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Reproductive Biology of the Ayu *Plecoglossus altivelis* (Temminck and Schlegel 1846) from its Southernmost Distribution Range

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Abstract

The ayu *Plecoglossus altivelis* (Temminck and Schlegel 1846), an annual and amphidromous fish, is generally distributed in the islands of Japan and along the Asian continental coast. Recently, it has been found in two rivers of northern Vietnam and the Tien Yen River is the southernmost distribution range of this species. Morphology of the ayu in Vietnam has been examined; however, its reproductive biology is poorly known. Therefore, this study attempted to describe the fecundity and the egg size of the ayu from this river. Gonadosomatic index (GSI) of the female ayu (160.0–194.7 mm standard length, SL) was 19.7–25.9 %. The number of eggs ranged from 33,548 to 45,114. Egg size ranged from 0.1 to 1.5 mm, with an average diameter of 0.8–0.9 mm. The GSI of the male ayu (153.3–194.7 mm SL) was 2.5–8.3 %. The present findings indicate that the ayu in its southernmost distribution region could spawn at least twice a year, and the breeding period occurs between November and February. This study provides reproductive data on the ayu species, thus contributing to the conservation of endangered ayu populations in Vietnam.

Key words: ayu, *Plecoglossus altivelis,* fecundity, egg size, northern Vietnam

Introduction

The ayu, *Plecoglossus altivelis* (Temminck & Schlegel, 1846) (Osmeriformes: Plecoglossidae), is distributed over the Japanese Archipelago, Korea, Taiwan, and along the Asian continental coast from Liaoning, China to northern Vietnam (Hosoya 2002; Shan et al. 2005; Tran et al. 2012a, b; Nguyen et al. 2015). It is a commercially important food fish and a target of recreational fishing, especially in Japan (Takahashi 2005).

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Ayu have an annual and amphidromous life history. They 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 the larval and early juvenile stages. In spring, juveniles swim upriver, 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), the taxonomy of this species throughout its distribution range has not been verified yet.

Besides the semelparous strategy, some studies have revealed that the amphidromous type of ayu can spawn twice a year and the land-locked form can spawn three times a year (Iguchi 1996; Iguchi 2012a, b), implying that this fish can adapt well to different environments. Among reproductive features, the fecundity and the egg size are two of the most important traits, reflecting maternal investment that affects offspring fitness in fish. The environment varies throughout the ayu distribution range; thus, it is reasonable to conclude that there are different patterns of fecundity and egg size in this species (Nishida 1986; Iguchi 2012a, b). Although reproductive characteristics of ayu in Japan are well known, those of the Vietnamese ayu populations remain unclear.

Populations in Taiwan and Okinawa Island, Japan are extinct (Nishida et al. 1992; Tachihara 2015), suggesting that ayu populations in lower latitudes are in a vulnerable state. In Vietnam, ayu have been found only in the Kalong and Tien Yen rivers from Quang Ninh, a province facing the northern part of the Gulf of Tonkin (Fig. 1) (Tran et al. 2012a, b; Tran et al. 2017). It has been reported that the ayu population in the Tien Yen River is relatively small (Tran et al. 2012b; Nguyen et al. 2015), and this river is at the most southwestern distribution range of this species (Tran et al. 2017). It is stated that biological characteristics are valuable data for the adaptation of this fish in the tropical region. Thus, this study investigates some important characteristics of the breeding biology of the ayu in Vietnam for conservation and enhancement of the population.

Materials and Methods

The study site

Samplings were conducted from the upper reaches of the Tien Yen River (ca. lat. 21° 25′N) in Ha Lau commune, Quang Ninh Province (Fig. 1). The Tien Yen River (82 km in length), the largest river in the province, flows into the Gulf of Tonkin, and one source (the western branch) of the river is located in Vietnam, while the other (the eastern branch) is in China. The average depth and width of the sampling sites varied from 2 to 6 m and 7 to 15 m, respectively (Fig. 1).

