

Early Life Stages and Habitats of Ayu *Plecoglossus altivelis* (Temminck and Schlegel 1846) from Two River-estuary Systems in Vietnam

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Abstract

The geographic and spatial distribution of ayu *Plecoglossus altivelis* (Temminck and Schlegel 1846) larvae were determined in the rivers of northern Vietnam. Collections were made with a larval net and a small seine net along the Tien Yen and Kalong Rivers from 2010 to 2015. Thirty individuals were collected in the Tien Yen and 160 from the Kalong. This is the first record of larval and juvenile ayu from the Tien Yen, which constitutes the southernmost range of this species. Collected fish were primarily at the preflexion stage (4.0–59.1 mm body length, BL). Mean temperature, salinity, turbidity and dissolved oxygen where larvae and juveniles were collected ranged from ca. 15.7–20.0 °C, ca. 5.6–20.7 psu, 2.7–14.6 NTU, and 7.7–10.0 mg.L⁻¹, respectively. Larvae began to appear in November for the Kalong and in December for the Tien Yen, primarily occurring at stations far from the river mouth. Larvae <7.0 mm BL were more widely distributed across both estuaries, migrating to shoreline waters after reaching a size of ca. 15.0 mm BL in February. With relatively few larvae being observed in the southernmost region, the Tien Yen estuary may be a focus of conservation efforts of this annual amphidromous fish.

Keywords: ayu, *Plecoglossus altivelis*, water column, distribution, backwaters, Vietnamese estuaries

Introduction

The ayu *Plecoglossus altivelis* (Temminck and Schlegel 1846) [Osmeriformes: Plecoglossidae] is an annual amphidromous species that is widely distributed over the Japanese Archipelago, Korea, and along the Asian continental coast from Liaoning, China to northern Vietnam (Hosoya 2002).

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It is a commercially important species as a food resource and as a target of recreational fishing, especially in Japan. Ayu spawn in the lower reaches of rivers in autumn. After hatching, the newly hatched embryos are carried downstream to the sea, where they spend the winter months until migrating upriver as juveniles in the spring (Senta and Kinoshita 1985; Takahashi et al. 1990; Yagi et al. 2006). Three subspecies of *P. altivelis* have been recognised. These are *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). However, the taxonomy of this species throughout its distribution still remains unresolved.

Ayu larvae exhibit significant plasticity in their downstream rearing along coastal marine waters and within estuaries (Takahashi et al. 1990; Aljamali et al. 2006; Tran et al. 2012a), whereas other larvae arrive after drifting from upriver spawning grounds. Yolk-sac larvae of around 7.0 mm body length (BL) are distributed throughout the estuary (Takahashi et al. 1998; Yoshimoto et al. 2006). Larvae after absorbing the yolk-sac tend to aggregate near the bottom of the estuary and remain there until reaching 10 mm BL (Takahashi et al. 1998), then they migrate to the littoral zone along the estuary (Yoshimoto et al. 2006); larvae >14.0 mm BL occur only in the littoral zone (Takahashi et al. 1998; Tran et al. 2012a). At approximately 20 mm BL, juvenile ayu begin to migrate from the littoral zone to deeper water and main channel habitats (Takahashi et al. 2002).

The dispersal of larval ayu *P. altivelis altivelis* and ryukyu-ayu *P. altivelis ryukyuensis* within estuarine systems is considered to be predominantly driven by tidal currents, precipitation, river discharge and wind (Tachihara and Kimura 1991; Azuma et al. 2003), where larvae appear to be more vertically distributed toward the bottom, which can enhance retention in the estuary while being dispersed by tidal currents (Takahashi et al. 1998; Kishino and Shinomiya 2005; Aljamali et al. 2006). The water column possibly determines the distributional pattern of larval ayu during the pelagic stage (Aljamali et al. 2006; Yagi et al. 2006; Tran et al. 2012a), where larger larvae continue to be more distributed at the surface when the water is more stratified but become more vertically dispersed when the column becomes more mixed (Aljamali et al. 2006).

In Vietnam, ayu have been recorded 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; 2014), the existence of ayu was recently reported from the Tien Yen River, 60 km west of the Kalong River (Tran et al. 2012b; Nguyen et al. 2015; Tran et al. 2017a–b) (Fig. 1). It has been reported that the Tien Yen River ayu population is very small (Tran et al. 2012b; Tran et al. 2017b). However, little is known about ayu larval and juvenile dispersal in the estuary, a critical period for growth and recruitment success. Information on their habitat for early stage recruitment success is therefore essential for management and conservation of this species.

Ayu have become extinct from several regions, mainly in subtropical regions such as Okinawa Island, Taiwan, and Hong Kong (Nishida et al. 1992; Tachihara 2015). As such, it has been suggested that subtropical populations may be more vulnerable to extinction.

In the current study, ayu larvae were collected from the Tien Yen and Kalong estuaries, representing the two systems that encompass the very southern edge of the populations of this species. In this study, we report on the occurrence of larval and juvenile ayu and the hydrological and biological differences between the two systems, the information on which can shed significant light on the drivers of ayu distribution, abundance and recruitment success.

Materials and Methods

Sampling sites and methods

Intensive surveys for ayu were conducted within two rivers (Tien Yen and Kalong) from northern Vietnam. The Tien Yen River (ca. 82 km in length) flows through Lang Son (Dinh Lap District) and Quang Ninh (Binh Lieu and Tien Yen Districts) Provinces. The river has two main tributaries, the eastern tributary with headwaters originating from China, which is also dammed, and the western tributary with headwaters within Vietnam without impeded flow, but with a weir (Fig. 1). Another weir is located between the Khe Soong Dam and the fork between the western and eastern tributaries (Fig. 1). The headwaters of the Kalong River originate in China, which runs along the border with Vietnam and flows into the northernmost coastal region within the Gulf of Tonkin. The Kalong estuary has two distributaries, the western and the eastern branches, with the western branch having a deeper current than the eastern branch (Fig. 1). The lower part of the two rivers is a typical estuarine system, having diurnal macro tides of ~3 to 4 m during spring tides and containing a diverse range of marine and brackish-water fish species.

Horizontal and vertical net tows and seine-based surveys were conducted within the two rivers (Fig. 1). Twenty (TL1–TL11 and L1–L9) and 24 sampling stations (TS1–TS14 and S1–S10) were selected in the centre of the main current and in the littoral zone of the western branches of the Tien Yen and Kalong Rivers, respectively (Fig. 1). In the Tien Yen River, the uppermost station, TS14, was located approximately 15 km from the river mouth and the lowest station, TS1, was located off the river mouth. The salt wedge usually reaches just below the station TS14. In the Kalong River, the highest upriver station (L9) was located just below the national border between Vietnam and China, with station S1 being the lowest at the surf zone of the sandy beach (Fig. 1).

For the pelagic stage, we made 10-minute horizontal tows monthly by boat at the surface and in the centre of the current with a larval net (1 m mouth-diameter, 0.5 mm mesh-aperture). For the immigrated larvae, a small seine net (1 × 4 m, 1 mm mesh-aperture) was used in the littoral zone. A single day's sampling usually consisted of one to four hauls at each littoral station. Sampling of upstream migrating juveniles was carried out monthly by cast-net along littoral waters of the river from March–July 2015. However, no ayu were collected during the study period; thus, the catch and environmental results from these surveys are not reported herein.

