Tropical Natural History 16(2): 107–117, October 2016

Occurrence of *Hypoatherina valenciennei* (Bleeker, 1854) Post-larvae and Juveniles Collected at Estuarine Habitats of Northern Vietnam

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Received: 7 December 2015; Accepted: 4 September 2016

ABSTRACT.– Development and distribution of *Hypoatherina valenciennei* are given based on 376 specimens of 6.6–24.1 mm body length caught from the Tien Yen and Kalong estuaries, Northern Vietnam from March 2013 to September 2015. Post-larvae and juveniles of *H. valenciennei* collected from Vietnam are elongated laterally compressed body, short trunk, compact gut and long tail. There were no spines on the head. Post-larvae had heavy pigment over the midbrain, on the dorsal surface of the gut and on mid-lateral surface, and did not arrange in a broken line, whereas juveniles had melanophores arranged in a broken line. Meristic values: D IV~V, I-8~10; A I,11~13; P_1 I,5; P_2 12–15. Preliminary data of otolith was first record in the present study. Sagitta was the largest otolith, whereas dimension of asteriscus became larger than lapillus with the development. The post-larvae and juveniles occurred primarily in rainy season from April to November when water temperature was from 21.7 to 30.2°C, and used outer parts of the Tien Yen and Kalong estuaries where salinity ranged from 5.2 to 21.0 psu as their nursery areas.

KEY WORDS: ***Hypoatherina valenciennei*, ontogeny, distribution, estuary, otolith**

INTRODUCTION

Sumatran silverside, *Hypoatherina valenciennei* a common atherinid fish inhabiting near coastal waters and estuaries (White *et al.*, 1984), and has a widely distributional range in Southwest Pacific and in Western Indian Ocean (Ivantsoff & Kottelat, 1988). Even Sumatran silverside has been recorded from various sites of Vietnam (Nguyen, 2005), early stages and distribution pattern during these periods have not received any attention. Early stages of this species and its congeners (e.g. *H. tsurugae*) were described by Leis & Rennis (1983), White *et al.* (1984), Takita & Nakamura (1986) and Tsukamoto & Kimura (1993), but most of these studies used laboratory-reared specimens. In the wild, early stages of *H. valenciennei* were examined morphologically for specimens ranging 3.7 mm to 20.2 mm in total length (Takita & Kondo, 1984). In the laboratory, the embryonic development and pre-larva of this fish was described based on the wild ripe males and females that were successful in artificial insemination and eggs collections (Takita & Nakamura, 1986). The two mentioned works shared resemble features of this fish under different environments. In particular, the former provided information about distribution of the larvae and juveniles in coastal water of Omura Bay. The young larva stage tended to be distributed in the surface layer, but well-grown larvae and juveniles were assembled along the sea-wall (Takita & Kondo, 1984). However, relationship between its occurrence and water conditions