Sampling and preservation of fish

Fish were sampled using a gill net (mesh size 20 mm) at the sampling sites (Fig. 1). The sampling was conducted during the spawning period of the Vietnamese ayu, from November to February (Tran et al. 2012a, b). Since the ayu were rare in the region (Tran et al. 2012b), only 11 specimens (4 females and 7 males) could be collected in the present study from the Tien Yen River in December 2014 and January 2015. Although many samplings were performed in the other months of the year, no fish was captured. Sampled fish were stored in a cool box with ice and brought to the laboratory for further analysis. Water temperatures, measured by a Water Quality Checker (WQC-22A, TOA DDK), at the sampling sites ranged from 19.0 to 20.7 °C during the collections.

Fish analysis

The individual and gonad weight were measured to the nearest 0.01 g by using electronic balance Type BL-320H, Japan, and standard length, SL to the nearest 0.1 mm by using vernier caliper (Mitutoyo), before dissecting the fish and removing the gonads. All eggs of an individual fish were placed in a petri dish to determine the batch fecundity (F = number of eggs/individual). The eggs of a female were well mixed before selecting for size measurements. The diameters of a random sample of 200 eggs from a female were measured to the nearest 0.01 mm using an ocular micrometer attached to a binocular microscope. Gonadosomatic index (GSI) was calculated as gonad weight/total fish weight x 100.

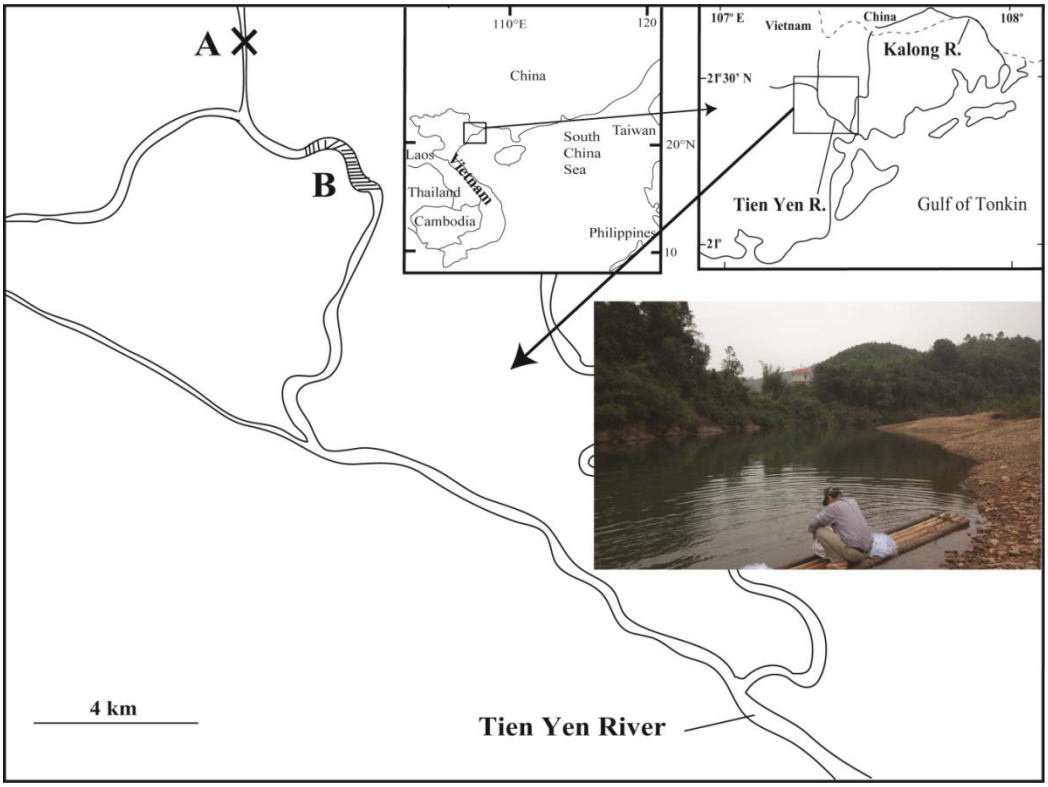

Fig. 1. Sampling sites (*cross lines*, B) where ayu adults were collected in the Tien Yen River, northern Vietnam. *A*, the site where a mature ayu was collected previously (Tran et al. 2012b). Photo showing a fishermen used a gill net to catch ayu at B.