Most stations were surveyed regularly, but stations TL2, TL10 and TL11 for the larval net and stations TS1, TS3, TS12, TS14 and S5 for the seine net had a modified sampling regime where collections were made according to the tidal cycle and in some instances carried out to avoid severe weather conditions (Fig. 1).

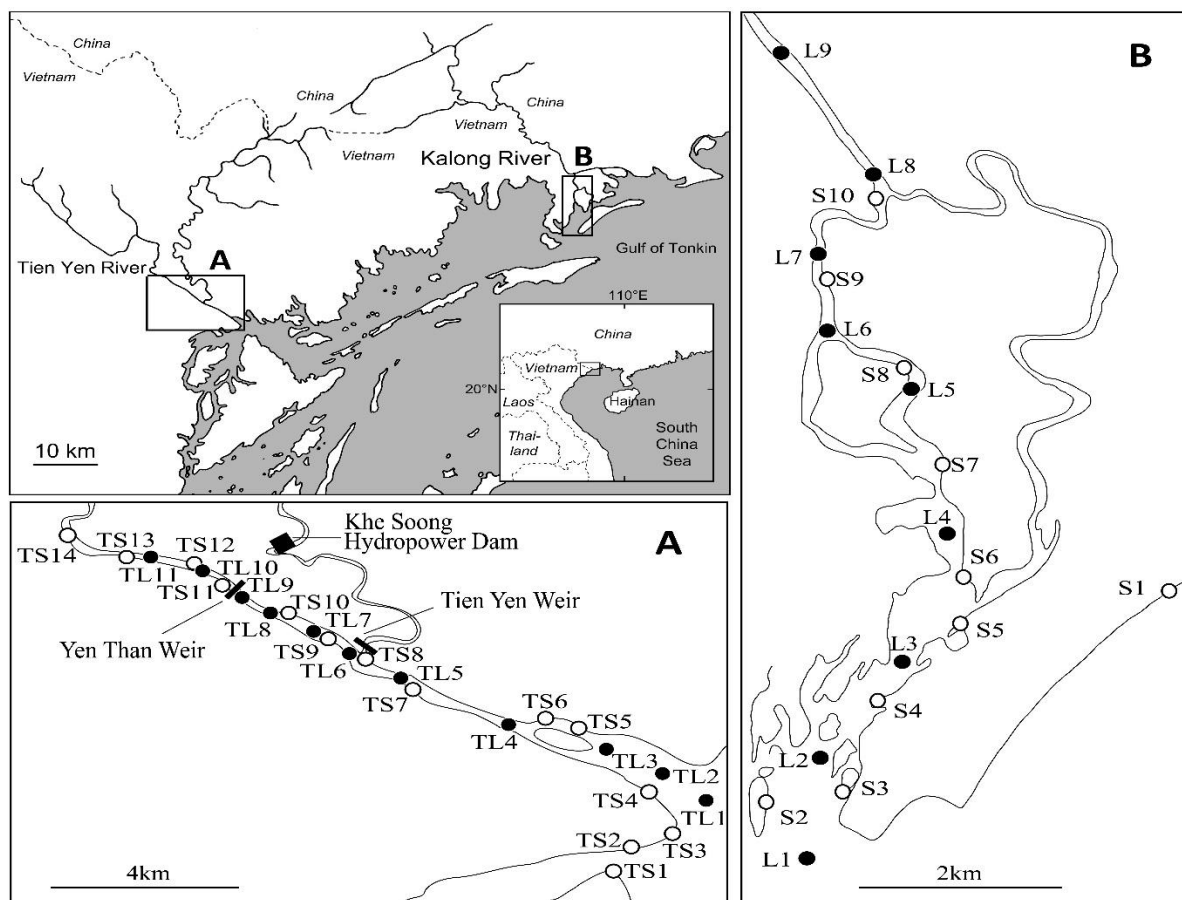


Fig. 1. Sampling stations for collection of ayu (*Plecoglossus altivelis*) larvae and juveniles in the Tien Yen and Kalong River systems, northern Vietnam, during the years 2010–2015. Stations where ayu larvae and juveniles were sampled are indicated by solid circles (stations TL1–TL11 and L1–L9, larva net) and open circles (stations TS1–TS14 and S1–S10, seine net), respectively. Sampling sites in the Kalong River were modified from Tran et al. (2012a).

Sampling periods

Between 2010 and 2011: The larval net was used at stations TL1–TL4 from November 2010 to February 2011 in the Tien Yen River (Fig. 1). Additionally, the seine net was used in the bank waters of the Tien Yen River, stations TS1–TS6 (Fig. 1). The present study used the water conditions recorded both in the centre of the current and the littoral zone in the 2010–2011 surveys.

Between 2013 and 2014: The seine net alone was used in the littoral zone of the 2013–2014 spawning time in the Tien Yen River. No surveys were conducted in the Kalong River in the sampling period of this year.

Between 2014 and 2015: Both the aforementioned larval net and seine net were used to collect ayu larvae and juveniles. In the centre of the Tien Yen River, collections were made monthly at stations TL4–TL11 in November 2014 and March 2015, and bimonthly between December 2014 and February 2015. At stations TL4, TL6 and TL9, we also made oblique and 3–4 m depth tows to investigate the vertical distribution of larvae and juveniles. Because of a weir across the Tien Yen River, sampling was limited upriver to surface tows at stations TL10 and TL11 and was carried out only in mid-December 2015 (Fig. 1; Table 1). In the littoral zone of the Tien Yen River, the same sampling times were used at stations TS7–TS14 (Fig. 1).

Sampling sites within the Kalong estuary were partly modified after Tran et al. (2012b), with two added stations (L8, L9) in the upper reach of the Kalong River system (Fig. 1). Surveys were carried out monthly from November 2014 to March 2015 in the centre (stations L0–L9) and littoral waters (stations S1–S10) within the Kalong estuary.

Measurement of environment and larvae

Samples collected by net tow were fixed in 5 % formalin, with ayu specimens immediately sorted and transferred to 80 % ethanol. In some months, fishes were preserved directly in ca. 80 % ethanol for molecular analyses. Body length BL (notochord length in preflexion and flexion larvae), and standard length for postflexion larvae and juveniles) was measured to the nearest 0.1 mm with a binocular microscope and camera lucida, with the exception of juveniles which were measured with a digital caliper.

During sampling, water temperature (°C), salinity (psu), and turbidity (NTU) were measured at each littoral station (stations S1–S10 and TS1–TS14) and at 1-m depth intervals from the surface to bottom at each station along the centre of the main channel (stations L1–L9 and TL1–TL11) using a water quality checker (WQC-22A, TOA DDK). Exceptions to this were the following: November 2014 at the Tien Yen River, measurements were obtained using a CTD (AST500-P, Alec Electronics); January 2015 survey in the Kalong River, turbidity was vertically measured only at stations L1–L3, and at the surface in stations L5 and L7–L9 by a water quality checker (WQC-22A, TOA DDK), and salinity only at the surface layer by Atago, except for station L9. From 2014 to 2015, at the surface of each main channel station dissolved oxygen (mg.L^{-1}) was measured using a WinLab® Data Line. Additional turbidity data from the main channel of the Kalong River between 2010 and 2011 were obtained with a water quality checker (WQC-22A, TOA DDK) which was also used in the present study.

