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Downstream migration of newly-hatched ayu (*Plecoglossus altivelis*) in the Tien Yen River of northern Vietnam

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Abstract The ayu (*Plecoglossus altivelis*) is an annual, amphidromous, plecoglossid fish, distributed in Vietnam, China, Taiwan, Korea, and Japan. To date, ayu have been found only in two rivers in northern Vietnam, where little is known about their life history. The Tien Yen River is believed to be the most southwestern habitat for this species. To determine whether newly hatched ayu larvae drift and to understand their downstream migration, intensive surveys were conducted in the Tien Yen River from October to March of 2013–2016. In total, 529 drifting ayu larvae were collected from four of six sampling stations along the river. Thus, ayu reproduction has been confirmed in this river for the first time, where only adult fish had been found previously. However, we did not successfully collect larvae in the eastern branch of the river, which has a hydroelectric dam, suggesting that ayu do not inhabit this branch or else do not reproduce there. The presence of drifting larvae in the western branch from mid-December to late January implies that they spawn from late November to mid-January. Drifting larvae were captured primarily at night, but peak occurrences varied depending upon the day and the sampling station. With the range of body sizes and variable diel abundance patterns, ayu in the Tien Yen River probably employ multiple spawning grounds. This study provides fundamental life history data for the vulnerable ayu populations in northern Vietnam.

Keywords Downstream migration · Drifting larvae · *Plecoglossus altivelis* · Spawning grounds · Vietnam

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Introduction

The ayu, *Plecoglossus altivelis* (Osmeriformes: Plecoglossidae), is distributed over the Japanese Archipelago, Korea, Taiwan, and along the Asian continental coast from Liaoning, China to northern Vietnam (Shan et al. 2005). It is a commercially important species as a food resource and as a target of recreational fishing, especially in Japan (Takahashi 2005). They have an annual, amphidromous life cycle. Ayu spawn in the lower reaches of rivers from fall to winter, where the demersal eggs are attached to pebbles on the riverbed. Just after hatching, the yolk-sac larvae drift downstream to coastal areas, where they stay throughout larval and early juvenile stages. In spring, juveniles ascend rivers, where they grow and mature (Ishida 1961; Takahashi et al. 1999; Kishino and Shinomiya 2004). Although three subspecies have been described for *P. altivelis*, including *P. altivelis altivelis* from

mainland Japan, *P. altivelis ryukyuensis* from the Ryukyu Archipelago in Japan, and *P. altivelis chinensis* from China (Nishida 1988; Shan et al. 2005), taxonomy of this species throughout its distribution has not yet been verified. So, the subspecies of these Vietnamese populations are not specified in this paper.

In Vietnam, ayu have been known only from Quang Ninh, a province facing the northern part of the Gulf of Tonkin. In addition to a population in the Kalong River, adjacent to the border between Vietnam and China (Tran et al. 2012a), the existence of ayu was recently reported from the Tien Yen River, 60 km west of the Kalong River (Tran et al. 2012b) (Fig. 1). As far as is presently known, ayu habitat in Vietnam is restricted to these two rivers, and the population in the Tien Yen River is very small (Tran et al. 2012b). Populations in Taiwan and Okinawa Island, Japan are extinct (Kawanabe 1972; Nishida et al. 1992; Tachihara 2015), suggesting that ayu populations in lower latitudes are vulnerable. The Vietnamese ayu populations are very important because they are the most southwestern populations of this species, but they are endangered due to modification of river structure, dam construction, land development in the basins, water pollution, and overfishing (Bui 2010; Dinh et al. 2014; Tran and Ta 2014). Although information about their life history is essential for conservation, little is known about these small ayu populations in Vietnam, except a few recent studies about larval occurrence and morphology in the Kalong Estuary (Tran et al. 2012a, 2014).

Early mortality of amphidromous species can occur in longer rivers, especially those with low flow rates. Because zooplankton are less abundant in rivers, more protracted downstream travel can result in larval starvation (McDowall 2009). Therefore, amphidromous fishes are more abundant in insular streams and rivers, but less common in large continental rivers (McDowall 2009; Maeda et al. 2015). Knowledge of larval drifting is important for conservation and management of stream ecosystems inhabited by amphidromous species (Luton