Results

The ovaries of the mature ayu females (160.0–194.7 mm in SL) collected in December and January filled up the abdominal cavity (Fig. 2). Ovaries were dark-yellow with visual blood vessels, and the eggs could be easily separated. The GSI of the collected fish ranged from 25.1–25.9 % in December, and about 19.7 % in January (Table 1). The number of eggs of ayu individuals, with SL of 160.0–177.0 mm, ranged from 36,013 to 45,114 in December, while that of ayu with SL of 194.7 mm was 33,548 in January (Table 1). Egg size ranged from 0.1 to 1.5 mm, with an average diameter of 0.79 mm to 0.90 mm (Table 1; Fig. 3). There was some overlap of the smallest and the largest egg sizes in two ayu individuals collected in December and in January (Fig. 3; female 1 and female 4).

Table 1. Body length and gonad parameters of female ayu in the Tien Yen River.

Sample time	Standard length (mm)	Body weight (g)	Ovarian weight (g)	Number of eggs	Egg diameter (mm) Mean (min-max)	GSI
10 Dec. 2014	160.0	-	-	36,497	0.84 (0.1-1.3)	-
10 Dec. 2014	177.0	78.42	20.31	45,114	0.79 (0.2-1.4)	25.9
10 Dec. 2014	163.7	69.66	17.50	36,013	0.86 (0.3-1.5)	25.1
02 Jan. 2015	194.7	85.78	16.86	33,548	0.90 (0.5-1.3)	19.7

- . These data were not available because this female was preserved directly in 10 % formalin.