Results

Environmental characteristics of the main channels

From 2010 to 2011, stations (TL1–TL4) from the Tien Yen River system were more stratified, particularly with respect to salinity and turbidity (Fig.2). Water temperatures were greater than 20 °C from November to early December, subsequently declining to around 17 °C at the inner stations (TL3 and TL4) and 16 °C at the outer stations (TL1 and TL2) in mid-February (Fig. 2).

Table 1. Number of individuals (n), densities/CPUE, stages, and body lengths (BL) of ayu *Plecoglossus altivelis* collected, with water temperature, salinity, turbidity and dissolved oxygen (DO) during sampling conducted with a larva net (1 m in diameter, 4.0 m in length with 0.5 mm mesh) and seine net (1 × 4 m, 1.0 mm mesh) along the Tien Yen River and Kalong River, northern Vietnam from 2014 to 2015.

Dates	Sampling station, type of tow	Tide	n	Stage	BL (mm) range	Water temperature (°C) range	Salinity (psu) range	Turbidity (NTU) range	DO (mg.L ⁻¹) range
Tien Yen River									
In the centre of current									
2014.12.22	TL5 ^{ob}	H, E	1 (8.5) ^a	B	6.7	17.6–17.7	10.1–24.4	2–4	8.6
	TL8 ^{su}	H, E	2 (7.3) ^a	B	7.4–9.5	16.9–18.7	5.9–13.9	2–3	8.6
	TL9 ^{su}	H, E	1 (3.9) ^a	B	7.7	16.7–18.2	4.6–10.2	0–2	9.6
	TL10 ^{su}	H, E	6 (24.6) ^a	B	7.2–9.4	16.2–16.9	3.2–5.2	0–2	N.D
	TL11 ^{su}	H, E	8 (38.5) ^a	A, B	5.8–9.0	15.7–16.5	0.5–12.0	3–4	N.D
2015.1.6	TL6 ^{su}	H, E	1 (3.5) ^a	B	5.6	18.1–18.2	7.2–20.3	1–4	8.9
	TL8 ^{su}	H, E	1 (3.2) ^a	B	5.4	18.1–18.2	5.6–15.2	0–3	8.6
	TL9 ^{su}	H, E	2 (7.8) ^a	B	5.3–5.5	18.0–18.2	3.8–10.4	1–3	8.9
	TL9 ^{ob}	H, E	1 (9.4) ^a	B	7.0	18.0–18.2	3.8–10.4	1–3	8.9
	TL6 ^{4-m}	H, E	1 (3.6) ^a	B	5.3	17.6–18.2	8.9–25.8	9–13	7.8
2015.1.23	TL9 ^{su}	L, E	1 (4.6) ^a	B	6.9	17.4–18.1	7.7–11.1	7–8	N.D
	TL9 ^{ob}	L, E	1 (5.9) ^a	B	7.6	17.4–18.1	7.7–11.1	7–8	N.D
In the littoral waters									
2015.2.8	TS8 ^{sc}	L, E	2 (0.8) ^b	D	15.0–22.2	17.3	6.8	55	N.D
2015.3.10	TS10 ^{sc}	L, E	2 (2.0) ^b	E	51.1–59.1	21.0	5.3	10	N.D
Kalong River									
In the centre of current									
2014.11.21	L1 ^{su}	L, E	2 (9.3) ^a	B	5.1–5.2	22.9	14.9–15.7	1–4	8.1
	L6 ^{su}	L, E	1 (6.8) ^a	A	5.5	22.1	3.8–3.9	3–4	8.2
	L7 ^{su}	L, E	1 (3.9) ^a	A	4.3	21.9	0.5–0.9	3–5	8.4
	L9 ^{su}	H, E	2 (10.4) ^a	B	6.0–6.1	21.2	0.0–0.1	0–2	7.4
2014.12.8	L1 ^{su}	L, E	3 (25.1) ^a	A	4.3–4.5	17.5	24.6–24.7	15–25	9.0
	L2 ^{su}	H, E	2 (13.8) ^a	A, B	4.4	17.3	28.4–28.5	11–15	9.2

Table 1 continued

Dates	Sampling station, type of tow	Tide	n	Stage	BL (mm) range	Water temperature (°C) range	Salinity (psu) range	Turbidity (NTU) range	DO (mg.L ⁻¹) range
2015.1.8	L5 ^{su}	H, E	13 (48.3) ^a	A, B	5.1–5.7	17.3–17.6	13.4–25.8	0–1	9.7
	L6 ^{su}	H, E	13 (49.8) ^a	A, B	4.4–6.0	17.6–18.0	8.1–13.7	0–2	10.2
	L7 ^{su}	H, E	17 (68.4) ^a	A, B	4.0–6.1	17.6–18.3	4.0–14.0	0–3	9.3
	L8 ^{su}	H, E	10 (36.5) ^a	A, B	5.2–6.4	18.0–18.6	0.7–13.8	0–7	10.1
	L9 ^{su}	H, E	13 (60.8) ^a	A, B	5.1–6.4	18.6	0.1–0.6	4–5	9.0
	L5 ^{su}	H, E	3 (11.0) ^a	A, B	4.3–5.3	17.1–17.5	21.0	2	N.D
	L6 ^{su}	H, E	6 (23.7) ^a	A, B	5.0–5.5	17.3–17.5	15.0	2	N.D
	L7 ^{su}	H, E	6 (40.3) ^a	A, B	5.1–6.0	17.4–17.5	10.0	2	N.D
	L8 ^{su}	H, E	23 (81.7) ^a	A, B	5.3–6.7	17.5–17.7	5.0	3–4	N.D
2015.2.9	L9 ^{su}	H, E	29 (171.9) ^a	A, B	5.5–6.6	18.0–18.2	N.D	3	N.D
	L3 ^{su}	H, E	1 (3.2) ^a	B	9.1	15.2–15.3	31.6–32.8	5–6	11.0
	L4 ^{su}	H, E	1 (2.8) ^a	B	5.1	15.2–15.4	26.7–31.2	4–5	9.7
	L6 ^{su}	H, E	2 (5.9) ^a	A, B	5.0–5.4	15.7	14.2–21.9	2–4	9.5
	L7 ^{su}	H, E	4 (7.9) ^a	A	4.0–4.5	16.0	8.8–11.7	1–2	9.4
	L8 ^{su}	H, E	5 (15.3) ^a	A, B, D	6.0–18.5	16.2–16.4	1.8–4.3	0–1	7.0
In the littoral waters									
2015.2.9	S2 ^{se}	L, E	2 (0.4) ^b	D	19.0–19.8	16.0	31.9	1	N.D
	S3 ^{se}	L, E	1 (0.7) ^b	D	19.5	16.4	32.9	76	N.D
	S4 ^{se}	L, E	1 (1.0) ^b	D	18.1	15.8	30.6	1	N.D