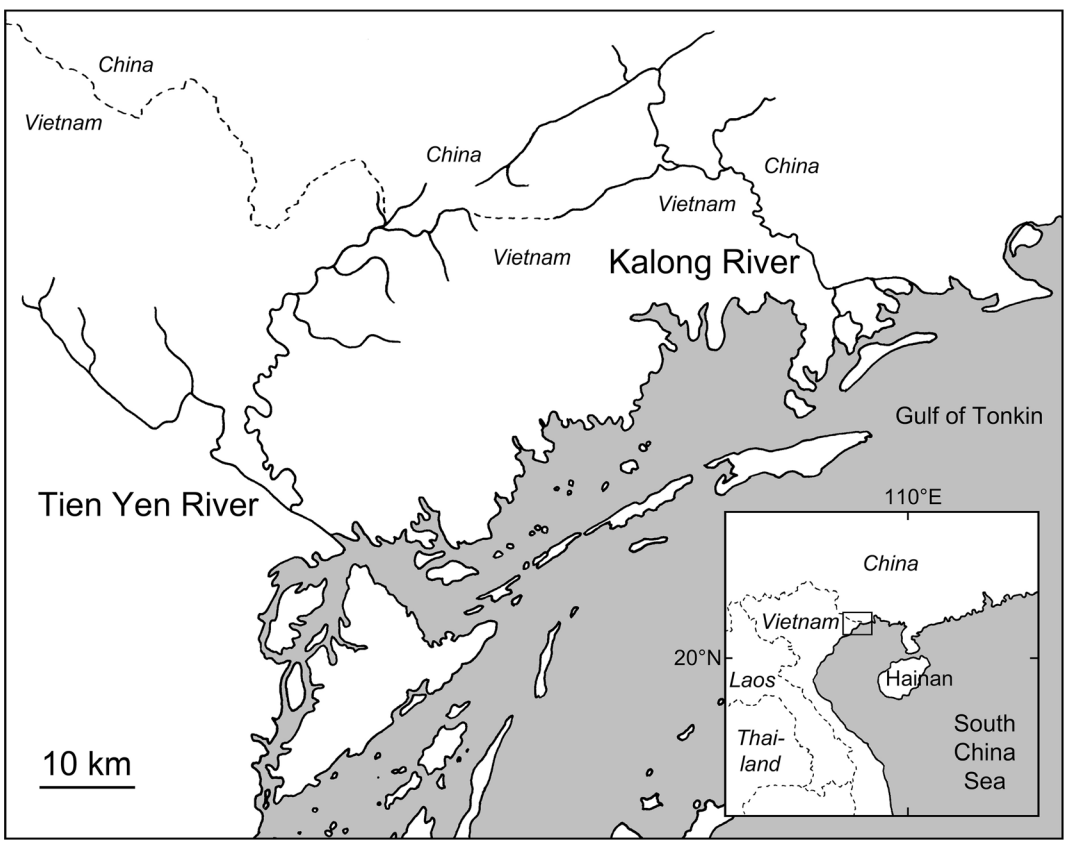

Fig. 1 Map showing the Kalong River and the Tien Yen River, where ayu have been reported in Vietnam. *Broken line*, border (rivers are also a border between Vietnam and China); *square on the inset map*, range of the magnified map

et al. 2005; Lechner et al. 2016). Nevertheless, no information is available for larval drifting of continental ayu populations, including those in Vietnam.

In the present study, drifting ayu larvae were collected along the Tien Yen River, Quang Ninh in northern Vietnam, to accomplish the following aims: 1) To determine whether ayu larvae are present, which would confirm their reproduction in the Tien Yen River. Although sampling to collect ayu larvae in estuaries of the Kalong and Tien Yen river systems was conducted from November 2010 to February 2011, only the former yielded ayu larvae (Tran et al. 2012a). Therefore, it was suggested that ayu might be absent in the Tien Yen River (Tran et al. 2012a). However, one adult ayu individual was collected in the upper reaches of the Tien Yen River in December of 2011, the first record of ayu in this river (Tran et al. 2012b). 2) The second purpose of this study was to determine whether ayu present in the eastern branch of the Tien Yen River. The western branch of the Tien Yen River, where adult ayu were found, has no major dam, whereas the eastern branch has the 8 m Khe Soong Dam. To date, no ayu have been recorded in the eastern branch. 3) The third purpose was to determine diel activity and seasonal abundance patterns of drifting ayu larvae in Vietnam. 4) The last purpose was to attempt to locate the ayu spawning grounds in the Tien Yen River and to understand their downstream migration, based on larval sampling at several sites along the river.

Materials and methods

Sampling sites

The Tien Yen River (ca. 82 km in length) originates at an elevation of 1175 m and has a basin size of 1070 km^2. In this area, the rainy season is from April to October, accounting for 85% of total annual precipitation. The dry season is from November to March. Annual precipitation ranges from 2000 to 2500 mm and differs between rainy and dry seasons. Annual water discharge averages from 10 to 100 m^3/s (Nguyen 1997).

The Tien Yen River has two major branches. Five sampling stations (D1–D5) were established along the western branch (Figs. 2 and 3), with the lowest station, D1, located approximately 15 km from the river mouth. The salt wedge usually reaches just below this station.