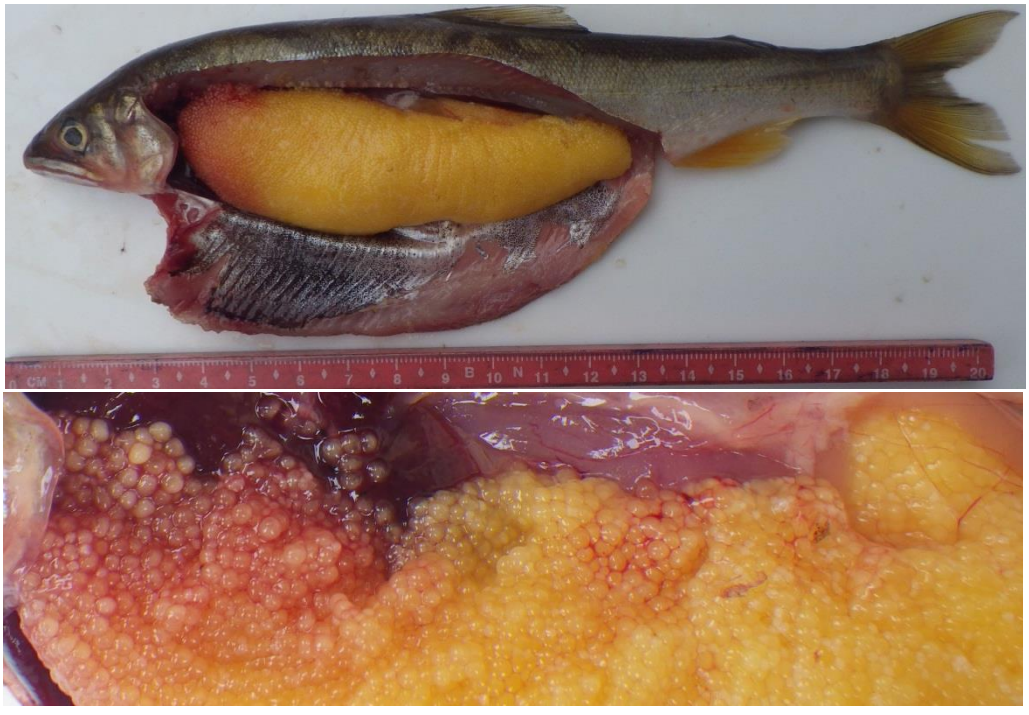

Fig. 2. Mature ovary of female ayu from the Tien Yen River (SL = 177.0 mm, collected on 10 December 2014), with a closeup view of the ovary

Sizes of male ayu ranged from 163.9 to 167.6 mm SL in December and from 153.3 to 194.7 mm SL in January (Table 2). The left testis was larger than the right one (Fig. 4). The weight of the testis ranged from 1.38 to 3.06 g in January and from 3.94 to 4.65 g in December (Table 2). In addition, the GSI of fish in January ranged from 2.5 to 4.9 %, while that in December was from 6.8 to 8.3 % (Table 2).