Developmental stage: *A* = Preflexion larva with yolk sac, *B* = Preflexion larva without yolk sac, *D* = Postflexion larva, *E* = Juvenile

Tide: *E* = Ebb tide, *L* = Low tide, *H* = High tide

Type of tow: ^{ob} Oblique tow by a larval net; ^{su} Surface tow by a larval net; ^{se} Seine net tow; ^{4-m} 4 m depth tow

^a Density (n.1,000 m⁻³) by a larva net in the centre of current; ^b CPUE [n.haul⁻¹ (ca. 50 m)] by a seine net in the littoral zone;

N.D: no data

From 2014 to 2015, the hydrological characteristics of the river showed similar patterns in salinity and turbidity, as shown in 2011 (Figs. 2, 3). The lowest temperature was recorded in early February 2015 at approximately 16–17 °C, and this increased to >20 °C by late February (Fig. 3). These values tended to be higher at the outer rather than the inner stations in most months, in contrast to late February and March 2015 (Fig. 3). Temperatures were higher in late February relative to March (Fig. 3). At stations TL10 and TL11, water temperature, salinity and turbidity ranged from 15.7 to 16.9 °C, 0.5–12.0 psu and 0–4 NTU, respectively (Table 1).

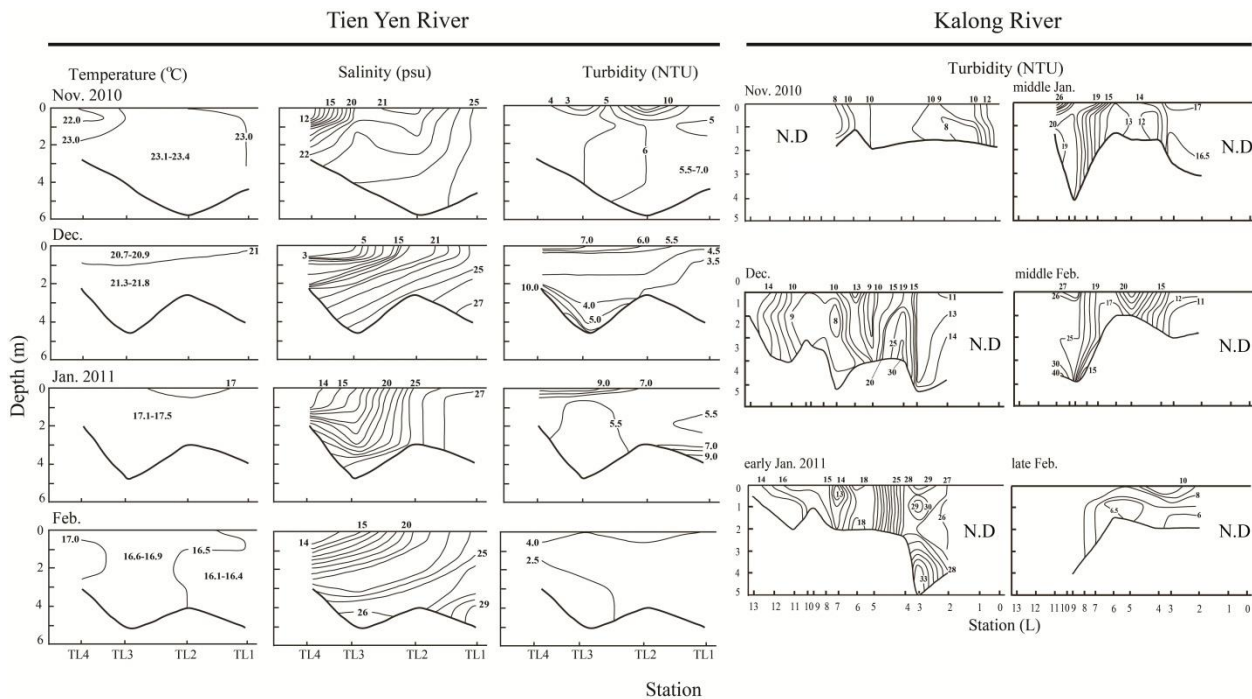


Fig. 2. Seasonal variation in vertical profiles of physical parameters along an axis from station TL1 to TL4 in the Tien Yen River (three parameters) and the Kalong River (turbidity) from November 2010 to February 2011 (see Fig. 1 in the present study, and Fig. 7 in Tran et al. 2012a). N.D: no data.

In contrast to the Tien Yen River, the Kalong River was more vertically well-mixed and received higher tidal exchange, with the exception of a measurable pycnocline in November and December and a stratification in turbidity in November (Fig. 4). Water temperatures were >20 °C from November to December, then declined to around 16 °C in February, and subsequently increased to approximately 18–19 °C by mid-March. These values tended to be higher from the inner stations with the exception of November, where this trend was reversed (Fig. 4). In contrast to the 2010–2011 survey, turbidity of the Kalong River from the 2014–2015 survey was lower, with the exception of stations L1–L3 in January and March (Figs. 2, 4).

Water temperature, salinity, and turbidity during the period ayu larvae occurred in the survey ranged from 15.7 to 18.7 °C, 0.5–25.8 psu and 0–13 NTU in the Tien Yen River, and 15.2–22.9 °C, 0.0–32.8 psu and 0–25 NTU in the Kalong River, respectively (Table 1). Dissolved oxygen in the surface layer ranged from 8.6–9.6 for Tien Yen and from 7.0–11.0 mg.L⁻¹ for the Kalong River system (Table 1).

