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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

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Abstract: Otoliths can be used to examine the migration history of fish individuals based on trace element concentrations in them. In order to verify migration patterns of *Clupanodon thrissa* and *Nematalosa nasus* (Clupeiformes: Clupeidae), Sr:Ca ratios in otoliths of 13 individuals (6 *C. thrissa* and 7 *N. nasus*) collected from Vietnam were examined. In addition, to provide guidelines for identification, the otolith morphology of the two species was comparatively illustrated. The otolith Sr:Ca ratios from the core to the edge ranged from 0 to 5.7×10^{-3} and 0 to 4.9×10^{-3} for *C. thrissa* and *N. nasus*, with an average of 1.8×10^{-3} and 2.3×10^{-3}, respectively. The present data do not show typical anadromous profiles as the Sr:Ca ratio of the two species mostly fluctuates evenly from 0 to 4×10^{-3}. These data suggest that the life cycles of the studied populations of *C. thrissa* and *N. nasus* are entirely completed in estuarine habitats.

Key words: Migratory pattern of shads; Sr:Ca ratios; estuarine habitat; comparative morphology of otoliths; Vietnam

Introduction

Chinese gizzard shad *Clupanodon thrissa* (Linnaeus, 1758) and Bloch's gizzard shad *Nematalosa nasus* (Bloch, 1795) (Clupeiformes: Clupeidae) are distributed in sea, brackish and occasionally entering fresh waters in the tropical region (Whitehead 1985, Nguyen 2005, Tran & Ta 2014, Ramya et al. 2016). *Clupanodon* is a monotypic genus and *Nematalosa* consists of 11 species (Froese & Pauly 2019). *Clupanodon thrissa* is found in the northwestern Pacific, while the other species seems to have a boarder range from the Indian Ocean to the northwestern Pacific. In general, these two species are of local interest as fisheries resources (Whitehead 1985).

Otolith is an otoconium structure located in the inner ear of all bony fishes, functioning as a balance organ, which is also important for the acoustics. Its morphology is species-specific and, therefore, identification of fish species can be performed with sufficient comparative data for otoliths (Secor et al. 1992, Baker 2006, Lin & Chang 2012, Ta et al. 2015). Trace element concentrations in fish otoliths also have been widely used to understand the life history of individuals because the variations in otolith elemental chemistry primarily reflect changes in the ambient water (Thorrold et

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al. 1998, Campana 1999, Sturrock et al. 2015). To examine the migratory history of fish, microchemistry analysis of otoliths, such as the Sr:Ca ratios, have been applied effectively (Arai & Tsukamoto 1998, Campana & Thorrold 2001, Tsukamoto & Arai 2001, Kuroki et al. 2006, Sturrock et al. 2015, Murase & Iguchi 2019).

The term "diadromy" refers to fish species that migrate between the ocean and freshwaters. There are three types of diadromy: (1) catadromy, as in some freshwater eels (Anguillidae), which are born in the ocean, then migrate to freshwaters and later return to the ocean to spawn; (2) anadromy, as in some salmons (Salmonidae), with the opposite pattern to the catadromy (Gross et al. 1988); and (3) amphidromy, as in ayu (Plecoglossidae), which are born in freshwater, then their newly-hatched larvae or early juveniles feed in the sea (usually for a few months) before a next period of feeding and growing in freshwater (Kuroki et al. 2006, McDowall 2007, Tran et al. 2012). The two species examined in the present study have been classified as typical anadromous fishes (David 1999, Nguyen 2005, Froese & Pauly 2019), implying that they stay in the sea for foraging until the reproductive season when they upstream to freshwater for spawning; their offsprings drift down to brackish water and the sea. However, none of these studies applied microchemistry analyses (e.g., Sr:Ca ratio) to verify the migratory patterns of the two species.

In Vietnam, *Clupanodon thrissa* and *Nematalosa nasus* are mainly recorded in coastal and estuarine habitats along the country and are of high commercial value (Nguyen 2005, Ministry of Science and Technology of Vietnam 2007). Some studies indicate that the two species occasionally occur in estuaries from central and northern Vietnam (Nguyen 2005, Tran & Ta 2014, Nguyen et al. 2015, 2019). Their occurrence in freshwater habitats is still unknown (Whitehead 1985).