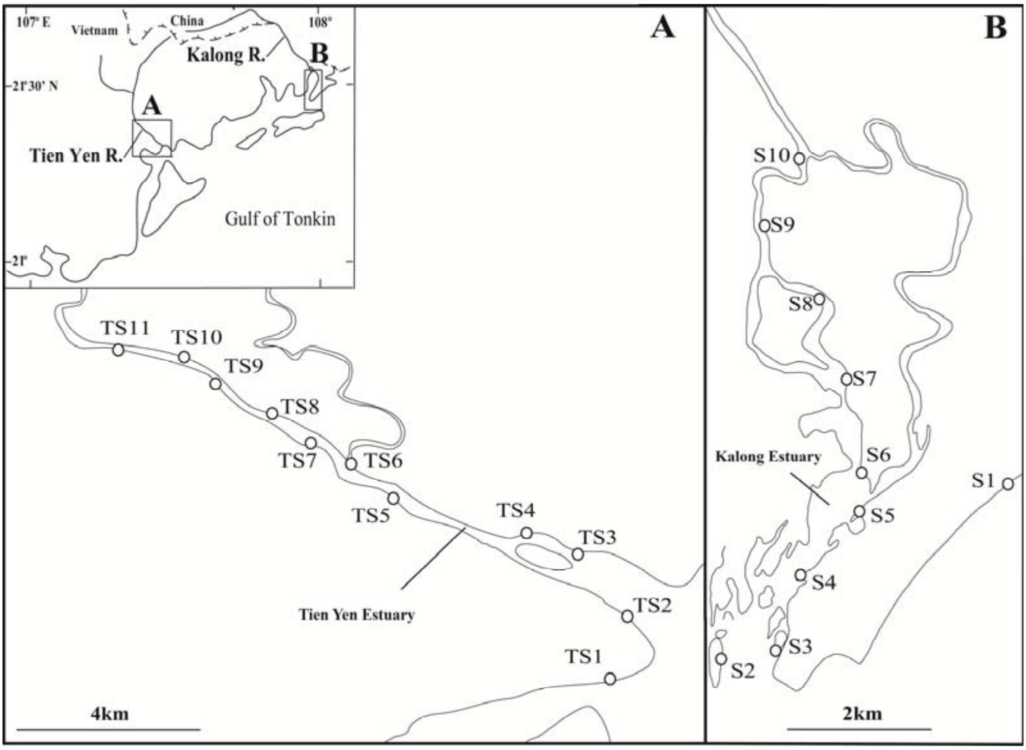

FIGURE 1. Chart showing stations (open circles) where larvae and juveniles of *Hypoatherina valenciennei* were collected in the shallow waters of the Tien Yen and Kalong estuaries in northern Vietnam from March 2013 to September 2015.

was not mentioned yet (Takita & Kondo, 1984).

Recently, otolith is considered to be a powerful tool for fish identification because of its species-specific although it sometimes appears variable morphology among species. Some studies of otolith have been carried out to estimate age of *Hypoatherina* sp. larvae (Subiyanto *et al.*, 2009) and to examine the rate of increment formation of *Hypoatherina tropicalis* (Schmitt, 1984). Recent evidences suggest that temperature, food availability, photoperiod, and developmental stage would have affected on the increment formation of fish. Thus, data on otolith of *H. valenciennei* collected from estuarine habitats would be fundamental for fish identification and preliminary aspects for studies on geographic variations.

Surveys conducted from the Tien Yen and Kalong estuaries in northern Vietnam collected a number of post-larvae and juveniles of *H. valenciennei*, whose adults were recorded at the former river (Tran & Ta, 2014a). Recently, much work have been studied of the importance of the two above estuaries for early stages of fishes, such as *Oryzias curvinotus*, *Nuchequula nuchalis* and species of genus *Lateolabrax* (Tran & Ta, 2014b and 2016; Tran *et al.*, 2016). These studies suggested that the center part of the estuarine environments plays a significant role for larvae and juveniles of the three given species. Herein, the present study attempts to elucidate the distributional pattern of post-larvae and juveniles of *H. valenciennei* in an estuarine habitat, and to provide an external and internal (otolith) morphological development of this fish in Vietnam.

MATERIALS AND METHODS

We collected early stages of *H. valenciennei* in the bank waters of the Tien Yen and Kalong estuaries, Quang Ninh Province in Northern Vietnam from March 2013 to September 2015 (Fig. 1). Collections were made monthly using a small seine net (1 × 4 m, 1 mm mesh-aperture). Two persons kept the net stretched, and waded backward in the waters, from ankle- to neck-depth along the shore-line for a distance of ca. 50 m (2 min.). A day's collection usually consisted of one to four hauls at each bank water station. Catch Per Unit of Effort (CPUE) is the number of individuals in each haul (ca. 2 minutes or 50 m). Water temperatures (°C), salinities (psu) and turbidity (NTU) were measured at each station during the sampling periods using a Water Quality Checker (WQC-22A, TOA DKK – temperature: 0-50°C ± 0.1°C; salinity: 0-4psu ± 2.5%; turbidity: 0–800NTU ± 0.1%).