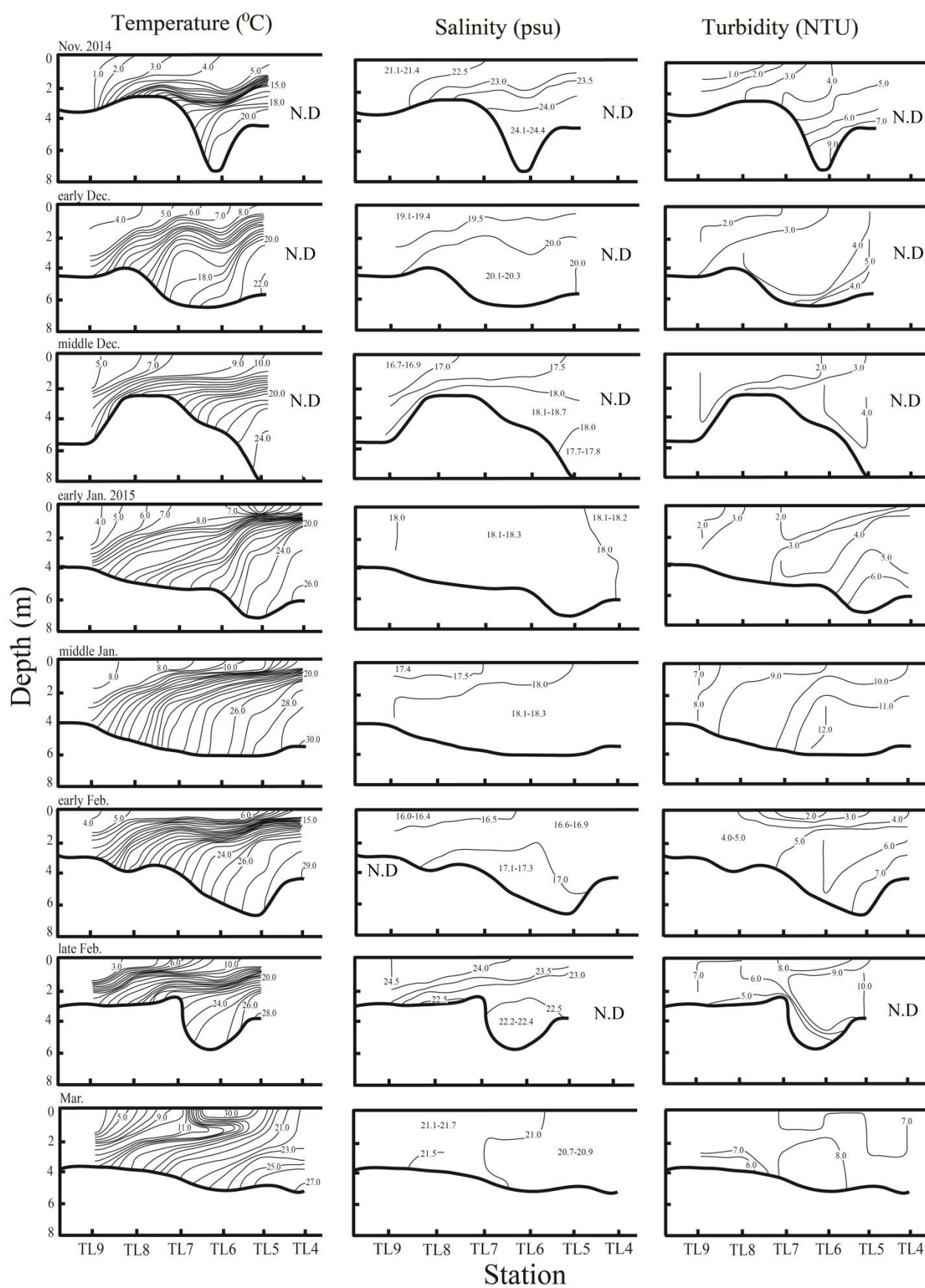


Fig. 3. Seasonal variation in the vertical profiles of water temperature (°C), salinity (psu) and turbidity (NTU) along an axis from station TL4 to TL9 in the Tien Yen River from November 2014 to March 2015 (see Fig. 1). N.D: no data.

Distribution of larvae in main channels

In the Tien Yen River, a total of 26 ayu larvae were collected between December 2014 to January 2015, with densities ranging from 3.2 to 38.5 individuals.1000 m⁻³ (Table 1). No ayu larvae were collected at stations TL1–TL4 during surveys of years 2010–2011 (Table 1; Fig. 1).

All larvae were at the preflexion stage, ranging from 5.3 to 9.5 mm BL, with three of the larvae having a yolk sac. Larvae were collected only at stations TL5, TL6, TL8–TL11 from 2014 to 2015, with no collections from 2010 to 2011 (Fig. 1; Table 1). Fish were collected primarily at the surface and were rarely found at any depth, with only one instance in which one larva was captured at 4-m depth at station TL6 (Table 1). Although sampling occurred only at the surface in one month at station TL11, upwards of 50 % of the preflexion larvae had a yolk sac, with the size of preflexion larvae without a yolk sac (7.9–9.0 mm BL) being larger than those collected from other sites (Table 1).

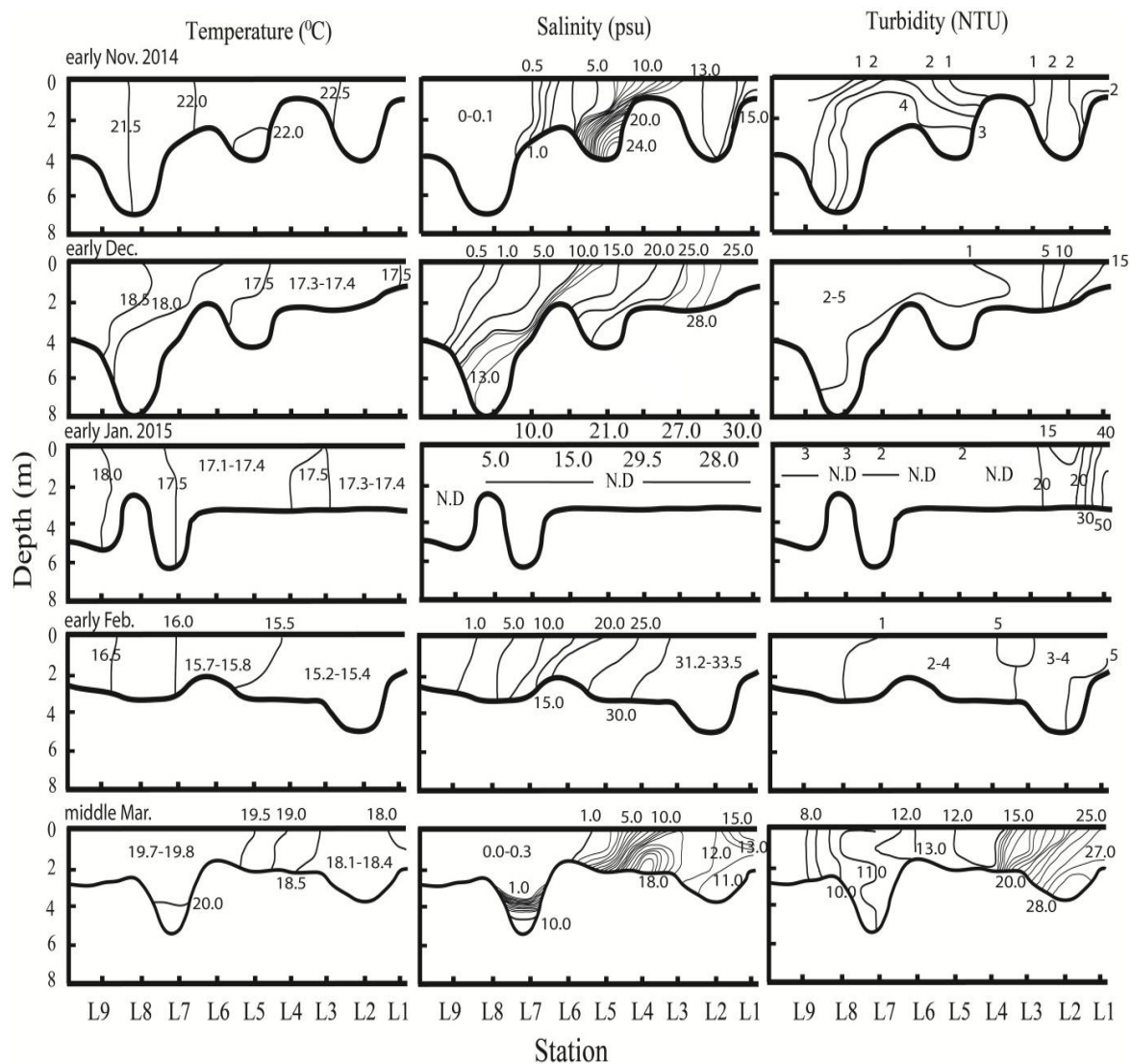


Fig. 4. Seasonal variation in the vertical profiles of physical parameters along an axis from station L1 to L9 in the Kalong River from November 2014 to March 2015 (see Fig. 1). N.D: no data.