In the present study, the migratory patterns of *Clupanodon thrissa* and *Nematalosa nasus* are examined in Vietnam using microchemistry analysis, which is important for the further exploitation and conservation of these two species.

Materials and Methods

Study area

Fishes used in the present study were collected from the Nhat Le River (c. 17° 38′N) and the Red River (Ba Lat Estuary, c. 20° 18′N) in Vietnam (Fig. 1). The Nhat Le River (c. 85 km in length), which is located in the central part of Vietnam, has two branches (Kien Giang and Long Dai, Fig. 1). The salt wedge usually reaches up to c. 30 km from the sea during dry seasons (April – August). Located in northern Vietnam, the Red River originates from China and enters Vietnam at Lao Cai Province, flowing into the Gulf of Tonkin through several estuaries. Amongst these estuaries, Ba Lat is the largest one (Fig. 1); it is characterised by having a large tidal flat and mangrove forests in the river mouth, totally different from the Nhat Le Estuary (Fig. 1).

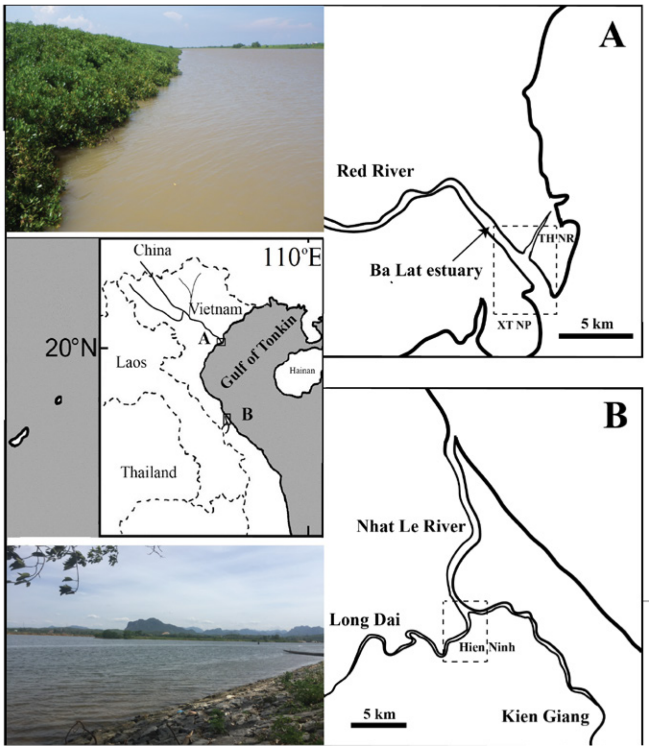

Fig. 1. Chart showing stations where gizzard shad samples were collected from 2018 to 2020 in central and northern Vietnam. The two dash rectangles indicate the sampling areas. *TH NR* and *XT NP* refer to Tien Hai Nature Reserve and Xuan Thuy National Park, respectively, which are characterised by mangrove forests. Photos show scenes around the sampling sites in the two rivers.

Fish collection and identification

In the Nhat Le River, both species were bought in the local market, from April to September 2018, and February 2020 (Fig. 1, Table 1). Initially, fish were frozen in a refrigerator prior to fixation in 70% ethanol. Fin rays of Chinese gizzard shad from Ba Lat Estuary of the Red River were fixed immediately after collection by local fishermen (Fig. 1, Table 1). These specimens were kept in a cool box until preservation in 70% ethanol for further analysis. From the two sites, totally 25 specimens of *Clupanodon thrissa* (60.4–165.3 mm SL) and 30 of *Nematalosa a* (91.4–150.0 mm SL) were used

Table 1. Data of *Clupanodon thrissa* and *Nematalosa nasus* collected from central and northern Vietnam used for the otolith research.

