnski (1989) and Kimura et al. (2008). The larvae and juveniles in this study were

TABLE 1. Data of *Nuchequula nuchalis* larvae and juveniles collected from the Tien Yen estuary, northern Vietnam and used for the otolith morphological research.

Body length range (mm)	Mean (SD) of fish length	No. of specimens	No. of otolith removed		
			Sagittae	Lapilli	Asterisci
6.3–8.7	7.84 (0.70)	30	36	22	2*
9.0–11.8	10.26 (0.86)	30	33	40	0
12.0–14.9	12.94 (0.88)	15	18	12	0
16.2–17.8	17.00 (1.13)	2	3	1	0
18.1–24.9	21.17 (2.51)	7	12	9	2
25.9		1	1	1	0
29.1		1	2	2	0
37.1		1	2	1	2
Total		**87**	**107**	**88**	**6**

*. Asteriscus broken but still had countable rings.

characterized by their laterally compressed and moderate to deep body depth (BD), where the BD was 28.6–41.9% of the standard length (SL); a coiled and compact gut; short gut (preanal length of 40.6–50.1% of the SL); head spination, particularly for the preopercular and supraoccipital spination; large ascending premaxillary process; ventral pigment on the tail; an extremely protrusible mouth (Leis and Trnski, 1989); 25 myomeres; D VIII, 16–17; A III, 14–15, P 15–19; V I, 5 (Kinoshita, 1988; Leis and Trnski, 1989); no pigmentation on the lower jaw symphysis; postflexion larvae with five spines on the superaocular ridge and slightly embed and wedge-shaped melanophores along the anal fin base (Haque and Ozawa, 1995). In addition, a conspicuous dark blotch located distally on the spinous dorsal fin started to appear in fish of 14.0 mm SL and larger (Kimura et al., 2008), and became darker in 31.7 mm SL juveniles.

RESULTS

Sagittae

The sagittae were oval-shaped, moderately thick, and changed remarkably in shape with growth (Fig. 4). The change in the sagittal morphology with growth was clearly evident in the mesial surface, dorsal margin, ventral margin, anterior margin, excisural notch, rostrum, antirostrum, postrostrum and the deep of sulcus (Table 2). A detailed description of the sagittal morphology is given in Table 2. Larvae of 6.3–7.8 mm BL (Fig. 4A, B) had a flat and hyaline mesial surface, and the rings on the sagittae could be clearly observed. There were 14 and 24 rings in the 6.3 and 8.5 mm BL specimens, respectively (Table 2). The dorsal, ventral and anterior margins were still smooth (Fig. 4B). The excisural notch was at an obtuse angle and the sulcus was somewhat shallow. The morphology of the rostrum, antirostrum and postrostrum was not determined (Table 2).

Larvae from 11.7–37.1 mm BL (Fig. 4C-K) had a rough and opaque mesial surface, and so the sagittal rings could not be directly observed. Thus, this method of aging fish could not be used on specimens larger than 11.7 mm BL (Table 2). The margin was sinuated (rounded with a regular wavelike curve), and in larvae of 11.7 mm BL the curve appeared at the anterior margin with the dorsal and ventral margins still being smooth (Fig. 4C). When the larvae attained a size of 16.2 mm BL, the curve appeared at the dorsal margin, and at the