From the Kalong River, a total of 156 ayu larvae were collected between November 2014 and February 2015, with a peak abundance in early January (Table 1; Fig. 6). Densities of ayu ranged from 2.8 to 171.9 individuals.1000 m⁻³, which comprised 82 of 154 (53 %) at preflexion with a yolk sac ranging from 4.0 to 9.1 mm BL, and 2 postflexion larvae from 15.2 to 18.5 mm BL (Table 1). The distribution of larvae tended to occur at stations L5–L9. However, larvae were more widely distributed and found at most of the sampling stations in December (Table 1).

Environmental characteristics in the littoral zone

Of the three water quality parameters measured from littoral waters, the average turbidity showed the highest monthly variation, especially between the years of 2014 to 2015 (Fig. 5). Mean salinity values were highest in the years 2010 to 2011 from the Tien Yen River, which exceeded all other years from both the Tien Yen and Kalong Rivers. This was mainly due to the fact that sampling sites in 2010–2011 were predominantly located in outer parts of the Tien Yen estuary (stations TS1–TS6) (Figs. 1, 5). The average salinity was <10 psu from the Tien Yen River from November 2014 to February 2015, and the average temperature was <20 °C between January to early February of 2015 (Fig. 5).

Distribution of larvae and juveniles in littoral waters

From the Tien Yen River, only two postflexion larvae (15.0–22.2 mm BL) and two juveniles (51.1–59.1 mm BL) were collected, which occurred at stations TS8 and TS10 between February and March 2015, respectively (Table 1). No larvae or juveniles were collected at stations TS1–TS6 between 2010 and 2011, and at stations TS2 and TS4–TS14 in 2013 to 2014, and stations TS7, TS9 and TS10–TS14 during the 2014–2015 survey (Fig. 1; Table 1).

At collection sites within the Tien Yen, water temperature, salinity and turbidity were from 17.3 to 21.0 °C, from 5.3 to 6.8 psu, and from 10 to 55 NTU, respectively (Table 1). During the cast-net sampling period, no upstream-migrating juvenile stages were collected. In the Kalong River, four postflexion larvae (18.1–19.8 mm BL) were collected at stations S2–S4 in early February 2015 (Table 1). At these collection sites, the water temperature, salinity and turbidity ranged from 15.8 to 16.4 °C, from 30.6 to 32.9 psu and from 1 to 76 NTU, respectively (Table 1).

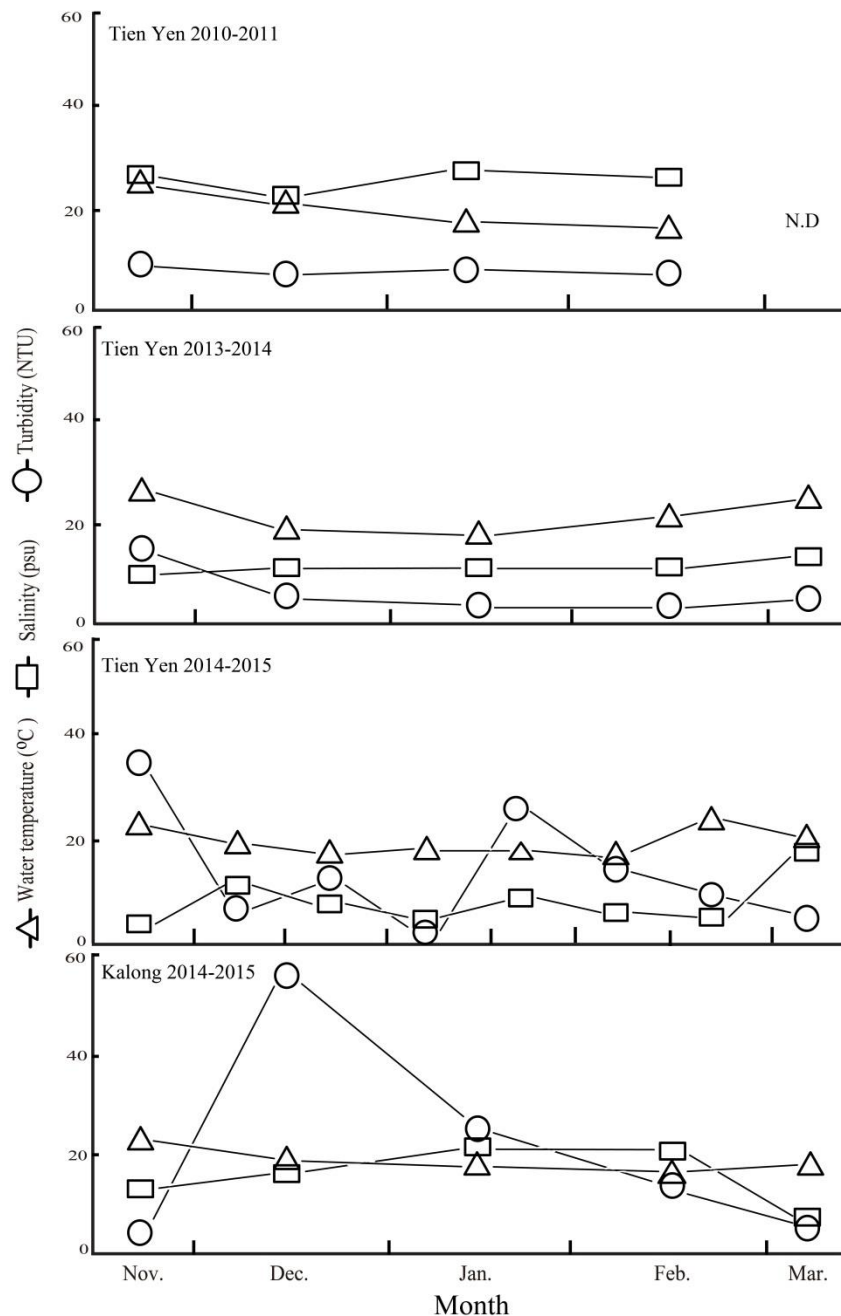


Fig. 5. Seasonal variation of mean temperature, salinity and turbidity in littoral waters around the Tien Yen River in the years 2010–2015 and the Kalong River in 2014–2015. N.D: no data.