Samples were fixed in 4-5% (w/v) buffered formalin solution in the field and then sorted specimens were preserved in 70% (v/v) ethanol, subsequently being measured their sizes by developmental stages (Kendall *et al.*, 1984). In this study, unlabeled lengths indicate body length (BL) (notochord length for flexion larvae and standard length for postflexion larvae and juveniles); in fin ray counts "i" indicates a forming spine. Specimens used in this study are deposited in Laboratory of Fish, Faculty of Biology, Hanoi National University of Education. The size ranges of the larvae on which the descriptions were based were: 75 flexion larvae, 6.6–7.8 mm; 268 postflexion larvae, 7.6–12.8 mm and 33 juveniles, 12.5–24.1 mm. Three specimens used for drawing were deposited at the above university (BHNUE 25101–25103). Observations and drawings of *H. valenciennei* were made with a binocular microscope and camera lucida.

Methods of removing and observation as well as description of otolith followed Ta *et al.* (2015). In the present study, to have an overview of otolith morphology, we removed both sides of otolith from 15 selected *H. valenciennei* individuals, resulting in a total of 24 sagittae, 17 lapilli and 7 asterisci were removed. All 24 sagittae were used to measure the dimension, the longest axis.

RESULTS

Identification of *Hypoatherina valenciennei* larvae and juveniles

Larvae and juveniles of the *H. valenciennei* were identified to species level based on the following combination characteristics: elongate laterally compressed body, short trunk, compact gut and long tail. The head length was longer than the body depth. There were no spines on the head. Larvae had heavy pigment over the midbrain, on the dorsal surface of the gut and on mid-lateral surface, not arranged in a broken line. However, juveniles had melanophores arranged in a broken line on mid-lateral surface and second dorsal fin origin almost above base of 4th anal fin ray. Meristic values: D IV~V, I-8~10; A I,11~13; P_1 I,5; P_2 12–15. These characteristics tally with description of Takita & Kondo (1984) and a key provided by Tsukamoto & Kimura (1984). In

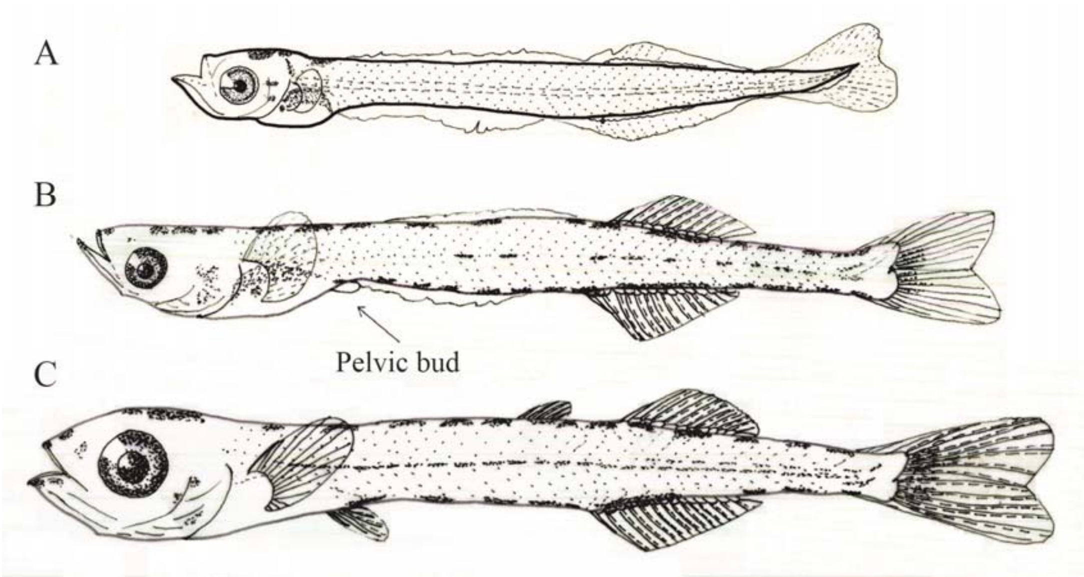

FIGURE 2. Development of *Hypoatherina valenciennei* in Vietnam. A. 6.6 mm flexion larva. B. 10.1 mm postflexion larva. C. 13.4 mm juvenile.

addition, meristic fins are important characteristics for identification to species level of *H. valenciennei* or *H. tsurugae* in juvenile stage (Kimura & Tsukamoto, 1993). They are similar in meristic values, but second dorsal fin origin is anterior to base of second anal fin ray in the latter species (Fig. 4 in Kimura & Tsukamoto, 1993) and above base of 4th anal fin ray in the former one (Fig. 2C).