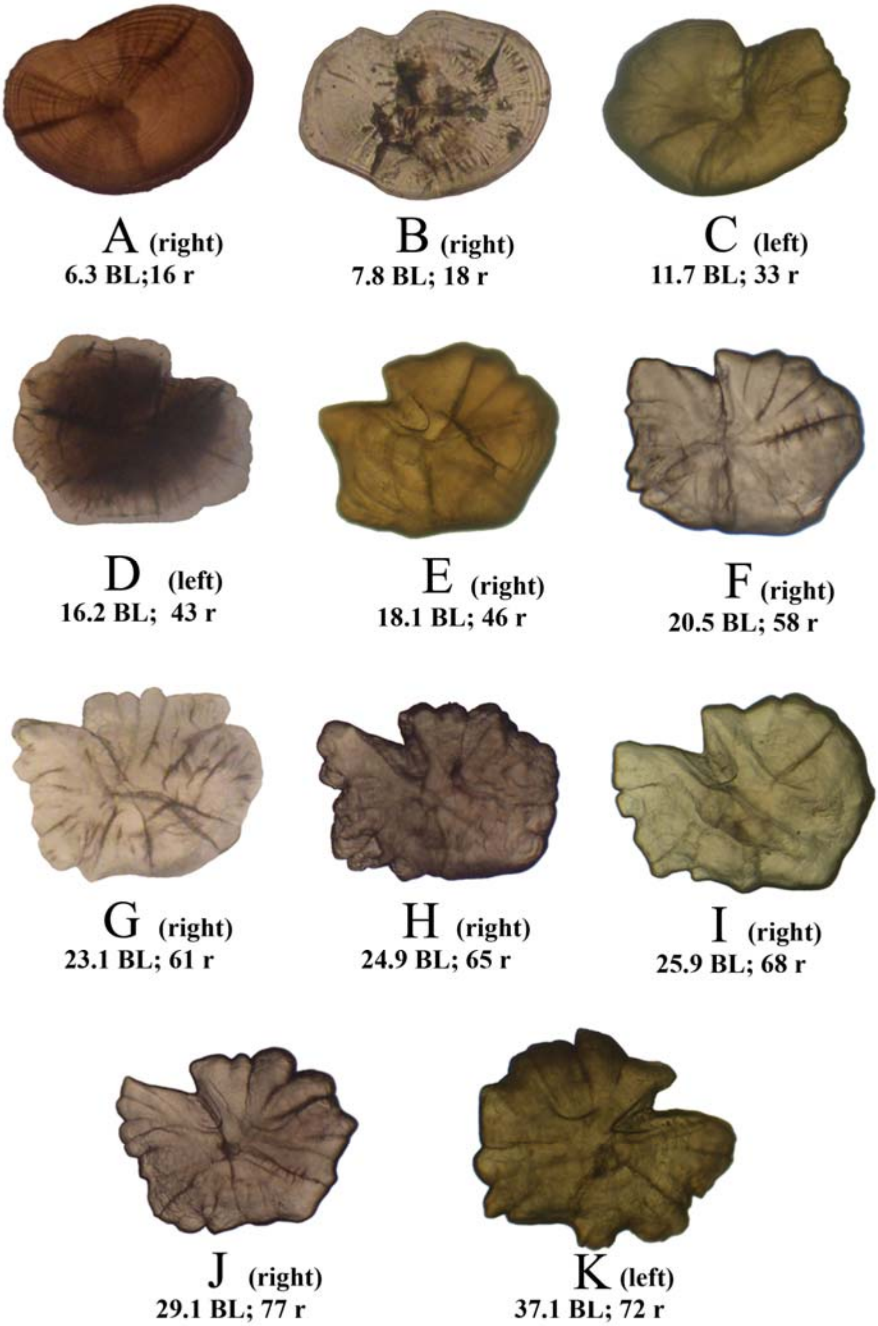

FIGURE 4. Morphology of the sagittae of collected from the Tien Yen estuary. The body length (BL) is shown for all parts in mm and the sagitta radius (r) is shown in µm.

TABLE 2. Key changes of sagittal morphology found in different sizes of *N. nuchalis* larvae and juveniles sampled from the Tien Yen River, northern Vietnam