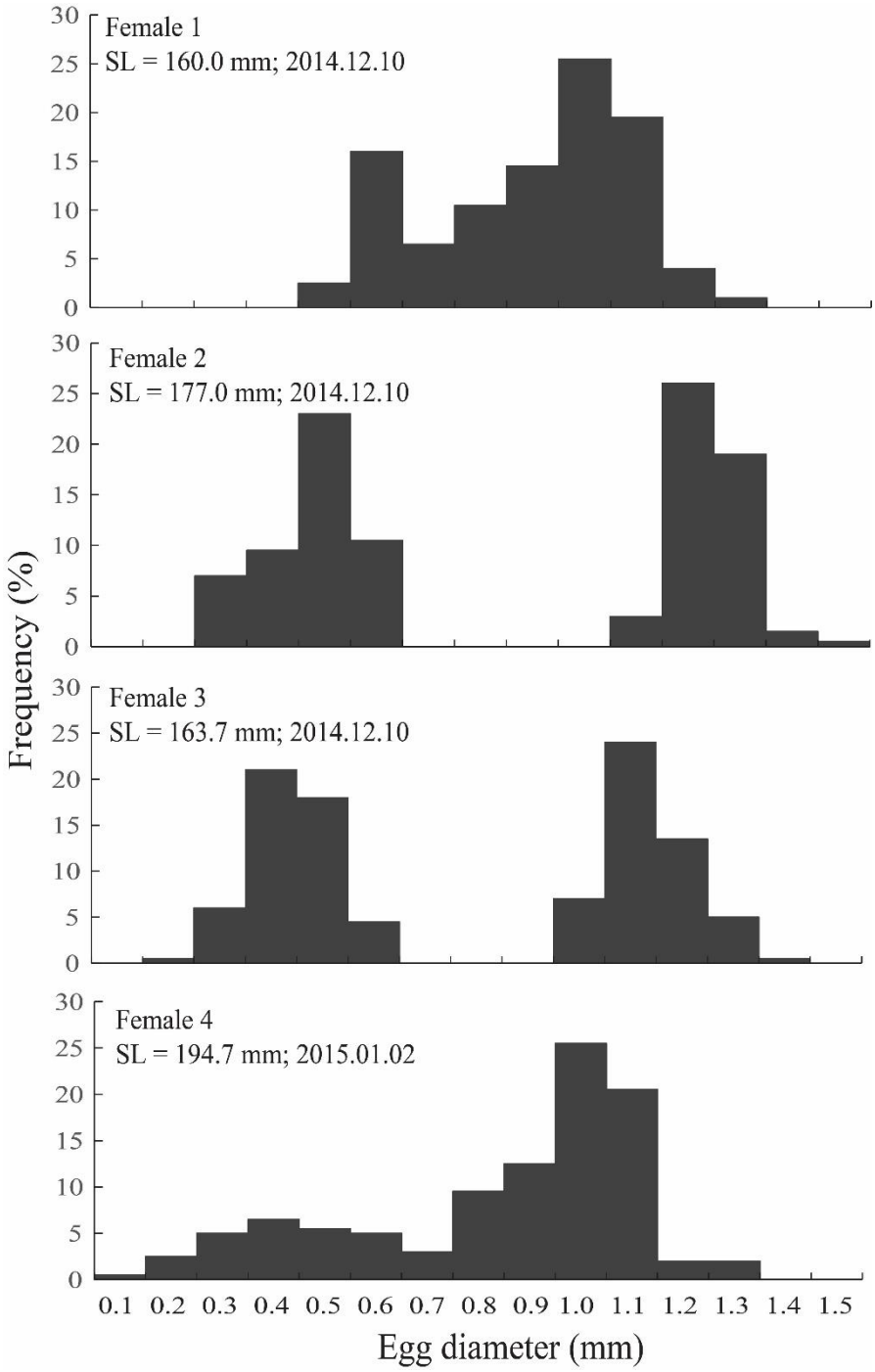

Fig. 3. Egg diameter of ayu collected in the Tien Yen River from December 2014 (females 1, 2 and 3) to January 2015 (female 4).

Table 2. Gonad weights of male ayu in the Tien Yen River.

Sample time	Standard length (mm)	Body weight (g)	Testis weight (g)	GSI
10 Dec. 2014	167.6	56.14	4.65	8.27
10 Dec. 2014	163.9	58.20	3.94	6.78
02 Jan. 2015	194.7	55.17	1.38	2.49
02 Jan. 2015	153.3	56.62	2.50	4.42
02 Jan. 2015	184.0	55.00	2.57	4.67
02 Jan. 2015	167.3	61.28	3.06	5.00
02 Jan. 2015	165.6	50.63	2.73	5.38