Sampling river	Sampling time	No. of fishes (SL in mm)	No. of otoliths for morphological observation	No. of otoliths for microchemistry analysis
Clupanodon thrissa				
Nhat Le River	April 2018	9 (126.0–146.3)	8 (left), 7 (right)	6
	May 2018	2 (111.3–135.4)	2 (left), 1 (right)	0
	August – September 2018	7 (60.4–135.5)	4 (left), 4 (right)	0
	February 2020	1 (150.1)	0	0
Red River (Ba Lat Estuary)	August 2019	6 (139.2–165.3)	4 (left), 3 (right)	0
Nematalosa nasus				
Nhat Le River	April 2018	6 (112.0–134.5)	6 (left), 2 (right)	2
	May 2018	5 (104.7–130.6)	5 (left), 4 (right)	4
	June 2018	11 (97.7–110.3)	10 (left), 9 (right)	0
	July 2018	5 (102.6–115.0)	5 (left), 5 (right)	1
	August 2018	1 (93.9)	1 (left)	0
	September 2018	1 (91.4)	1 (right)	0
	February 2020	1 (150.0)	0	0
Total		55	81	13

for the present study (Table 1). Identification was based on external morphology (Fig. 2), referring to works by Whitehead (1985) and Nguyen (2005).

Otolith analysis

Otoliths (sagittal) were extracted from 55 fish samples. All otoliths were observed by dissecting microscope and then stored in 70% ethanol. They were photographed by a digital camera attached to the microscope. Totally, 81 successfully removed sagittal otoliths from both sides were used for the morphological study (Table 1). Otolith length (OL) measurements were made along the longest axis across the core measured to the nearest 0.1 mm using an ocular micrometer attached to a binocular microscope (Fig. 3). The morphological description of otolith components followed Secor et al. (1992) and Lin & Chang (2012).

Each otolith was embedded in epoxy resin (Epofix, Struers, Tokyo, Japan), mounted on glass slides, then ground to near the core using a grinding machine equipped with a diamond cup-wheel (Discoplan-TS, Struers) and further polished to expose the core on an automated polishing wheel (Planopol-V, Struers) with OP-S suspension. After cleaning the surface of each otolith, they were vacuum coated with Pt–Pd.

Six sagittal otoliths of *C. thrissa* and seven *N. nasus* collected in the Nhat Le River were examined the migratory patterns based on analysis of Sr and Ca concentrations along a line down the longest axis of each otolith from the core to the posterior edge using a wavelength-dispersive X-ray electron microprobe (JEOL, JXA-8230). Strontianite ($SrTiO_3$) and calcite ($CaCO_3$) were used as standards. The accelerating voltage and beam current were 15 kV and 12 nA, respectively. The electron beam was focused on a point of 9 µm diameter, with measurements spaced at 10 µm intervals.

Results

Otolith morphology

The shape of sagittal otoliths of *C. thrissa* and *N. nasus* was oblong to rectangular and ovate, respectively (Figs. 4–6). Sagittal otoliths of the two species (from specimens ranging from 60.4 to 165.3 mm SL) were identical to others in the family Clupeidae by having an extendedly pointed rostrum and antirostrum as well as a deep notch (Figs. 4–6). In addition, the curves at the ventral margin were sinuate, being more obvious in *C. thrissa* (from ca. 140 to 160 mm SL) and in *N. nasus* (Figs. 4–6). Sagittal otoliths of *C. thrissa* from the Nhat Le River and Ba Lat Estuary were not much different (Figs. 4, 5). Otoliths of *C. thrissa* were different from *N. nasus* by having a longer rostrum and a longer postrostrum (Figs. 4–6). In a specimen 165.3 mm long (SL), the otolith surface of *C. thrissa* seemed to be smooth (Figs. 4, 5).