Description of development (Table 1 and Fig. 2)

Morphology: post-larvae and juveniles of *H. valenciennei* are elongate (Body depth = 7.4–17.9% Body length, BL), laterally compressed and shallow body with a broad and a very short head (Head length = 13.2–28.2% BL) (Table 1). The gut was compact, and it increases in length with growth (Preanal length = 19.1–45.3% BL) (Table 1). The gas bladder was not apparent (Fig. 2). The snout was short (Snout length = 1.4–7.7% BL) (Table 1). The eye was round (Orbit diameter = 5.9–11.3% BL) (Table 1). The gill membranes were free from the isthmus. No spines were on the head (Fig. 2). The flexion of the notochord was completed by 7.8 mm BL larvae. The scale appeared by 16.2 mm BL juvenile. The small teeth were formed along the edges of the upper and lower jaws.

Meristic: the finfold of dorsal, anal fins and the pectoral bud appeared during flexion-larva period (Fig. 2A). The pelvic bud occurred by 10.1 mm BL larvae (Fig. 2B). Adult compliments of D IV~V, I-8~10; A I, 11~13; P_1 I, 5; P_2 12–15 were achieved by 11.8 mm, 8.0 mm, 10.3 mm and 10.5 mm BL, respectively (Table 1). In the juvenile stage, the second dorsal fin origin

TABLE 1. Morphometric (mm) and meristic values in early stages of *Hypoatherina valenciennei* collected from the Tien Yen and Kalong estuaries of northern Vietnam

Body length	Preanal length	Predorsal length	Body depth	Head length	Orbit diameter	Snout length	Dorsal fin	Anal fin	Pelvic fin	Pectoral fin
Flexion larvae										
6.6	1.2		0.5	0.8	0.3	0.1	Finfold	Finfold		Bud
7.4	1.6		0.8	1.1	0.5	0.1	Finfold	Finfold		Bud
Postflexion larvae										
8.0	2.1		0.8	1.3	0.6	0.2	Finfold,I-9	I,12		Bud
8.4	2.1		0.8	1.3	0.6	0.2	Finfold,I-9	I,12		Bud
9.2	2.2		0.8	1.4	0.6	0.2	Finfold,I-9	I,11		Bud
9.5	2.3		0.8	1.5	0.7	0.2	Finfold,I-9	I,11		6
10.1	2.7	5.3	0.8	1.5	0.8	0.3	Bud,I-10	I,11	Bud	10
10.3	2.7	5.4	0.9	1.6	0.8	0.3	v,I-11	I,12	Bud	12
10.5	2.8	5.5	0.9	1.7	0.9	0.4	iv,I-10	I,11	I,5	12
11.0	3.3	5.6	1.1	2.0	0.9	0.4	IIii,I-10	I,12	I,5	12
11.2	3.5	5.7	1.2	1.9	1.0	0.4	IIIi,I-10	I,12	I,5	13
11.8	3.7	5.8	1.4	2.2	1.1	0.5	IV,1-9	I,11	I,5	14
12.5	4.4	6.1	1.5	2.7	1.1	0.5	V,I-9	I,12	I,5	15
Juveniles										
13.5	4.8	6.5	1.6	2.5	1.3	0.3	IV,I-10	I,13	I,5	13
13.7	5.2	6.7	1.7	2.7	1.3	0.5	IV,I-10	I,12	I,5	12
14.4	5.5	7.6	1.8	3.0	1.4	0.4	V,I-10	I,12	I,5	14
15.0	6.8	7.8	2.1	3.3	1.5	0.7	V,I-10	I,12	I,5	16
15.7	6.9	8.5	2.2	3.5	1.7	0.6	V,I-10	I,11	I,5	15
16.3	7.2	8.2	2.4	4.1	1.6	1.0	V,I-10	I,12	I,5	14
17.0	7.4	9.0	2.5	4.5	1.8	1.1	V,I-8	I,11	I,5	15
19.5	8.5	10.5	3.5	5.5	2.2	1.5	V,I-10	I,13	I,5	15
24.1	9.3	12.8	3.9	5.6	2.3	1.2	V,I-10	I,13	I,5	15

was located almost above base of 4th anal fin ray (Fig. 2C).