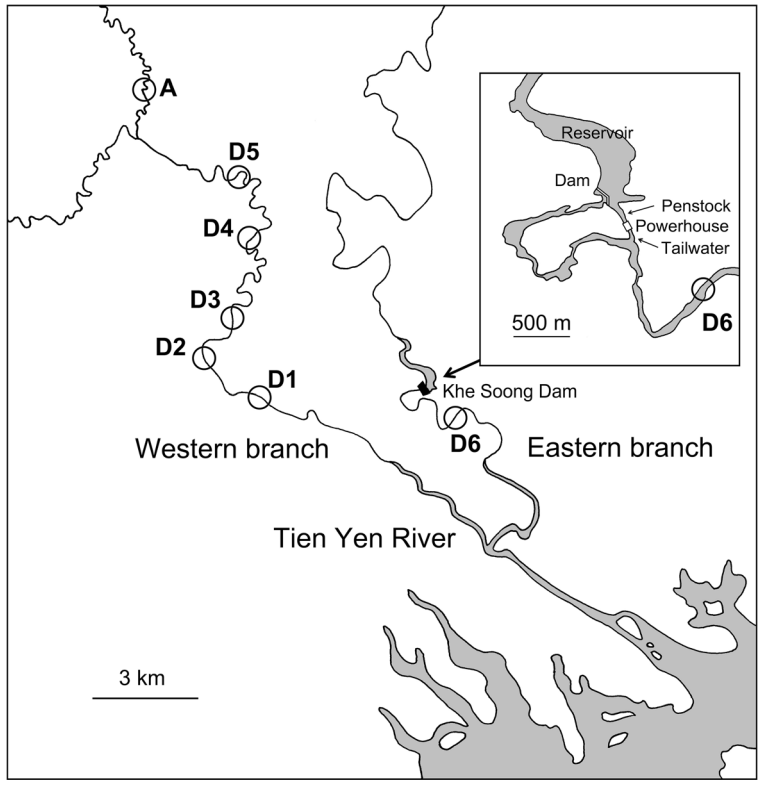

Fig. 2 Sampling stations (*open circles*, D1–D6) where drifting ayu larvae were collected in the Tien Yen River, northern Vietnam with a magnified insert showing the area around the Khe Soong Hydroelectric Dam. *A*, the site where a mature ayu was collected in Tran et al. (2012b)

Fig. 3 Photos showing scenes around sampling stations and a dam in the Tien Yen River, northern Vietnam. **a** a pool near station D1; **b** larger larva net (1 m diameter, 0.5 mm mesh size) set at station D3; **c** a rapid and pools around station D5; **d** site A in Fig. 2; **e** a rapid at station D6; **f** the Khe Soong Hydroelectric Dam

Stations D2–D4 were set between station D1 and the uppermost station, D5, located 13.5 km upstream from D1. The Tien Yen River has a repeating sequence of rapids and pools. For example, from stations D1 to D5 there are about 20 pools and 29 rapids (Fig. 3), at 100–900 m intervals (mainly 300 m). During the study period (from late October to March) in the dry season, the water level was not high, thus rapids and pools were easily recognized (Fig. 3). As mentioned above, an adult ayu was collected from the upper reaches of the Tien Yen River (A in Figs. 2 and 3d, Tran et al. 2012b). In addition, adult ayu were also collected around stations D4 and D5 in December 2014 and January 2015 during this study (Nguyen et al. 2015). The Khe Soong hydroelectric dam (Figs. 2 and 3) is located about 17 km from the river mouth on the eastern branch. The concrete dam has a height of ca. 8 m and a crest length of ca. 300 m. It generates 3.6 MW of hydroelectric power. Station D6

was set 3.5 km below the dam (Fig. 2). Some of the headwaters of the eastern branch are located in China. Water depth was 0.5–1.0 m and the stream width was 8–10 m at the sampling stations, except D6, which had a greater width (Fig. 3e). The substratum at all sites was gravel, containing pebbles to cobbles up to 20 cm in diameter.

Preliminary sampling during the 2013–2014 spawning season

Larval occurrence in the Kalong River estuary suggested that the spawning season of Vietnamese ayu is from late November to mid-February (Tran et al. 2012a). Therefore, preliminary sampling was conducted at D1 and D2 in the western branch from December 2013 to February 2014 to try to confirm larval presence in the Tien Yen River. Since many studies in Japan have revealed that drifting ayu larvae are mainly encountered at night and predominate in the evening (Tago 1999a; Takahashi 2005; Kishino and Shinomiya 2006), a larva net (1.0 m in diameter, 4.0 m in length with 0.5 mm mesh) was set at the center of the stream flow mainly from evening into the night on December 15th, 2013 (D1), January 4th (D1), and February 8th, 2014 (D2). Sampling duration varied from 10 to 30 min depending on water volume and weather conditions (Table 1). In addition to this irregular sampling, a 22-h sample was taken on 3–4 January 2014 at D2 to establish the diel activity pattern of ayu larvae. Ten-min sampling (but 5-min sampling at 20:00) was conducted every 2 h from 8:00 on January 3rd to 6:00 on January 4th with the same net (Table 1).

Regular sampling during the 2014–2015 and 2015–2016 spawning seasons

Whole-day (22-h) sampling was conducted at D1, D3, and D5 in November and December 2014, January, February, and March 2015, and January 2016 (Table 2). Larvae were collected every 2 h (eleven or twelve times per day) with the aforementioned larva net, but a smaller larva net (0.45 m in diameter, 2.0 m in length with 0.33 mm mesh) was used for 8:00 and 10:00 samples on 22 December 2014. Sampling duration was usually 10, 15, or 20 min, although the net was occasionally set for 30 or 60 min.