BL (mm)	Margin (dorsal margin, ventral margin, anterior margin, posterior margin)	Rostrum (rostrum, antirostrum, postrostrum)	Excisural notch	Rings
6.3	Still smooth	Not present	Not present	Clear observation (about 14 rings)
8.5	Still smooth	Not present	Still an obtuse angle, but become deeper	Clear observation (about 24 rings)
11.7	A rough of anterior margin	Not present	Deeper angle	Just clear observation for outer rings
14.9	Dorsal margin rounded with a regular wavelike curve	Not present	Deeper angle	Not clear observation
16.2	Ventral and posterior margins first appear wavelike curve	Not present	Seemed to be like a right angle	Not clear observation
18.1	Margin with a shallow wavelike curve	Rostrum and antirostrum were clearly formed	Started to form an acute angle	Not clear observation
20.5	The curve at margin became more distinctive	Rostrum and antirostrum were clearly formed	Formed as an acute angle	Not clear observation
25.9	At conjunction between ventral and posterior margin, a somewhat wavelike curve started to form	Postrostrum was first formed	Formed as an acute angle	Not clear observation
29.1 and 37.1	The curve at margin became more sinuate than the smaller specimens	Three rostrums became more distinctive	Formed as an acute angle	Not clear observation

ventral margin it appeared from the anterior and extended posterior with growth (Fig. 4D-K). At this size, the excisural notch was at a right angle and the sulcus was deeper (Fig. 4D). For larvae of 18.1 mm BL, the rostrum and antirostrum had started to form, and the excisural notch began to form an acute angle (Fig. 4E). In larvae of 18.1–24.9 mm BL, the curve at the margin of sagitta was more distinctive, but the postrostrum still had not appeared (Fig. 4E-H). The postrostrum began to form in larvae of 25.9 mm BL, and it was more distinctive in larvae of 29.1 mm BL (Fig. 4I, J). Because specimens between 29.1 and 37.1 mm BL were not collected in this study, no information about the morphology of sagittae was recorded for larvae in this range. For the largest size evaluated (37.1 mm BL, juvenile), the sagitta showed a different pattern to the smaller (< 29.1 mm BL) larvae, where the mesial surface was rougher, the margin of sagitta (dorsal, ventral and anterior) was more sinuate, the excisural notch was an acute angle, the rostrum, antirostrum and postrostrum were more distinctive and the sulcus was deeper and could easily be observed (Fig. 4K).

The relationship between the sagittal radius and BL of *N. nuchalis* over the 6.6 to 37.1 mm BL size range examined showed a good linear regression ($r = 0.96$) with the best fit equation of $y = 2.282x + 3.782$ (Fig. 5). Thus, there is likely to be a strong relationship between the sagittal radius and the BL in these larval-juvenile *N. nuchalis*.

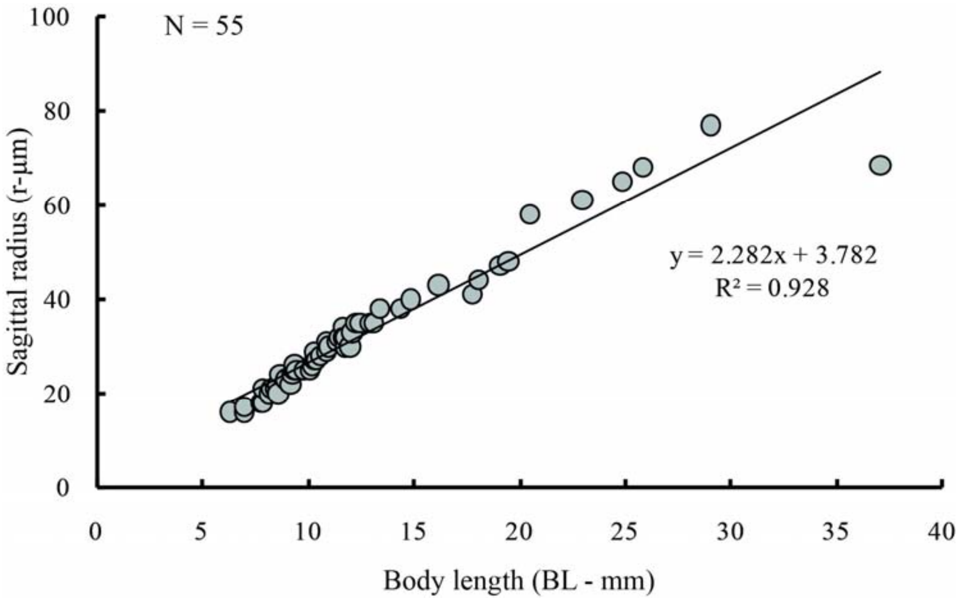

FIGURE 5. Relationship between the body length (BL) and sagittal radius (r) in *N. nuchalis* collected from the Tien Yen estuary. The equation of the linear regression line and correlation coefficient are shown.