Pigmentation: At 6.6 mm BL flexion-larva: three large conspicuous melanophores were present over the midbrain and one on the back of the neck. Two branched melanophores were distributed on the opercle. A patch of tiny melanophores appeared on the dorsal surface of the gut (Fig. 2A). At 10.1 mm BL postflexion larvae: branched melanophores appeared on the snout and posterior corner of the lower jaw; formed a single row along the dorsal contour between the head and the caudal peduncle, except along the second dorsal fin base, where they occurred in two rows. Melanophores formed a single row on the mid-lateral surface but not arranged in a

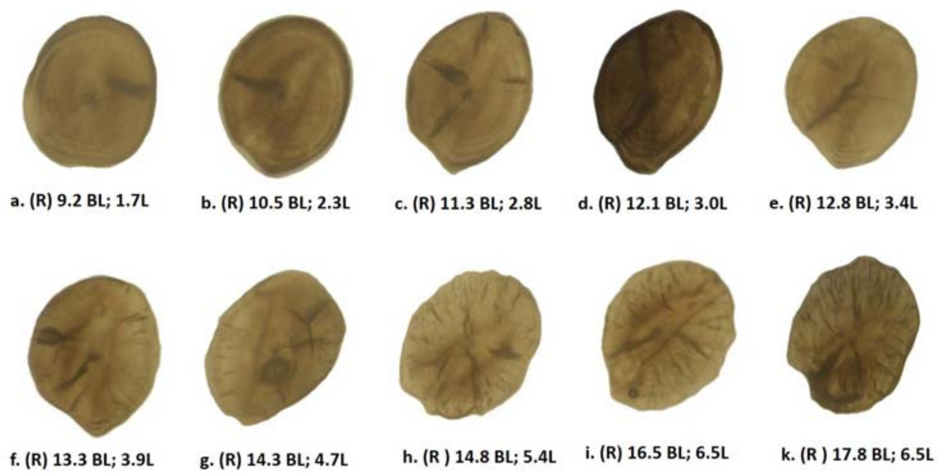

FIGURE 3. Morphology of sagittae of *Hypoatherina valenciennei* collected from the Tien Yen and Kalong estuaries. R. right sagittal, BL. Body length, L. the longest axis (dimension).

broken line. Amongst all fins, tiny melanophores appeared only on the caudal fin (Fig. 2B). At 13.4 mm BL juvenile, melanophores became denser and a series of punctuate melanophores appeared as a single black line from above the pectoral base to the caudal peduncle (Fig. 2C). Above this line, another of melanophores appeared as a parallel black line running from the third fin of the second dorsal ray to the caudal peduncle (Fig. 2C).

Description of otolith (Figs. 3, 4, 5)

Sagittae. The sagittae are round-shaped, moderately thick, and somewhat changed with growth (Fig. 3). The change of sagittal morphology was clearly seen for the excisural notch, rostrum, the depth of sulcus and the surface. The excisural notch, rostrum and sulcus were first found at the 10.5 mm BL specimen (Fig. 3b). The margin was still smooth until 14.4 mm BL specimen (Fig. 3g). The sagittal rings could be observed on specimens less than 12.8 mm BL (Fig. 3e). The relationship between the sagittal radius and BL of *H. valenciennei* ranging from 9.2 to 17.8 mm BL showed a good linear regression (r = 0.96), with the best fit equation of $y = 0.175x + 0.648$.

Lapilli. Morphology of the lapillus appeared to be relatively stable over the developmental period (Fig. 4). Lapilli were nearly round-shaped, and the nucleus was slanting. It was hard to observe rings on lapilli in this study (Fig. 4). During the development of the lapilli, the mesial surface was not changed, but the shape was somewhat varied (Fig. 4).