During the 2014–2015 season, several brief samples were also taken at D1, D3, D4, and D5 (Table 3). Larvae

Table 1 Numbers of individuals, densities, stages, and notochord lengths (NL) of ayu larvae collected, with water temperature and turbidity during preliminary sampling conducted with a larger larva net (1 m in diameter, 4.0 m in length with 0.5 mm mesh) at sampling stations D1 and D2 along the Tien Yen River, northern Vietnam during the 2013–2014 spawning season

Sampling date	First sampling	Last sampling	Approx. Interval (hours)	Number of sampling periods	Duration of each sampling (minutes)	No. Ayu	Mean density (n/10^3 m^3)	Peak density	Larval stages	NL (mm) mean (range)	Water temperature (°C) mean (range)	Turbidity (NTU) mean (range)
D1												
15 Dec. 2013	17:50–18:10	19:30–19:40	1	3	10 or 20	6	5.9	10.3	A, B	6.1 (5.5–6.8)	17.8 (17.6–17.9)	0
4 Jan. 2014	9:00–9:30	11:00–11:30	2	2	30	0	0	–	–	–	16.2 (15.6–16.7)	2.0 (0–4)
4 Jan. 2014	18:00–18:20	22:00–22:15	1	5	15 or 20	2	2.8	8.2	A, B	5.9 (5.4–6.5)	16.4 (16.1–17.0)	19.6 (17–22)
D2												
3–4 Jan. 2014	8:00–8:10	6:00–6:10	2	12	5 or 10	8	3.9	21.6	A	5.5 (5.4–5.6)	16.0 (15.2–17.0)	4.3 (3–6)
8 Feb. 2014	17:20–17:40	18:40–18:55	1	2	15 or 20	0	0	–	–	–	20.5 (20.4–20.6)	2

Larval stage: *A* preflexion larvae with yolk sacs; *B* preflexion larvae without yolk sacs

Table 2 Numbers of individuals, densities, stages, and notochord lengths (NL) of ayu larvae collected, with water temperature, turbidity, and dissolved oxygen (DO) during 22-h sampling conducted at sampling stations D1, D3, and D5 in the Tien Yen River, northern Vietnam during the 2014–2015 and 2015–2016 spawning seasons

Sampling date	Gear	First sampling	Last sampling	Approx. Interval (hours)	Number of sampling periods	Duration of sampling (minutes)	No. Ayu	Mean density ($n/10^3$ m^3)	Peak density	Larval stages	NL (mm) mean (range)	Water temperature (°C) mean (range)	Turbidity (NTU) mean (range)	DO (mg/l) mean (range)
D1														
22–23 Dec. 2014	L/S	8:00–8:20	6:10–6:30	2	12	10, 15 or 20	59	40.5	170.1	A, B	6.3 (5.5–7.3)	16.1 (15.0–17.4)	2.7 (2–4)	8.6 (6.0–9.7)
6–7 Jan. 2015	L	12:30–12:50	10:30–10:50	2	11[a]	20 or 30	277	117.1	389.3	A, B	5.9 (4.0–7.0)	18.5 (18.3–18.7)	1.3 (0–2)	8.6 (7.9–8.9)
23–24 Jan. 2015	L	14:20–14:40	11:40–12:00	2	12	20	13	4.8	18.6	A, B	6.0 (4.7–7.0)	18.1 (17.4–18.8)	2.1 (1–3)	N/A
7–8 Feb. 2015	L	8:10–8:30	6:00–6:30	2	12	15, 20 or 30	0	0	–	–	–	16.2 (15.6–16.7)	3.2 (2–5)	7.4 (6.8–8.8)
10–11 Mar. 2015	L	15:25–15:45	13:00–13:20	2	12	15, 20 or 30	0	0	–	–	–	20.9 (20.6–21.6)	1.6 (0–6)	N/A
9–10 Jan. 2016	L	14:40–15:00	12:00–12:20	2	11[b]	15 or 20	0	0	–	–	–	20.6 (20.4–21.1)	3.7 (1–11)	N/A
D3														
22–23 Nov. 2014	L	16:30–16:50	14:20–14:35	2	12	10, 15 or 20	0	0	–	–	–	22.9 (21.7–24.2)	3.7 (1–5)	8.9 (8.1–9.7)
10–11 Dec. 2014	L	15:00–15:20	12:45–13:05	2	12	10, 15, 20 or 60	0	0	–	–	–	19.6 (19.0–20.6)	4.4 (3–6)	8.7 (8.3–9.0)
23–24 Dec. 2014	L	13:00–13:20	10:50–11:10	2	12	20	152	47.8	118.0	A, B	6.6 (5.7–7.5)	16.4 (15.7–17.6)	3.7 (1–6)	9.1 (7.7–9.6)
D5														
9–10 Dec. 2014	L	16:10–16:30	13:50–14:10	2	12	10 or 20	0	0	–	–	–	19.3 (19.0–20.1)	3.5 (0–6)	9.3 (8.8–10.2)