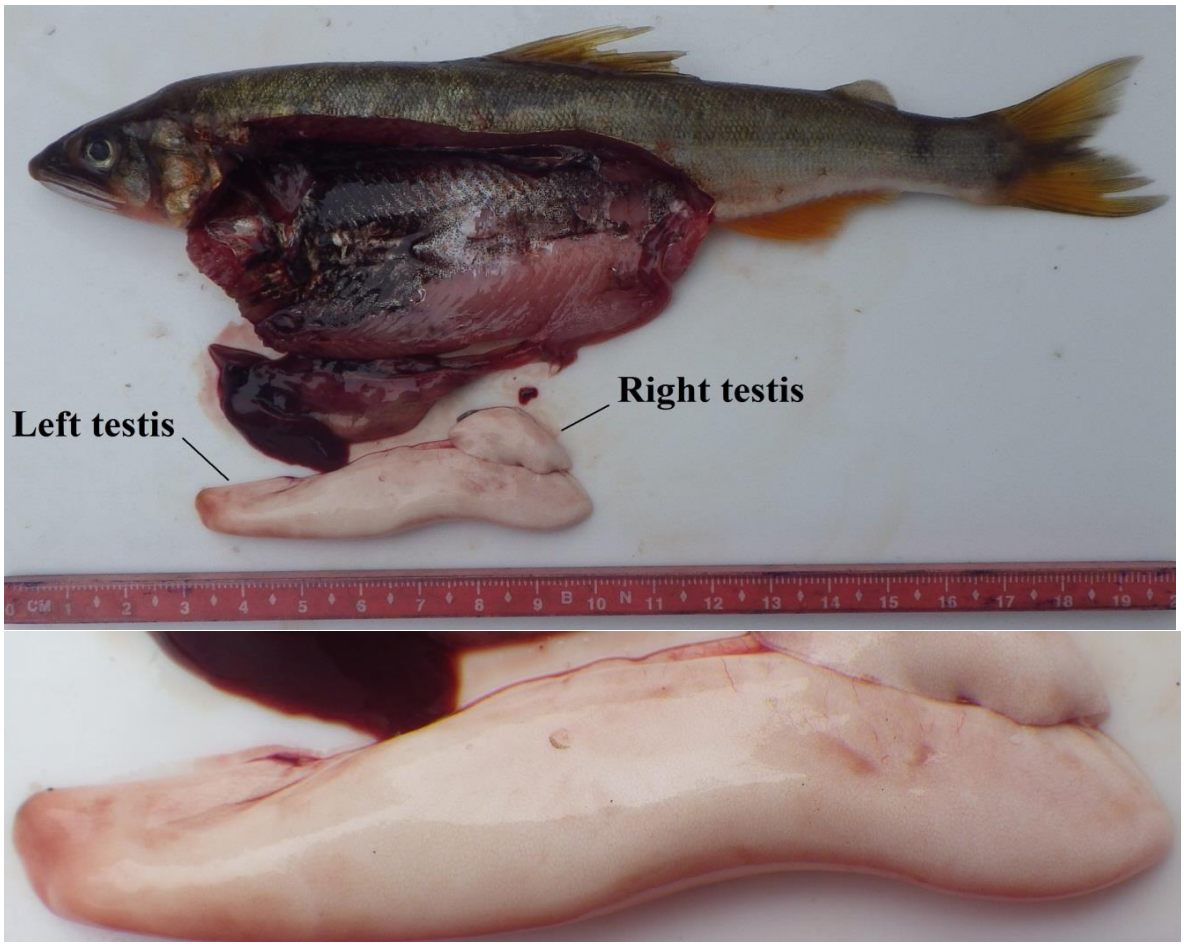

Fig. 4. Testis of ayu, with its closeup view, from the Tien Yen River (SL = 167.6 mm) collected on 10 December 2014.

Discussion

Ayu populations from different geographical regions have different reproductive strategies as an adaptative response to different environmental factors, such as photoperiod, water temperature, food conditions, predator pressure or mortality rates of offspring (Nishida, 1986).

The present study initially described the reproductive parameters of the Vietnamese ayu, as shown from the observation of eggs of samples collected in December and January (Fig. 2; Table 1). Based on icthyoplankton data from the Kalong estuary (Fig. 1), Tran et al. (2012a) presumed that ayu spawn from late November to mid-February. Furthermore, based on the character of the ovary of an adult ayu collected in late December from Tien Yen River, the spawning time of the ayu is estimated to be in January or February (Tran et al. 2012b). No adult ayu was collected in the present sampling sites in other months, suggesting that the breeding period of this species in the Tien Yen River would have been between December and February. This result agrees with the previous data of Tran et al. (2012a, b). In addition, according to collections of drifting ayu larvae at four stations below B (Fig. 1) along the Tien Yen, the ayu in this river spawn from late November to mid-January (Tran et al. 2017).

It is interesting that there was an evidence of multiple spawning of the ayu in the Tien Yen River. The GSI and egg diameters of ayu collected in December and January (Tables 1, 2) probably indicated that the samples captured in January would have already spawned. Furthermore, in an ovary, there were two main sizes of eggs (Table 1; Fig. 3), implying that these ayu could have spawned at least twice. Previously, rivers where the multiple spawning of ayu has been reported were relatively small (about 20 km in length) (Matsuyama and Matsuura 1985) or quite large (more than 100 km in length) (Shimizu et al. 2008). From the current data, this common phenomenon is also found in the Tien Yen River, which could support the previous work of Shimizu et al (2008), who suggested that multiple spawning was not related to the river system. Also, Matsuyama and Matsuura (1985) and Iguchi (1996) reported that multiple-spawners were only the smaller fish in the case of landlocked ayu of Lake Biwa, but Shimizu et al. (2008) did not agree with that inference since their largest specimens (SL > 160 mm) showed evidence of three spawning batches. The present study could support the latter as females collected from the Tien Yen River in December were all larger than 160 mm SL. Thus, the ayu in this river is a multiple-spawner, regardless of migration pattern, which is under-examined. However, different sizes of female ayu at maturation are needed for further understanding of its spawning patterns in this river.