Asterisci. The asterisci were duck mussel shell-shape (Fig. 5). Rings appeared clearly on asterisci of 14.3 to 16.5 mm BL specimens. In the present study, among the three pairs of otolith, the asterisci were the most difficult to remove successfully.

Distribution

The distribution of Sumatran silverside is described in Fig. 6 and Table 2. Post-larvae and juveniles of *H. valenciennei* mainly occurred in rainy season (from April to November) (Fig. 6). At that time, the water temperature was high, from 21.7 to 30.2°C

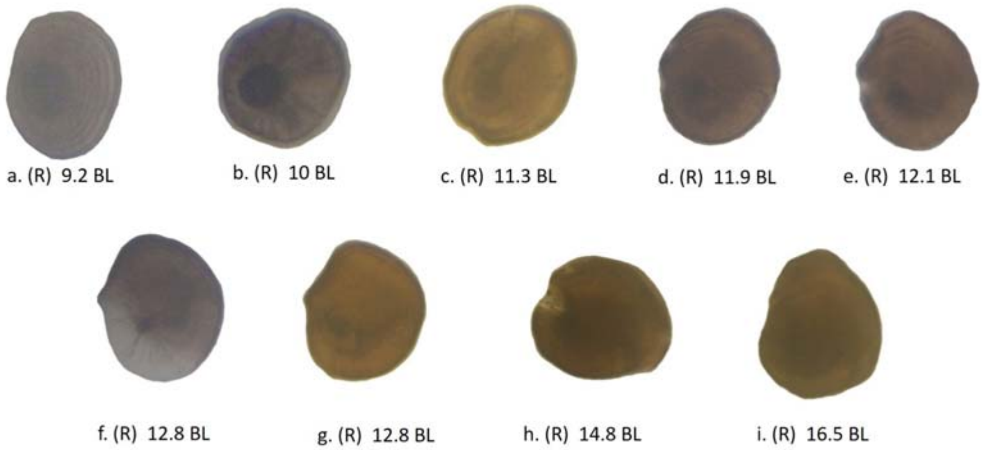

FIGURE 4. Morphology of lapilli of *Hypoatherina valenciennei* collected from the Tien Yen and Kalong estuaries. Otherwise same as Fig. 3.

(Table 2). They occurred at the outer part of the estuary, at which salinity was higher and fluctuated between 5.2 and 21.0 psu (Table 2). The turbidity at which the fish was collected was from 2.0 to 70.0 NTU (Table 2).

In the Tien Yen estuary, there were two periods of fish occurrences in 2014, i.e., April to May and September to October. In 2015 from the Tien Yen estuary, *H. valenciennei* were collected only in April (Fig. 6, Table 2). In the Kalong estuary, this fish were found in April and November (Fig. 6, Table 2). The flexion larvae were collected only from the Kalong estuary. The average of body length of fish in the first period (i.e., April and May) was smaller than that in the second one (August to November) in the both sites, except in September 2013 of the Tien Yen estuary (Fig. 6).

DISCUSSION

When *H. valenciennei* post-larvae and juveniles from Vietnam were compared with early stages of this species from Japan (Takita & Kondo, 1984; Takita & Nakamura, 1986), there were few differences in pigmentation. Menanophores on the mid-lateral surface of the body and on the edge of caudal appeared earlier in the specimens from Japan than that in Vietnam (4.6 vs. 7.0 mm BL) (Takita & Kondo, 1984) contrary to that on the dorsal and ventral edges of the body. At 10.1 mm BL larva of the present study, there were two clear rows of branched melanophores along the second dorsal fin base and 6 branched melanophores on the ventral edges of the abdomen (Fig. 2B). However, similar melanophores appeared at larvae beyond 12 mm BL in Japan (Takita & Kondo, 1984). Post-larvae and juveniles from the Tien Yen and Kalong estuaries had no menalophores on the top of fin ray of the pectoral fin which is presented at 16 mm BL larva in Japan (Fig. 2, Takita & Kondo, 1984). Generally, laboratory-reared fish larvae had been shown to have greater pigmentation than wild-caught larvae (Miyashita *et al.*, 2001), but the post-larvae of this fish under the laboratory did not bear two rows of