Gear: *L* Larger larva net (1 m in diameter, 4.0 m in length with 0.5 mm mesh); *S* Smaller larva net (0.45 m in diameter, 2.0 m in length with 0.33 mm mesh). Larval stage: *A* preflexion larvae with yolk sacs; *B* preflexion larvae without yolk sacs

N/A no data

[a] A sample (2:30–) was skipped

[b] A sample (2:40–) was skipped

Table 3 Numbers of individuals, densities, stages, and notochord lengths (NL) of ayu larvae collected, with water temperature, turbidity, and dissolved oxygen (DO) during short-period sampling conducted at sampling stations D1, D3, D4, D5 and D6 in the Tien Yen River, northern Vietnam during the 2014–2015 spawning season

Sampling date	Gear	First sampling	Last sampling	Approx. Interval (hours)	Number of sampling periods	Duration of each sampling (minutes)	No. Ayu	Mean density ($n/10^3$ m^3)	Peak density	Larval stage	NL (mm) mean (range)	Water temperature (°C) mean (range)	Turbidity (NTU) mean (range)	DO (mg/l) mean (range)
D1														
11 Dec. 2014	L	13:50–14:10	20:10–20:40	1	6	10, 20 or 30	4	2.1	8.2	A	5.3 (5.1–5.5)	19.5 (19.4–20.2)	3.7 (1–5)	8.9 (8.3–9.2)
D3														
28 Oct. 2014	L	15:10–15:25	17:05–17:15	2	2	10 or 15	0	0	–	–	–	27.3 (27.1–27.4)	7	N/A
6 Jan. 2015	S	14:00–14:30		–	1	30	0	0	–	–	–	21.1	N/A	8.7
23 Jan. 2015	S	19:00–19:20	21:00–21:20	1	3	20	1	12.7	38.1	A	6.3	N/A	N/A	N/A
D4														
24 Dec. 2014	L	12:50–13:10	18:30–18:50	1	4	20	7	18.8	33.0	A, B	6.7 (5.7–7.2)	17.3 (17.0–17.6)	5.0 (4–6)	8.3 (6.7–9.8)
D5														
6 Jan. 2015	S	16:00–16:30		–	1	30	0	0	–	–	–	20.7	N/A	8.7
D6														
22 Dec. 2014	S	17:25–17:40	20:30–20:50	1	4	15 or 20	0	0	–	–	–	14.5 (14.1–14.9)	N/A	10.1 (9.4–10.5)
7 Jan. 2015	S	15:10–15:20	20:00–20:10	1	6	10 or 15	0	0	–	–	–	18.3 (18.2–18.5)	2.0 (0–5)	8.3 (8.2–8.4)
24 Jan. 2015	S	15:55–16:05	20:25–20:35	1	6	10	0	0	–	–	–	18.2 (17.9–18.4)	2.0 (0–4)	N/A
7 Feb. 2015	S	15:30–15:45	17:30–17:40	1	3	10 or 15	0	0	–	–	–	17.6 (17.4–17.7)	3.0 (2–4)	9.1 (8.4–10.5)
10 Mar. 2014	S	15:00–15:10	18:00–18:10	1	4	10	0	0	–	–	–	20.9 (20.6–21.0)	N/A	N/A

Gear: *L* Larger larva net (1 m in diameter, 4.0 m in length with 0.5 mm mesh); *S* Smaller larva net (0.45 m in diameter, 2.0 m in length with 0.33 mm mesh). Larval stage: *A* preflexion larvae with yolk sacs; *B* preflexion larvae without yolk sacs

N/A no data

were collected for 10–30 min with either larva net from afternoon to night. Sampling was carried out 1–6 times per day at intervals of one or two hr. To confirm the occurrence of ayu below the dam in the eastern branch, sampling was conducted at D6 on December 2014, early and late January, February, and March 2015 (Table 3). The smaller net was set for 10–20 min every hour from afternoon to night (3–6 times per day).

Measurement of environment and larvae

During all sampling, a flow meter (2030R, General Oceanics) was mounted at the mouth of each net. Water temperatures (°C) and turbidity (NTU) were measured using a Water Quality Checker (WQC-22A, TOA DDK). Dissolved oxygen (mg/l) was measured with a WinLab® Data Line during some sample periods.

Samples were fixed in 3–5% formalin immediately after collection. Ayu specimens were sorted immediately and transferred to 80% ethanol. Identification of ayu larvae followed Tran et al. (2012a). After fixation, notochord lengths (NL) were subsequently measured to the nearest 0.1 mm using an ocular micrometer attached to a binocular microscope. Larvae were classified as to whether they possessed a yolk sac. Voucher specimens were deposited at the Museum of Biology, Hanoi National University of Education (BHNUE-194001001–194001005). Densities of ayu larvae (larvae per 1000 m^3 water) were calculated by the volume of water filtered, which was determined from flow meter measurements.