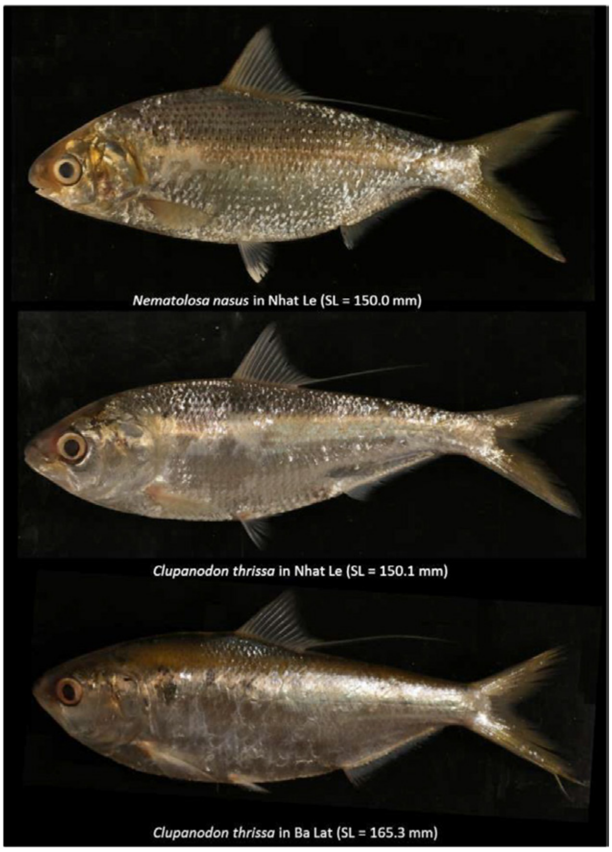

Fig. 2. *Clupanodon thrissa* and *Nematalosa nasus* collected from the Nhat Le River (central Vietnam) and the Red River (Ba Lat Estuary, northern Vietnam).

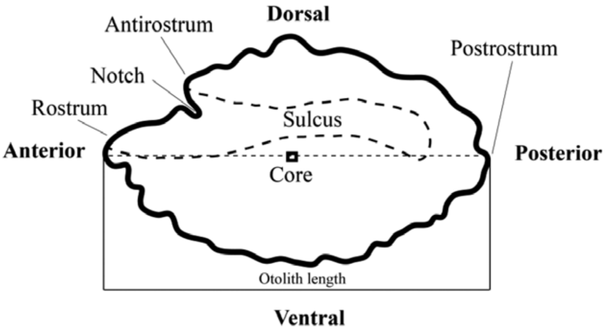

Fig. 3. Medial view of the right sagitta of a typical teleost (after Secor et al. 1992 and Lin & Chang 2012, modified).

The relationship between the standard lengths and otolith lengths of *C. thrissa* and *N. nasus* showed a linear regression (r = 0.95 and 0.91), with the best-fit equation of y = 0.0201x + 0.8133 and y = 0.0266x + 0.06, respectively.

Otolith Sr:Ca ratios

The profiles of otolith Sr:Ca ratios showed a similar pattern in the two examined fish species (Figs. 7, 8). The Sr:Ca ratios from the core to the edge ranged from 0 to 5.7 × 10^{-3} and 0 to 4.9 × 10^{-3} for *C. thrissa* and *N. nasus*, averaging 1.8 × 10^{-3} and 2.3 × 10^{-3}, respectively. In addition, the Sr:Ca ratio in *C. thrissa* frequently approached almost zero, which was observed visibly at smaller 800 µm from the core (e.g., individuals VNC10, VNC9, VNC6 and VNC3) (Fig. 7).

Discussion

Identification by otolith morphology

Different morphology between the two studied species confirms the usefulness of using morphology of otoliths as an identification character at the species level in teleost fishes (Baker 2006, Lin & Chang 2012, Ta et al. 2015). It can be also seen that sagittal otoliths of the two current fish species are unique in having a deep excisural notch and a long and pointed rostrum (Figs. 4–6), somewhat similar to other clupeid species (Homauni et al. 2011, Lin & Chang 2012). The sagittal otolith of *C. thrissa* seems closer to that of *Amblygaster sirm* and *Herklotsichthys quadrimaculatus* of the same family (Lin & Chang 2012). However, the current otolith at 3.7 mm OL (Fig. 5) is different from that of *A. sirm* (3.88 mm OL) by having a smoother ventral margin and a rounded postrostrum edge (plate 3, 71 in Lin & Chang 2012). Furthermore, the shape of otoliths of specimens between 2.4 and 3.0 mm OL is somewhat different from that in *H. quadrimaculatus* (2.74 mm OL), especially of dorsal and posterior sides (plate 3, 71 in Lin & Chang 2012).