TABLE 2. Distribution of *Hypoatherina valenciennei* larvae and juveniles from the Tien Yen and Kalong estuaries of northern Vietnam

Station	Time	CPUE	Stage	Temperature (°C)	Salinity (psu)	Turbidity (NTU)
Tien Yen Estuary						
TS1	Apr. 2013	8.7	D, E	22.2	21.0	10.0
	May 2013	2.0	D	27.3	18.6	2.0
	Aug. 2013	1.0	E	30.2	13.7	2.0
	Oct. 2013	1.5	D, E	25.9	20.3	70.0
TS3	Apr. 2013	4.0	D	21.7	12.1	22.0
TS4	Sep. 2013	2.0	D	28.3	5.2	3.0
TS6	Apr. 2015	89.0	D, E	23.6	11.3	2.0
Kalong Estuary						
S2	Aug. 2015	21.4	C, D, E	30.9	9.3	14.0
S3		66.9	C, D, E			
S6	Nov. 2015	2.0	E	22.4	9.9	5.0

CPUE: individuals per haul (about two minutes net towing); Developmental stage: C. Flexion larvae, D. Post-flexion larvae, E. Juveniles

melanophores on the lateral lines that are the specific traits in the wild (Takita & Kondo, 1984; Takita and Nakamura, 1986). Consequently, it could be concluded that melanophores should be considered to be more potential variations among different localities rather than other external morphological traits in Sumatran silverside during early stages. It needs to be further examined to prove the above presumption. Additionally, it is stated that the rearing temperature influences on body shape and meristic characters in fish juveniles (Georgakopoulou *et al.*, 2007; Sfakianakis *et al.*, 2011). The sea bass (*Dicentrarchus labrax*) had more slender body at 15°C than that at 20°C (Georgakopoulou *et al.*, 2007). The body depth of post-larvae and juveniles herein were similar to those in Takita & Kondo (1984) in spite of somewhat different in water temperatures when the fish was collected among the two studies, which ranged from 21.7°C to 30.9°C (Table 2) and from 19.0 to 31.6°C (Takita & Kondo, 1984).

Otolith morphology of this genus species has been poorly known, and the present data is the first record for *H. valenciennei* in post-larval and juvenile stages. Like other fishes, the sagitta was the largest otolith.

FIGURE 5. Asterisci of *Hypoatherina valenciennei* collected from the Tien Yen and Kalong estuaries. Otherwise same as Fig. 3.

However, dimensions of lapillus and asteriscus in 12.8, 14.3, 14.8 and 16.5 mm BL fish were 1.5 and 1.4, 1.7 and 1.7, 1.9 and 2.1, and 2.3 and 2.5 µm, respectively, unlike that in *Nuchequula nuchalis* collected from the Tien Yen estuary (Ta *et al.*, 2015). Of the three pairs of otolith components in *H. valenciennei* larvae and juveniles, the asteriscus was a different shape (Figs. 3, 4, 5). In 16.2 mm BL *H. tropicalis*, the shape of sagitta seems to be similar to that in the 16.5 mm BL of the current study (Schmitt, 1984), with respect to the rostrum (Fig. 3i), but the margin of the former one seems smoother (Schmitt, 1984). In comparison with otolith of *Nuchequula nuchalis* larvae and juveniles collected in the Tien Yen estuary (Ta *et al.*, 2015), the present samples showed a more stable pattern of morphological change throughout the larval development (Figs. 3, 4, 5). Otolith is considered to be different among species, thus the current data on the otolith shape and ontogeny would have been used as a crucial key for further identification into species level of this genus.