Collected larvae were statistically analyzed. The Mann-Whitney *U*-test was used for comparing larval NL between stations D1 and D3, where many larvae were collected, and a Fisher's exact probability test was used for a ratio of larvae with and without yolk sacs at D1 and D3. Prism 6.0b (GraphPad Software Inc.) was used for these analyses. Significant differences were determined at the 0.05 probability level.

Results

Water temperatures during sampling ranged from 14.1 to 27.4 °C (Tables 1, 2, and 3). Turbidity (0–11 NTU) did not change drastically during sampling except on 4 January 2014 (19.6 on average) (Tables 1, 2, and 3). Dissolved oxygen showed similar values (7.4–10.1 on average) throughout sampling (Tables 1, 2, and 3). The water current was generally faster in the upper reaches and slower in the lower reaches. The mean volume of water filtered per minute at the major sampling stations of D5 (upper) was 36.0 L, 17.7 L at D3 and 2.3 L at D1 (lower) during four sampling times in December 2014 of peak downstream migration periods.

A total of 529 ayu larvae were collected (Tables 1, 2, and 3). During preliminary sampling in the 2013–2014 spawning season, 16 larvae were collected at D1 and D2 from mid-December to early January (Table 1). No larvae were collected during sampling in early February at D2. During regular sampling periods from October 2014 to March 2015, ayu larvae were found from mid-December to late January, with a peak in late December and early January (Tables 2 and 3). No larvae were collected during the next spawning season, even though an entire day of sampling was conducted at D1 in early January 2016 (Table 2). The average water temperature when ayu larvae were present, ranged from 16.0–19.5 °C (Tables 1, 2, and 3).

Whole-day sampling was conducted once during preliminary sampling at D2 and 10 times during regular sampling at D1, D3, and D5. Larvae were collected in five samples (3–4 January 2014, 22–23 and 23–24 December 2014, 6–7 and 23–24 January 2015) at D1, D2, and D3 (Tables 1 and 2). These five whole-day samples revealed that drifting ayu larvae occurred primarily at night (17:00–6:00; Fig. 4). Among the three samples that involved relatively large larval numbers, abundance peaked before dawn in the samples from D1 and D3 in late December 2014, and peaked at dusk in the sample from D1 in early January 2015 (Fig. 4). Water temperatures usually fluctuated within a range of about 2 °C, higher from 14:00–16:00 and lower from 0:00–6:00, but almost stable at 18.5 °C at D1 on 6–7 January 2015 (Fig. 4).

Ayu larvae occurred at D1, D2, D3, and D4, but not at D5, the uppermost station in the western branch. Notably, no larvae were collected at D6 below the Khe Soong Dam along the eastern branch during five sampling periods from December 2014 to March 2015, though many larvae were collected at D1 on the western branch on the same days in December and January.

Larval length varied from 4.0–7.5 mm NL. During the study, 394 preflexion larvae with yolk sacs (4.0–7.2 mm NL) and 135 preflexion larvae without (4.7–7.5 mm NL) were collected. NLs of larvae were significantly larger at station D3 than at D1 (*U*-test, $p < 0.0001$) (Fig. 5). The ratio of preflexion larvae

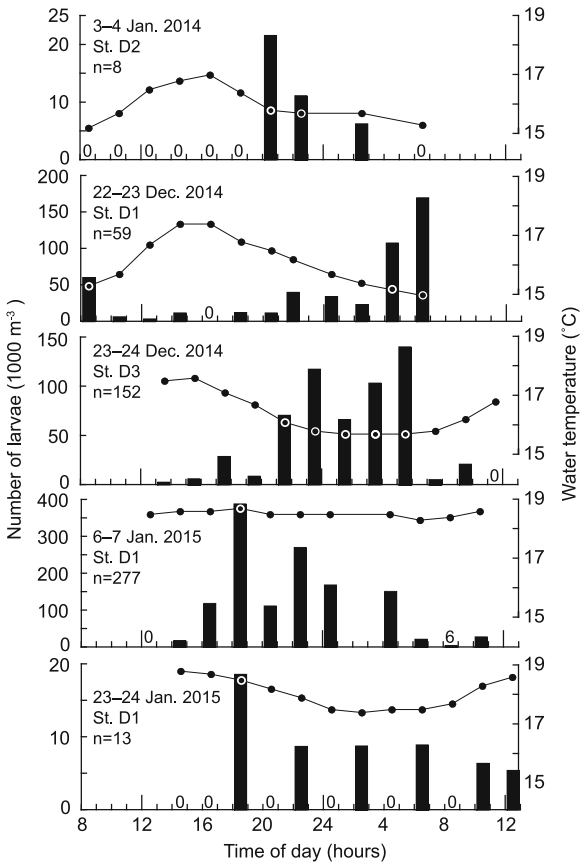

Fig. 4 Diel changes in abundance of drifting ayu larvae (per 1000 m^3) in the Tien Yen River at sampling stations D1, D2, and D3 in January and December 2014 and January 2015. *Bars* and *lines* represent larval density and water temperature, respectively