Lapilli

Compared to the sagitta and the asteriscus, the morphology of the lapillus appeared to be relatively stable over the developmental period (Fig. 6). Lapilli were mussel shell-shaped with a slanting nucleus. Rings also appeared in the lapilli and the number of rings corresponded to those on the sagittae of the same sized specimens in the fish smaller than 11 mm BL. For instance, the number of rings on both the sagitta and lapillus of 7.8 and 10.9 mm BL fish was about 17 and 32, respectively. It was also impossible to read the rings on the lapilli for fish of 11.7 to 31.7 mm BL. With the growth (development) of the fish, the lapilli became thicker, the mesial surface was rougher, and the margin was somewhat more sinuate.

Asterisci

The asterisci were the smallest of the otoliths and were found to morphologically change with growth (Fig. 7). One side of the asteriscus appeared as a hook in 20.5 mm BL larva (Fig. 7A), and at 37.1 mm BL (juvenile) fish the asteriscus margin was sinuate (Fig. 7B). Rings appeared clearly on the asterisci of 8.7 mm BL larvae, but in the specimens of 20.5 and 37.1 mm BL the rings could not be observed directly. In the present study, among the three different otoliths, the asterisci were the most difficult to remove successfully (Table 1).

DISCUSSION

Of the three pairs of otolith components in *N. nuchalis* larvae and juveniles, the sagitta was the largest otolith, like in all other teleost fish, but the asteriscus was the smallest, in contrast to most teleost fishes where the lapillus is the smallest (Paxton, 2000). This apparent exception requires further verification and discussion. Although this study preliminarily revealed the sagittal and lapillus development with growth, the morphological development of