Discussion

This is the first record of ayu larvae and juveniles in the Tien Yen estuary, though previously the occurrence and downstream migration of newly hatched larvae and ayu reproductive traits were reported by Tran et al. (2017a, b). Within our study, preflexion larvae started to occur in late December in the Tien Yen estuary (Table 1) as ayu started to drift downstream in mid-December (Tran et al. 2017a). Thus, the ayu in the Tien Yen River may start to spawn in late November, approximately 1–2 months later than *Plecoglossus altivelis altivelis* and *P. a. ryukyuensis* from Japan (Kishino and Shinomiya 2004). It has been reported that ayu start spawning when water temperatures fall below 20 °C (Kishino and Shinomiya 2004).

In the Tien Yen estuary, the water temperature range during successful sampling was always below 20 °C (Table 1; Fig. 3), suggesting that ayu in the Tien Yen also begin to reproduce when water temperatures are below 20 °C. The spawning season starts later in warmer, low latitude regions. Due to this delay, larval aggregation in the Tien Yen estuary occurred ca. 2 months later in the littoral zone (Senta and Kinoshita 1985; Takahashi et al. 1990; Tago 2002; Aljamali et al. 2006) and their CPUE was much lower during the study period (Table 1; Fig. 6). Although we conducted surveys throughout the year, no larvae were collected in littoral waters after March, and this was also reported by Tran et al. (2012a).

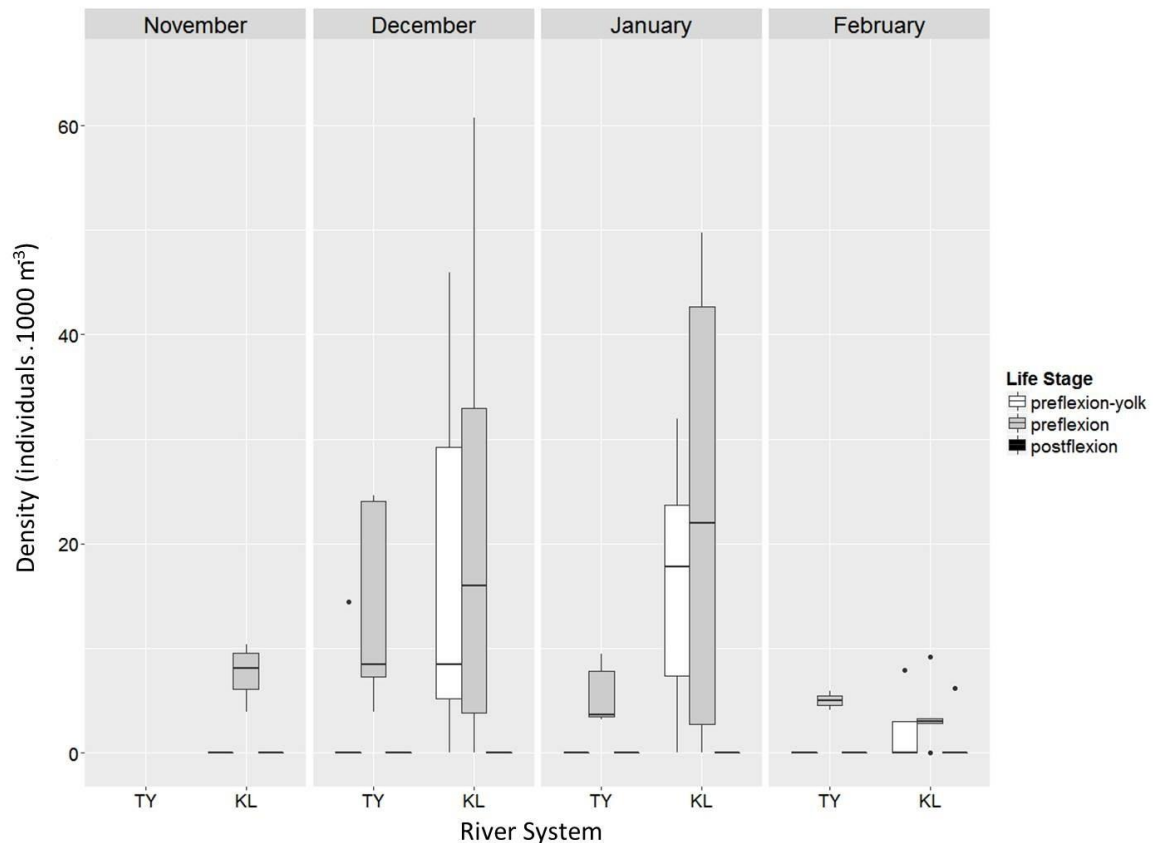


Fig. 6. Boxplot of monthly mean ($\pm$ SD) densities of larval ayu *Plecoglossus altivelis* collected from net hauls in main channels of the Kalong (KL) and Tien Yen (TY) river systems. Collections by month were from November and December 2014, and January to February 2015.

Similar to the findings of a study by Tran et al. (2012a), ayu larvae were abundant at the station far from the river mouth (Table 1). In Japan, likewise, a number of larval remainders of ayu were found in some estuaries (Takahashi et al. 1990; Aljamali et al. 2006). In the Tien Yen river system, a number of newly-hatched ayu larvae during downstream migration were collected at station TS14 (Fig. 1; Tran et al. 2017a), with few being collected in the estuary. The remaining larvae in the Tien Yen were likely due to a number of factors. Tran et al. (2017a) have suggested that ayu spawn beyond station TS14, and with seven pools between TS14 and the Yen Than Weir (Fig. 1), this could have reduced downstream drift rate of larvae to the estuary. This has similarly been observed in the case of landlocked larval Ryukyu-ayu (Kawakami and Tachihara 2011).

Additionally, ayu larvae may remain in the estuary to avoid cooler less-favourable ocean conditions (Tran et al. 2012a), and this is consistent with the water temperature trends from the Kalong River estuary observed here (Fig. 4). However, the opposite trend can be seen from the Tien Yen River, where the river water temperature was lower than that of the sea (Figs. 2, 3). According to Saruwatari (1995), the higher temperature of adjacent coastal waters to Lake Hinuma would have triggered a seaward migration of ayu larvae. Thus, the overall gradient in water temperature possibly determines the distribution pattern of ayu larvae in an estuary.

It has been observed that a bigger number of larger-sized ayu larvae are collected during more stratified conditions in the water column (Aljamali et al. 2006; Yagi et al. 2006). In this study, the two rivers contrasted in their vertical profiles, with the Tien Yen being more stratified and the Kalong River being more mixed (Figs. 3, 4). Despite observed levels of stratification, however, no larvae >10 mm BL were collected from the main channels of both rivers during the study period (Table 1). In the Shimanto River of Japan, ayu larvae begin to migrate from the near-bottom layer to the littoral zone at ca. 10 mm BL (Takahashi et al. 1998). In the Tien Yen River, we also made oblique and deeper layer tows to examine their spatial and depth-specific distribution; however, we failed to collect additional ayu (Table 1). Thus, a more comprehensive understanding of the distribution of early life stage ayu over space and time in coastal systems of Vietnam remains elusive. However, it can be assumed that smaller larvae <7.0 mm BL were well-dispersed throughout the two estuaries (Table 1), similar to observations reported by Takahashi et al. (1998), and that they subsequently migrate to littoral waters when they attain a postflexion stage >15 mm BL.