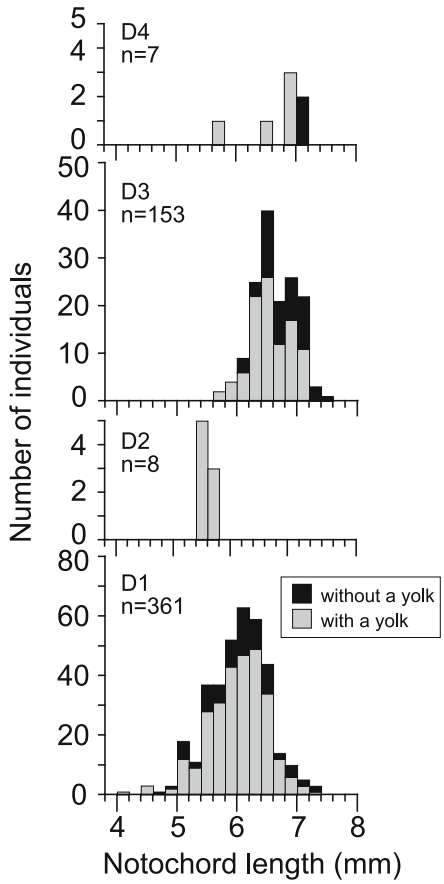

Fig. 5 Length-frequency distribution of drifting ayu larvae collected at four stations (D1, D2, D3 and D4) in the Tien Yen River from 2013 to 2015. *Gray* and *black* bars indicate larvae with and without yolk sacs, respectively

with/without yolk sacs was significantly different between D1 and D3 (Fisher's exact probability test, $p < 0.01$) (Fig. 5).

Discussion

We confirmed that ayu larvae are present in the Tien Yen River of northern Vietnam. More than half of the larvae collected had yolk sacs. Because ayu larvae absorb the yolk within 3–9 days of hatching (Hyodo and Seki 1985; Tachihara and Kawaguchi 2003), larvae collected in this study had to have been born in this river. It also means that the larvae recorded in this river were not transported from other river systems during the larval period. The Tien Yen River is the most southwestern habitat in which this species is able to complete its life cycle. The Tien Yen River is located 60 km farther west than the Kalong River, previously believed to be the western limit of the species (Tran et al. 2012a) (Fig. 1).

Drifting ayu larvae were collected from mid-December to late January, with a peak in late December and early January, suggesting that the ayu spawning season in the Tien Yen River is from late November to mid-January, at least during the 2014–2015 season. This is similar to the spawning season in the Kalong River (from late November to mid-February; Tran et al. 2012a). The ayu spawning season in Vietnam is later than in mainland Japan (September to December; Matsuyama and Matsuura 1982; Hyodo et al. 1984; Takahashi and Niimi 1998; Tago 1999b). Later initiation of spawning is also known for ayu in the Ryukyu Archipelago, subtropical islands in Japan, where spawning was recorded from late November to early March at Okinawa and Amami-oshima Islands (Kishino and Shinomiya 2004; Kawakami and Tachihara 2010).

It has been reported that ayu start spawning when water temperatures fall below 20 °C (Kishino and Shinomiya 2004). In the Tien Yen River, average water temperature during successful sampling was always below 20 °C (Tables 1, 2, and 3), implying that Vietnamese ayu also start spawning when the water temperature falls below ~20 °C and that the spawning season starts later in warmer, low latitude regions, including Vietnam and the Ryukyu Archipelago.

During the surveys, local fishermen often reported ayu in the upper reaches of the western branch of the Tien Yen River, but during the winter of 2015–2016, we received no such reports. That is why we conducted larval sampling on 9–10 January 2016 at D1, where many larvae were collected in previous years. However, no larvae were collected (Table 2), even though early January was the time of peak abundance in previous years. The mean air temperature in Tien Yen town along the river was warmer in the winter of 2015–2016 (17.9 °C in December 2015 and 17.5 °C in January 2016) (https://www.worldweatheronline.com), compared to the mean monthly air temperature there from 1980 to 2010 (16.6 °C in December and 15.4 °C in January) (Quang Ninh Province Department of Natural Resources and Environment 2012). Water temperature during sampling was also warmer, exceeding 20 °C, even in January. It may be that few ayu spawned this year because of warm temperatures. Warmer winters may threaten the southernmost populations of this annual fish species. If global warming causes continuous warm winters, the ayu populations in Vietnam will disappear.

We failed to collect ayu larvae at site D6 below the Khe Soong Dam on the eastern branch of the Tien Yen River in every sampling period. The absence of drifting larvae during the peak downstream migration season suggests that ayu do not inhabit this branch, or at least, that they do not reproduce there. Though there may be other reasons for their absence in the eastern branch, such as habitat degradation or overfishing, the presence of the dam, which blocks ayu migration, is likely the major factor. Because ayu live in the western branch and because the eastern and western branches provide a common habitat for pelagic larvae (the estuary and the sea), ayu might be able to recover in the eastern branch if a functional fishway could be built to bypass the Khe Soong Dam. Expansion of possible habitat is very important for conservation of the small, and vulnerable Vietnamese ayu populations.