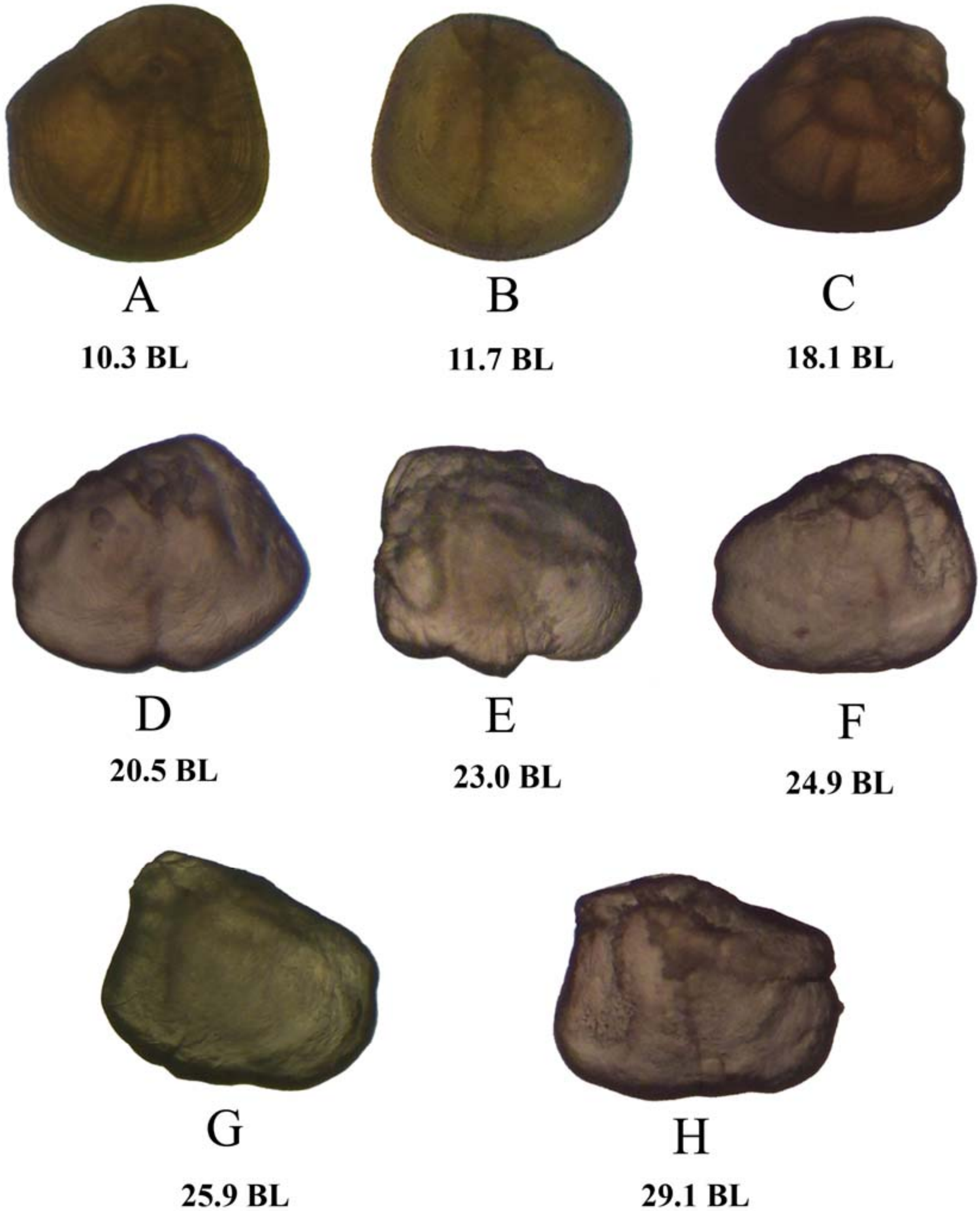

FIGURE 6. Morphology of the lapilli of *N. nuchalis* collected from the Tien Yen estuary. The body lengths (BL) are shown in mm.

the asterisci needs to be examined further. In addition, the sagitta showed a more distinguished pattern of morphological change throughout the larval development than the two other otoliths, implying that this structure could be a useful and informative character for studying the early life history of this fish species. The asteriscus was formed at 20 d after hatching in the ayu, *Plecoglossus altivelis*, (Tsukamoto and Kajihara, 1987), but was not identified in *N. nuchalis* larvae of 10.42 to 12.00 mm TL (Chen and Jan, 2001). In the present study, the asteriscus could be found in fish of 8.7 mm BL (~ 11 mm total length) fish, but not in smaller specimens. Therefore, among the three otoliths, the astericus would have formed last in this fish species.

Due to the paucity of available studies on the developmental morphology of otoliths from the early larval to adult fish stages, the present study attempted to evaluate the morphological changes in otoliths with growth (development) in *N. nuchalis*. Baker (2006) illustrated the morphology of sagittae in larger sized fish specimens, and found changes in the morphology in leiognathid fish, but not in the morphology and especially the shape of sagittae in *Sillago* spp. or *Ambassis* spp. For *N. nuchalis* in this study, general changes in the sagittal shape were found in fish within the size range of ~12–20 mm BL (Fig. 4, Table 2), like those in 13–24 mm BL *S. ruconius* and 15–30 mm BL *L. equulus* (Baker, 2006). The above size range is during the transition from postflexion larval to the juvenile stage, which represents a size of ~ 14 mm BL in the present *N. nuchalis* samples (Tran et al., 2014). This metamorphosis is from a pelagic life to settlement and so there may be a difference between these developmental stages in their locomotion and habitat use.