Sagittal otolith of *N. nasus* collected from Vietnam facing the Pacific Ocean resembles to that from the Persian Gulf facing the Indian Ocean as described by Homauni et al. (2011). Morphology of otoliths has slight geographical variations within species because it is located inside the body, probably leading to a low impact by the ambient water environment. In addition, the otolith of *N. nasus* from the two sites is similar since a linear relationship between otolith size and body length was observed, with r being near 0.9 (Homauni et al. 2011). Morphological comparisons between *N. nasus* (present study) and two close species collected from Taiwan, *Nematalosa come* (3.31 mm OL) and *N. japonica* (3.82 mm OL) (Lin & Chang 2012), show few differences. Otoliths of *N. nasus* (3.4–3.5 mm OL) (Fig. 6) seem to have a shorter rostrum (a distance from tip of the rostrum to the notch), which is 1/3 smaller than OL in both species from

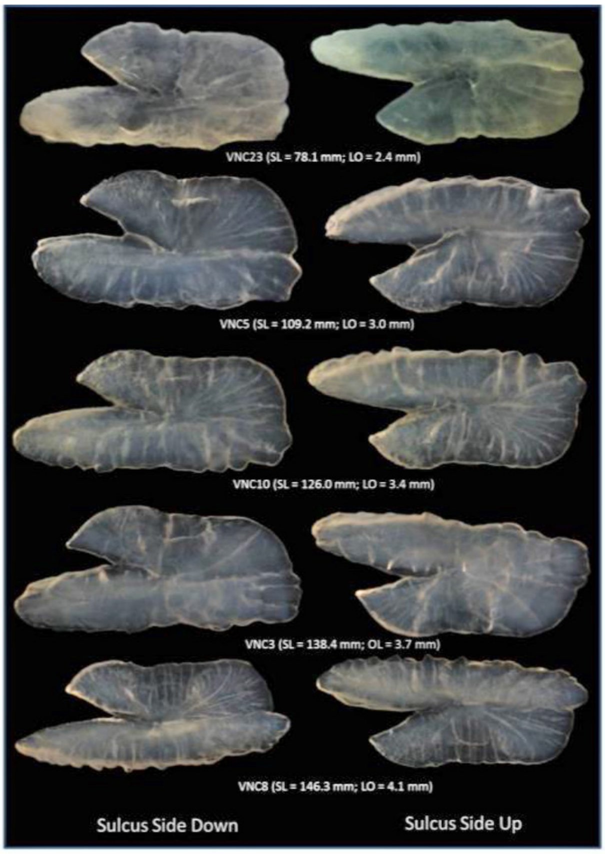

Fig. 4. Morphology of the sagittal otoliths of *Clupanodon thrissa* collected from the Nhat Le River, central Vietnam.

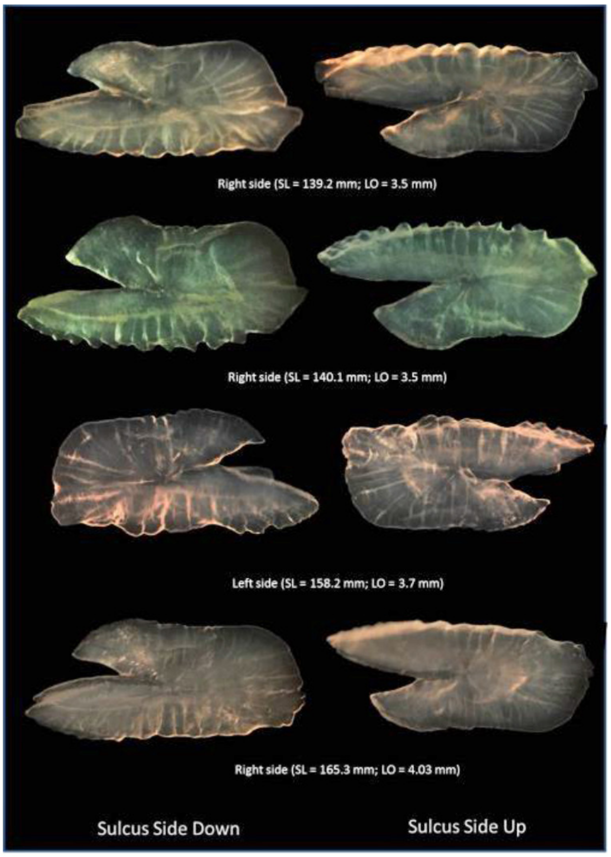

Fig. 5. Morphology of the sagittal otoliths of *Clupanodon thrissa* collected from the Red River, northern Vietnam.