Egg size is a critical characteristic at the early developmental stage of fish that can maximise maternal fitness (Iguchi 2012b). In the case of the amphidromous type of ayu, there seems to be a north-south cline of fecundity. The amphidromous populations in the north have more eggs of smaller size and maximise the number of offspring surviving under local environmental circumstances (Nishida 1986). The egg size of ayu is influenced by several factors, such as genetics, predator pressure, food availability (Nishida 1986; Iguchi 2012a) and the incubation temperature or stream length (Iguchi 2012b). In the present study, the fecundity of the Vietnamese ayu seemed to be higher than those from Japan (from 33,548 to 45,114 vs. 1,030 to 28,200 eggs) (Iguchi 1996; Nishida 1986) although only four females were collected and examined. The current result is out of the north-south cline of the fecundity as amphidromous populations in the north have more eggs of smaller size (Matsuyama and Matsuura 1985; Iguchi 1996; Nishida 1986).

Although we do not know whether the Vietnamese ayu are of the migratory or non-migratory type, they are definitely not landlocked because there is no any physical barrier in the branch of the Tien Yen River (Fig. 1). It has been reported that the egg size and the fecundity generally increased with maternal body size (Iguchi 1996, 2012a). Likewise, hatchings from larger eggs had larger larvae and a faster growth rate, and had higher survival to maturity (Iguchi and Yamaguchi 1994). However, an increase in egg size usually results in reduced fecundity. For example, the fecundity of the Lake Biwa population was extremely high, but the eggs of the Lake Biwa ayu were by far the smallest in size (Iguchi 1996). The maximal egg diameter of the Vietnamese ayu was 1.5 mm, which is still greater than that of the Japanese ayu whose egg diameter was always smaller than 1.0 mm (Iguchi 1996, 2012a). According to Iguchi (2012a), ayu that have larger egg sizes are predisposed to early survival in competitive environments. This seems to support the result of Tran et al. (2014), who compared the growth rate of ayu larvae among cohorts in Vietnam and Japan, and concluded that the ayu has a potential adaptability that allows it to disperse from cool temperate to tropical regions.

The Tien Yen ayu population is threatened (Tran et al. 2012a; Nguyen et al. 2015) due to the modification of the river structure, dam construction, water pollution, overfishing, and land development in the basins (Dinh et al. 2014; Tran and Ta 2014). The reduction in the Tien Yen ayu population was responsible for the small size of samples collected in the present study. This confirms the uncertain state of ayu populations at lower latitudes, which they could disappear if global warming causes winters to become warmer (Tran et al. 2017). Tran et al. (2017) also pointed out that the ayu in this river probably have multiple spawning grounds. Thus, it is expected that collecting samples in different spawning seasons and in different sections of the Tien Yen River can increase the sample size, which would be helpful to fully understand the fecundity of this species in the tropical region. The reproductive characteristics play an important role in affecting the population size of this fish. The findings from the present study are necessary for the conservation and enhancement of a threatened ayu population in Vietnam in spite of the limitations of sample sizes as mentioned earlier.

Conclusion

This study represents the first report on ayu reproduction characteristics in the Tien Yen River in Vietnam, the southernmost population of this species. The Tien Yen ayu population could spawn at least twice a year, with larger egg size in comparison to ayu from Japan. Thus, the results of this study have revealed the adaptation of this fish to the local habitat in a tropical region.

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