In Omusa Bay (Japan), larvae and juveniles of this species occurred abundantly from June to early September (Takita & Kondo, 1984). However, in northern Vietnam, their seasonal occurrences tended to be longer. They appeared from April to May and from August to November (Table 2; Fig. 6) at various sizes and development stages in the research area. It can be clearly seen from the figure 6, in the Tien Yen estuary, there were different between 2014 and 2015 results. This is probably due to difference in sampling stations during the study period. During the former year, collections were made only outer stations of the estuary. On the contrary, TS6 was the outermost sampling sites in the latter year (Fig. 1). This is first information of this fish temporal and spatial occurrences during the post-larval and juvenile stages in estuarine environments in the world. They tended to be distributed at outer parts of the two estuaries (Table 2; Fig. 1) and the salinity when the post-larvae and juveniles occurred ranged from 5.2 to 21.0 psu (Table 2). The spatial occurrence of the present post-larvae and juveniles are somewhat consistent with that in *Sillago sihama*, which were found abundantly at the outer station of the Tien Yen esturay where the salinity ranged from 10 to 20 psu (Tran *et al.*, 2015) and different from *Oryzias curvinotus* that were distributed abundantly in the range of 0.6 to 2.5 psu (Tran & Ta, 2016). The stages prior to the flexion were not collected in this study (Table 1), hence, this fish should be a marine migrant species. However, a further

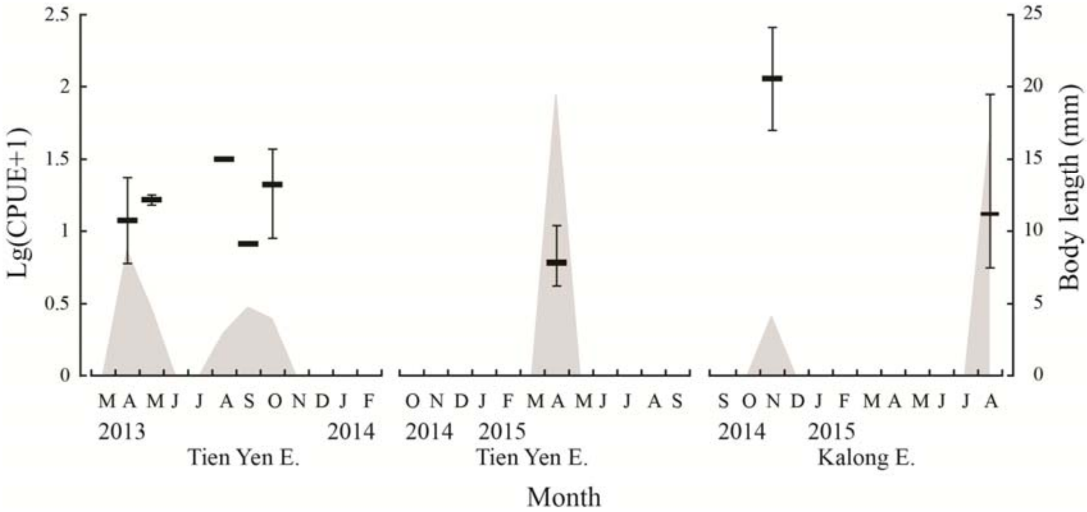

FIGURE 6. Seasonal occurrence of *Hypoatherina valenciennei* larvae and juveniles in the shallow waters of the Tien Yen and Kalong estuaries. Horizontal and vertical bars indicate the mean and the range of the body length, respectively.

investigation needs to be examined to complete its life cycle in the research area.

The body length of collections was irregular changes (Fig. 6), but its sizes tended to increase during their occurrence periods from April to May and from September to October 2013 (Fig. 6). Both post-larva and juvenile stages appeared in some months together (Fig. 6, Table 2). These proofs prove that the above stages of *H. valenciennei* reside in shallow waters of the Tien Yen and Kalong estuaries but swelling river water made the estuarine environment unstable and seemed to inhibit stable recruits into there. This is similar to species of group C in Fujita *et al.* (2002) and criteria of Heupel *et al.* (2007) who determined the types of fish either residents or migrants. Therefore, it is supposed that the outer parts of the Tien Yen and Kalong estuaries are an important area for the post-larvae and juveniles of *H. valenciennei*.

ACKNOWLEDGEMENTS

This study is funded by the following grants: Nagao Natural Environment Foundation (Japan), Vietnam National Foundation for Science and Technology Development (NAFOSTED, Vietnam – grant number: 106-NN.05-2014.03) and International Foundation for Science (IFS, Sweden – grant number: A/5532-1). We also express our thanks to Ms. To Thuy Dung for her support in laboratory and processing images of otoliths.

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