Drifting ayu larvae were found primarily at night during this study (Fig. 4). Nocturnal larval drifting is common for amphidromous fish, including gobies, pipefishes, and ayu (Iguchi and Mizuno 1990; Tomiyama et al. 1999; Takahashi 2005; Maeda and Tachihara 2010; Iida et al. 2013). As with other amphidromous fishes, ayu larvae hatch at dusk when the light intensity drops and they start drifting immediately (Kimura 1953; Tago 1999a). Locations of ayu spawning grounds vary with the size and topography of the river (Ishida 1964). The diel abundance pattern of larval drifting is influenced by distance and flow rate of the rivers between spawning grounds and sampling sites. In small streams (typically shorter than 20 km), ayu usually spawn at a single spawning ground close to the upper limit of the estuary and larvae reach the estuary immediately after hatching. Drifting larvae occur from dusk to midnight at sampling sites just below the spawning ground and peak for some hours after sunset (Kishino and Shinomiya 2004; Kawakami and Tachihara 2010). Even in larger rivers, the majority of drifting larvae are captured in the first half of the night, if the spawning grounds are located no more than several kilometers above the estuaries (Hyodo et al. 1984; Tago 1999a, b; Takahashi 2005). On the other hand, in large rivers having longer, gently flowing lower reaches, ayu spawning grounds are distributed over dozens of kilometers. For example, in the 322-km Tone River in Japan, spawning grounds were distributed over 58 km (175–233 km from the river mouth) (Ishida 1964; Takahashi 2005). In such large rivers, diel abundance patterns of the larvae are usually unclear or irregular because both larvae that hatched near the sampling site and larvae that drifted long distances are collected together (Hyodo et al. 1984; Takahashi and Niimi 1999). Even though larvae are sometimes captured during the day, the majority of larvae are usually captured at night. In large rivers, larvae take several days to drift from spawning grounds to estuaries or the sea (Hyodo et al. 1986; Tsukamoto 1991). It has been suggested that larvae pause drifting in the bottom layers of pools or slow-moving river segments in the morning, and that they resume drifting in the evening (Takahashi 2005; Kishino and Shinomiya 2006). If so, downstream travel must necessarily take longer than would occur with continuous drifting.

Spawning grounds have not been located in the Tien Yen River, although mature ayu have been observed as far upstream as A (Fig. 2) (Tran et al.

2012b) and around D4 and D5 (Nguyen et al. 2015). In the western branch of the Tien Yen River, ayu larvae were found at every sampling station except D5, where sampling was conducted twice. One of these samples at D5 was collected over 22 h in early December when the ayu downstream season probably had not yet started. The other, a short-period sample in late December at D5 (16:00–16:30), was conducted earlier than the expected beginning of nocturnal drifting. Ayu larvae might have been collected at D5 if sampling had been conducted at night during the peak drifting season (late December to early January). In any case, these data suggest that ayu spawn more than 10 km upstream from D1, which is located just above the upper limit of the tidal salt wedge. Larval activity peaked from midnight to dawn at D1 and D3 (22–23 and 23–24 December 2014; Fig. 4), suggesting that many larvae hatched in the far upper reaches of the river, such as at site A, at dusk and reached D3 and D1 between midnight and early morning. However, the majority of ayu larvae probably hatched farther downstream, not far from D1 on 6–7 January 2015, as the peak occurrence was seen that evening (Fig. 4). Thus, the diel pattern of drifting ayu larvae in the Tien Yen River is different from that in small streams on Okinawa and Amami-oshima Islands, where ayu spawn at single spawning ground near the upper limit of the estuary (Kishino and Shinomiya 2004; Kawakami and Tachihara 2010). The Tien Yen River is not as large as the Tone River, where ayu spawning grounds are distributed over 58 km (Ishida 1964). It is likely that ayu in the Tien Yen River have multiple spawning grounds between site D1 and the upper reaches around site A, and that these shift, depending on the year and the condition of the river.

Body sizes of collected larvae were larger at D3 than downstream at D1. Larger percentages of larvae with yolk sacs at D1 would be consistent with smaller notochord lengths of larvae captured there. The majority of larvae from D1 were collected in January while most larvae were collected in December at D3. The body size of newly hatched larvae varies depending on the water temperature during their incubation (Komada 1977; Iguchi and Yamaguchi 1994). Different body sizes between D1 and D3 may be explained by different environmental conditions during incubation. However, it is also possible that the larger body and smaller ratio of yolk-sac larvae collected in late night and early morning in December are caused by longer drifting from spawning grounds upstream. Larvae should take several days to absorb the yolk (Hyodo and Seki 1985). Nevertheless, we cannot estimate the time spent for downstream migration, since spawning ground locations and drift times are unknown. Further studies of the ages of drifting larvae should resolve this conundrum. Also, intensive research will be required to locate ayu spawning grounds in the Tien Yen River. Such knowledge is important to understand ayu downstream migration in this river, and it is also important for conservation of this vulnerable ayu population.

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