Taiwan (Lin & Chang 2012). The otoliths studied by us differ from those of *N. come* and *N. japonica* by the characteristics of the postrostrum and the dorsal margins, respectively, as described by Lin & Chang (2012). Although *N. nasus* in the Indo-Pacific waters may be a complex of species and the taxonomic status of the populations species should be further confirmed in a wider geographical range (Whitehead 1985), otoliths of *N. nasus* from the central Vietnam can be used to distinguish it from the two closely related species. This indicates that the populations identified as *N. nasus* in Vietnam should be examined for the possible confirmation of their status of a distinct species as revealed based on the otolith morphology.

Migratory patterns

The typical catadromous and anadromous fish species show clearly different patterns of the otolith Sr:Ca ratios; this ratio in the sea is usually higher than that in freshwaters. In case of catadromous fishes such as river eels, this value is higher than 10 x 10^{-3} during the ocean life and then decreases to ca. 0-2.5 × 10^{-3} when fishes migrate and stay in freshwater (Tsukamoto & Arai 2001). Opposite data could be found in anadromous fishes such as salmons; in them, the Sr:Ca ratio is below 3 × 10^{-3} and tends to increase with the fish migration to the ocean (Arai & Tsukamoto 1998).

Fig. 6. Morphology of the sagittal otoliths of *Nematalosa nasus* collected from the Nhat Le River, central Vietnam.

The present data exhibit a different pattern as the two fish species seem to spawn in freshwater

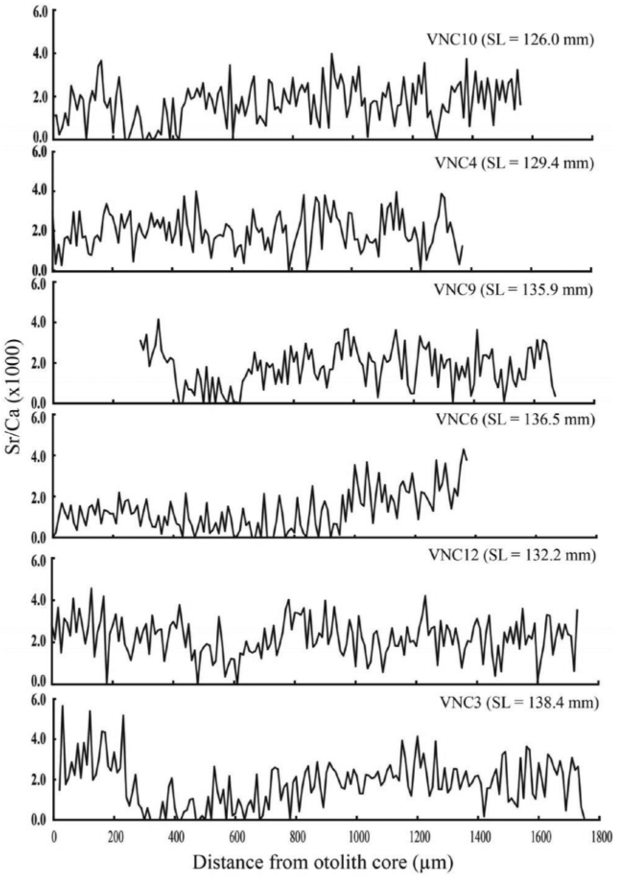

Fig. 7. Profiles of Sr/Ca ratios from the core to the edge of the sagittal otolith in *Clupanodon thrissa* collected in April 2018 from the Nhat Le River, central Vietnam.