Conclusion

From this study, the distribution of larval ayu within two estuaries of Vietnam can be explained by topography, gradients in water temperature and water column stratification. To fully understand ayu migration patterns in these systems, further investigations incorporating greater spatio-temporal sampling are needed and the application of other methods such as otolith microchemistry would provide significant additional insight. It is significant to note that the present study confirmed the existence of a reproducing population of ayu in the southernmost range of its global distribution. Although few larvae were observed from the southernmost region of the Tien Yen estuary, the results provide critical information for future research on population and distributional dynamics and viability of the population, which ultimately can be applied to conservation efforts.

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References

- Aljamali, E., I. Kinoshita, M. Sashida, T. Hashimoto and J. Nunobe. 2006. Do the ayu (*Plecoglossus altivelis altivelis*) born in the river with an inlet or larger estuary in its mouth perform a homing? *La Mer* 44:145–155.
- Azuma, K., H. Hiraga and I. Kinoshita. 2003. Influence by precipitation to spatial dispersion of ayu larvae swept downstream to sea. *Nippon Suisan Gakkaishi* 69:352–358.
- Hosoya, K. 2002. *Plecoglossidae*. In: *Fishes of Japan with pictorial keys to the species*, English edition (ed. T. Nakabo), p. 297. Tokai University Press, Tokyo.
- Kawakami, T. and K. Tachihara. 2011. Dispersal of land-locked larval Ryukyu-ayu, *Plecoglossus altivelis ryukyuensis*, in the Fukuji Reservoir, Okinawa Island. *Cybiu* 35:337–343.
- Kishino, T. and A. Shinomiya. 2004. Seasonal appearance and size of newly-hatched larvae of Ryukyu-ayu *Plecoglossus altivelis ryukyuensis* in the Yakugachi and Kawauchi Rivers, Amamioshima Island, southern Japan. *Japanese Journal of Ichthyology* 51:149–156.
- Kishino, T. and A. Shinomiya. 2005. Migration and recruitment of amphidromous Ryukyu-ayu (*Plecoglossus altivelis ryukyuensis*) larvae and juveniles in Sumiyo and Yakugachi bays and neighboring waters, Anami-oshima Island, southern Japan. *Japanese Journal of Ichthyology* 52:115–124.
- Nguyen, T.T., H.D. Tran and T.T.H. Tran. 2015. Additional data on morphology and distribution of ayu (*Plecoglossus altivelis*) in Vietnam. *VNU Journal of Science* 31:422–428.
- Nishida, M. 1988. A new subspecies of the ayu, *Plecoglossus altivelis* (*Plecoglossidae*) from the Ryukyu Islands. *Japanese Journal of Ichthyology* 35:236–243.
- Nishida, M., Y. Sawashi, S. Nishijima, M. Azuma and H. Fujimoto. 1992. Distribution and abundance of the ryukyuan subspecies of the ayu *Plecoglossus altivelis ryukyuensis*: results of a survey made in 1986. *Nippon Suisan Gakkaishi* 58:199–206.
- Saruwatari, T. 1995. Temporal utilization of a brackish water lake, Lake Hinuma, as a nursery ground by amphidromous ayu, *Plecoglossus altivelis* (*Plecoglossidae*) larvae. *Environmental Biology of Fishes* 43:371–380.
- Senta, T. and I. Kinoshita. 1985. Larval and juvenile fishes occurring in surf zones of western Japan. *Transactions of the American Fisheries Society* 114:609–618.
- Shan, X.J., Y.F. Wu and B. Kang. 2005. Morphological comparison between Chinese ayu and Japanese ayu and establishment of *Plecoglossus altivelis chinensis* Wu & Shan subsp.nov. *Journal of Ocean University of China* 4:61–66.
- Tachihara, K. 2015. *Plecoglossus altivelis ryukyuensis*. In: *Red data book 2014, Threatened Wildlife of Japan* (ed. Ministry of Environment), pp. 52–53. Gyosei Corporation, Tokyo.

- Tachihara, K. and S. Kimura. 1991. Embryonic development and morphological changes with growth of the larval and juvenile ayu *Plecoglossus altivelis altivelis* of Lake Ikeda in southern Kyushu. Nippon Suisan Gakkaishi 57:789–795.
- Tago, Y. 2002. Occurrence and size distribution of ayu larvae around the surf zones facing Toyama Bay related to habitat shift. Nippon Suisan Gakkaishi 68:144–150.
- Takahashi, I., I. Kinoshita, K. Azuma, S. Fujita and M. Tanaka. 1990. Larval ayu *Plecoglossus altivelis* occurring in the Shimanto estuary. Nippon Suisan Gakkaishi 56:871–878.
- Takahashi, I., K. Azuma, S. Fujita and I. Kinoshita. 1998. Spatial distribution of larval ayu *Plecoglossus altivelis* in the Shimanto estuary. Fisheries Science 64:522–525.
- Takahashi, I., K. Azuma, S. Fujita and I. Kinoshita. 2002. Habitat shift of ayu *Plecoglossus altivelis altivelis* in early stages from waters adjacent to the bank to the center of flow in the Shimanto Estuary. Fisheries Science 68:554–559.
- Tran, D.H., I. Kinoshita, T.T. Ta and K. Azuma. 2012a. Occurrence of Ayu (*Plecoglossus altivelis*) larvae in northern Vietnam. Ichthyological Research 59:169–178.
- Tran, D.H., T.T. Ta and D.H. Nguyen. 2012b. Preliminary data on the ayu (*Plecoglossus altivelis*) in Vietnam. In Proceedings of the 1st national scientific conference on biological research and teaching in Vietnam, pp. 94–99. Agricultural Publishing House.
- Tran, D.H., I. Kinoshita, K. Azuma, T. Iseki, Y. Yagi, J. Nunobe and T.T. Ta. 2014. The potential biodiversity of Ayu, as evidenced by differences in its early development and growth between Vietnam and Japan. Environmental Biology of Fishes 97:1387–1396.
- Tran, D.H., M. Iida and K. Maeda. 2017a. Downstream migration of newly-hatched ayu (*Plecoglossus altivelis*) in the Tien Yen River of northern Vietnam. Environmental Biology of Fishes. <https://doi.org/10.1007/s10641-017-0646-6>.
- Tran, D.H., P.H. Nguyen and X.H. Nguyen. 2017b. Reproductive biology of the ayu (*Plecoglossus altivelis*) from its southernmost distribution range. Asian Fisheries Science 30:274–283.
- Yagi, Y., C. Bito, T. Funakoshi, I. Kinoshita and I. Takahashi. 2006. Distribution and feeding habits of ayu *Plecoglossus altivelis altivelis* larvae in coastal waters of Tosa Bay. Nippon Suisan Gakkaishi 72:1057–1067.
- Yoshimoto, Y., H. Fuji and H. Nakanishi. 2006. Occurrence of Ayu *Plecoglossus altivelis altivelis* larvae in the Hidaka estuary. Aquaculture Science 54:543–551.

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