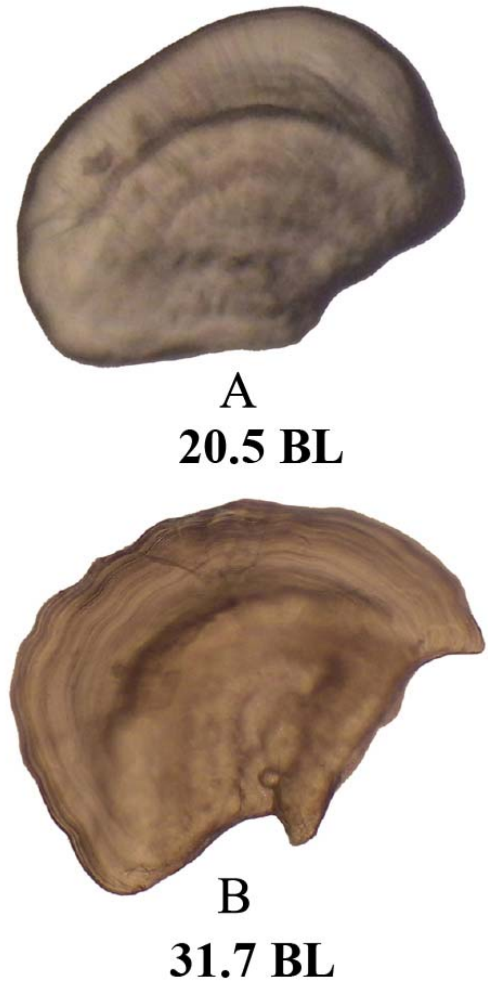

FIGURE 7. Asterisci of *N. nuchalis* collected from the Tien Yen estuary. Body lengths (BL) are shown in mm.

The main function of the otoliths is for balance in fish and there seems to be a relationship between the sagittal shape and the pelagic to settlement metamorphosis in this fish species. This is also supported by previous reports on the association between otoliths and habitat use in fish (Modin et al., 1996; Jaramillo et al., 2014).

When the sagittal morphological development in *N. nuchalis* collected from the

Tien Yen estuary, northern Vietnam was compared with that of some leiognathid fish observed from Australia (Baker, 2006), there were general similarities in their shapes and the changes in the curve of margin, especially of the excisural notch (Fig. 4). For larvae of ~ 24 mm BL, the sagittae in this study were noticeably different from those of *Gazza minuta* and *S. recunius* (Baker, 2006), in that they had a longer rostrum and a rougher curve, but they look more similar to those in species of the genus *Leiognathus*. Sagittal morphology is known to vary among species, and the otolith morphology is often species- or genera-specific, allowing confident identification to at least the generic level (Baker, 2006). Consequently, this study supports the potential of using otolith morphology as a taxonomic character in teleost fish. In addition, this study supplements the earlier works on the development of sagittae in leiognathid fish (Baker, 2006).

Amongst the total of 107 sagittae removed from 87 *N. nuchalis* specimens of 6.3–37.1 mm BL, sagittal rings could be distinctly observed in larvae up to 11.0 mm BL (Fig. 4A, B). This is consistent with the photographs of sagittae derived from the samples obtained from the digestive tract of predators (Baker, 2006). For the 88 lapilli examined, rings could be clearly observed in larvae up to 11.7 mm BL (Fig. A, B), where the number of rings on the lapilli corresponded to those in the sagittae of the same sized larvae. Rings also appeared on the asterisci, but the number of rings did not correspond to those on the sagittae and lapilli of larvae smaller than 12 mm BL. The lapillus has been reported to be the best ageing structure for juvenile dolphin fish, *Coryphaena hippurus*, (Morales-Nin et al., 1999) and larval and juvenile cyprinid *Engraulicypris sardella* (Morioka and Kaunda, 2005). However, the asteriscus and lapillus are not currently used for age determination, but rather the sagitta is, in aging ayu fish (*P. altivelis*) up to 300 d old (Tsukamoto and Kajihara, 1987). Thus, further work is required to determine whether the sagitta, lapillus or asteriscus are more reliable for age estimation in *N. nuchalis* larvae and juveniles.

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