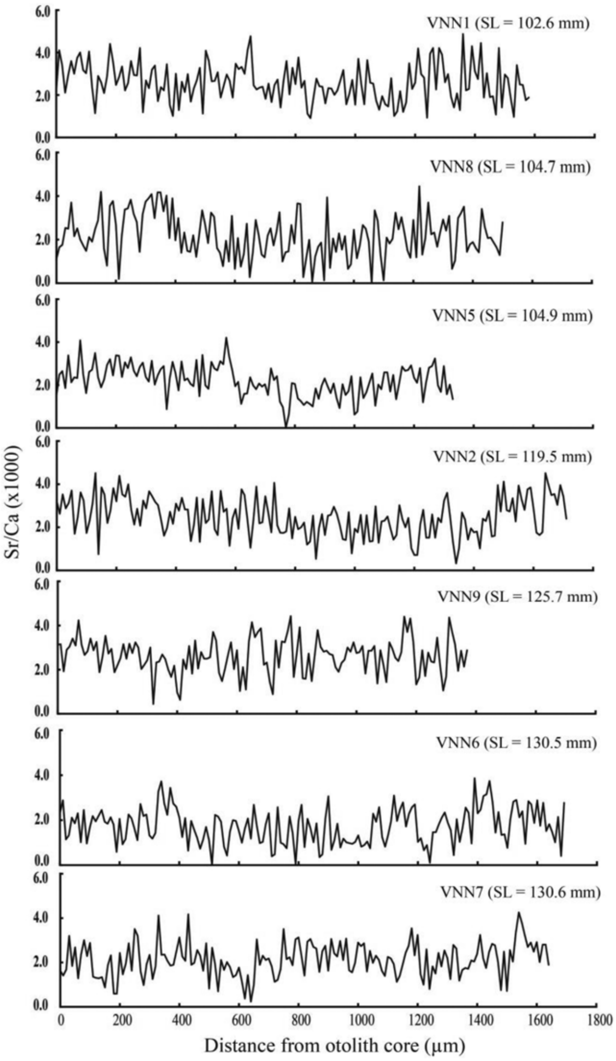

Fig. 8. Profiles of Sr/Ca ratios from the core to the edge of the sagittal otolith in *Nematalosa nasus* collected in 2018 from the Nhat Le River, central Vietnam. *VNN1* collected in July, *VNN2, 5, 7–8* in May, and *VNN6-7* in April.

or brackish water based on their otolith Sr:Ca ratio being at the core mostly from 1 to 3 × 10^{-3} (Figs. 7, 8). The otolith Sr:Ca value is affected by the environmental water Sr:Ca (Thorrol et al. 1998, Campana 1999, Brown & Severin 2009). The water Sr:Ca ratio often has high values in freshwaters in response to the soil content (Singh et al. 1998). This ratio is defined in brackish water as being 4.0–8.0 × 10^{-3}, in freshwater as < 4.0 x 10^{-3} and in seawater as > 8.0 × 10^{-3} for the case in the Yakugachi River, Amami Oshima Island, Japan (Murase & Iguchi 2019). From the hatching to the collection date, the Sr:Ca ratio of the two studied species mainly fluctuates between almost zero and 4 × 10^{-3} (Figs. 7, 8). Thus, these fishes might not usually stay in sea water during the period of feeding as do anadromous fish species (Arai & Tsukamoto 1988).

Of the two species, *C. thrissa* in central and northern Vietnam might show a higher tolerance to freshwater based on the present findings that the Sr:Ca ratio of several individuals is almost zero (Fig. 7). Nguyen (2005) revealed that this fish species migrates a long distance from the Red River mouth for spawning. Monthly ichthyoplankton collections from the Ba Lat Estuary recorded eggs and larvae of this species (Tran, unpublished data), which will add more data to understand its migration pattern.

It is of interest to note that the two species cooccurred in the central part of Vietnam in several months (Table 1) not only during reproductive seasons, which is from March to May for *C. thrissa* and from April to November for *N. nasus* (Nguyen 2005, Ministry of Science and Technology of Vietnam 2007). During field surveys from March 2018 to February 2020 (Tran, unpublished), we recorded a number of *C. thrissa* in Tien Hai Nature Reserve and Xuan Thuy National Park of the Ba Lat Estuary in all months. Additionally, almost all individuals of the two species from the Nhat Le River used in this study had small testes and ovaries (Tran, unpublished), indicating that these fishes might be foraging in estuaries.

The present results indicate that the two fish species tend to stay within estuarine habitats, differing from anadromous fish that enter the river for the spawning purpose only. This conclusion is in agreement with Gross et al. (1988) indicating that the productivity in lower latitudes is higher in freshwaters than in the ocean, thus determining the retaining of fish in estuaries for feeding. Similar observations can be found in other works from Vietnam (Tran et al. 2012, 2018, Tran & Ta 2016). This explanation is also in agreement with the evolution of diadromy (Gross et al. 1988, McDowall 1988).

The two studied species are endangered and listed in the Vietnam Red Data Book; their fishing has recently been reduced due to their over-exploitation (Nguyen 2005, Ministry of Science and Technology of Vietnam 2007). Thus, the migration patterns revealed by the present study are worthy for further measures for protection and sustainable exploitation of these two commercial fish species in Vietnam.

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