Dispersal of land-locked larval Ryukyu-ayu, *Plecoglossus altivelis ryukyuensis*, in the Fukuji Reservoir, Okinawa Island

by

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ABSTRACT. - The dispersal of larval land-locked Ryukyu-ayu, *Plecoglossus altivelis ryukyuensis*, which originated from an amphidromous form on Amami-oshima Island, was investigated from November 2000 to April 2001 in the freshwater Fukuji Reservoir. A total of 232 larvae were collected at three stations at different distances from the mouth of the San-numata River. They were most abundant at the station closest to the San-numata River (Station1; 76.3% of total catch) and their distribution in the reservoir was not uniform. Although larvae tended to disperse throughout the sampling area during the sampling period, their abundance decreased significantly as the distance from the river increased. The body length (BL) of larvae ranged from 5.1 to 23.0 mm. Yolk-sac larvae were collected only at Station1, and larvae smaller than 10 mm BL accounted for 80.2% of the all larvae collected at Station1, whereas they accounted for 56.3% of the all larvae collected at Station2. The body size and estimated age based on body length were highly similar between the largest larva in the reservoir (23.0 mm, about 60 days) and the smallest ascending larva (24.2 mm, about 63 days). These results indicate that larvae inhabit the area close to the river mouth after drifting, and staying there until they begin upstream migration without experiencing long-distance dispersal.

RÉSUMÉ. - Dispersion des larves landlocked de l'ayu des Ryukyu, *Plecoglossus altivelis ryukyuensis,* dans le réservoir de Fukuji, île d'Okinawa.

La dispersion des larves landlocked de l'ayu des Ryukyu, *Plecoglossus altivelis ryukyuensis,* issu d'une forme amphidrome de l'île d' Amami-oshima, a été étudiée de novembre 2000 à avril 2001 dans les eaux douces du réservoir de Fukuji. Les larves ont été collectées dans 3 stations à différentes distances de l'embouchure de la rivière San-numata. Au total, 232 larves ont été collectées. Elles sont plus abondantes à la station la plus proche de la rivière San-numata (Station 1 : 76,3% des captures) et leur distribution dans la retenue est non uniforme. Bien que les larves aient tendance à disperser dans la zone échantillonnée dans la période donnée, leur abondance décroît significativement lorsque la distance à la rivière augmente. La longueur du corps des larves se situe entre 5,1 et 23 mm quelle que soit la station. Cependant, les larves vésiculées ne sont collectées que sur la station 1 et les larves de taille inférieure à 10 mm y représentent 80,2% tandis qu'elles représentent 56,3% des collectes à la station 2. La longueur du corps et l'estimation de l'âge sont similaires entre les plus grosses larves de la retenue (23 mm, environ 60 jours) et les plus petites larves en montaison (24,2 mm, environ 63 jours). Ces résultats indiquent que les larves occupent la zone proche de l'embouchure après la dévalaison et y restent jusqu'au début de leur migration amont sans réaliser de dispersion à longue distance.

Key words. - *Plecoglossus altivelis ryukyuensis* - Fukuji Reservoir - Land-locked population - Larvae - Dispersal - Amphidromy.

The Ryukyu-ayu, *Plecoglossus altivelis ryukyuensis* Nishida, 1988, is an amphidromous fish with an annual life history (Shinomiya, 2003). It is classified as an endemic subspecies living in the Ryukyu Islands based on morphological and genetic differences from its typical subspecies, the ayu, *Plecoglossus altivelis altivelis* (Nishida, 1988). Although *P. a. ryukyuensis* had been recorded on both Okinawa Island and Amami-oshima Island, wild populations on Okinawa Island went extinct during the 1970s as a result of habitat destruction (Nishida *et al.*, 1992). Given that the extant wild populations on Amami-oshima Island have also decreased due to habitat destruction caused by deforestation, riverine development, and typhoons, the conservation status of this subspecies is regarded as "critically endangered" (Shinomiya, 2003). To restore *P. a. ryukyuensis* populations on Okinawa Island, artificially cultured juveniles originating from the Amami-oshima population have been released into rivers and reservoirs on the island since 1992 (Ikehara and Shokita, 1994), and a land-locked population of *P. a. ryukyuensis* has subsequently become established in the Fukuji Reservoir (26°39'N, 128°09'E) and its inlets. This land-locked population is considered to be a useful genetic stock for the conservation of the endemic subspecies because this population contains the genetic variability of the original population (Ikeda *et al.*, 2001). This population also has an annual and semelparous life history, which is the same as the

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amphidromous form.

The larval dispersal of *P. a. altivelis* in estuaries and the sea is considered to take place via passive transportation driven by tidal currents, rainfall, and river discharge (Azuma *et al*., 2003). Dispersal of larval land-locked *P. a. altivelis* in Lake Biwa (Azuma, 1970) and Lake Ikeda (Tachihara and Kimura, 1991) is also affected by environmental conditions such as wind and water currents. The downstream migration and dispersal of amphidromous *P. a. ryukyuensis* larvae, however, is reportedly determined by larval behaviour such as sinking to the bottom layer and diurnal vertical movement (Kishino and Shinomiya, 2006). Because of this behaviour, amphidromous *P. a. ryukyuensis* larvae can avoid being dispersed by tidal currents and remain in the brackish water, the most suitable habitat for survival throughout their entire period living in the brackish area (about 2-4 months; Kishino and Shinomiya, 2003, 2005, 2006).

Considering this distribution in the wild and because the survival rate of *P. a. ryukyuensis* larvae is highest when salinity is approximately 15 psu (Kishino *et al*., 2008), the seaward dispersal of larvae may be limited by salinity, although whether the larval retention behaviour is a response to the saline environment remains unknown. By studying a land-locked population, we can exclude the effects of tides and salinity, thus simplifying the examination of larval dispersal. Here, we investigated the horizontal distribution of land-locked *P. a. ryukyuensis* larvae in a part of the Fukuji Reservoir, located on northern Okinawa Island. We also discuss the factors limiting the larval dispersal of *P. a. ryukyuensis*.

MATERIALS AND METHODS

Fish collection

Fish collections were performed at three stations (Stn. 1-3) in a part of the reservoir located over the lower San-numata River from mid-November 2000 to late April 2001 (Fig. 1). This sampling period mostly overlaps with the spawning period of *P. a. ryukyuensis*. And in the San-numata River, spawning of *P. a. ryukyuensis* was observed at just above backwater during the sampling period. Among four inlet rivers, *P. a. ryukyuensis* juveniles were released only into the San-numata River and now they mostly inhabit the San-numata River probably because of suitable habitat (Ikehara and Shokita, 1994), while a small number of adults found in other inlet rivers. St. 1, St. 2, and St. 3 were located approximately 900 m, 1,400 m, and 2,200 m downstream from the backwater of the San-numata River (where the river enters the reservoir), respectively. In surveys of distribution of *P. a. ryukyuensis* larvae in 1992 and 1993, a few larvae (0 in 1992 and 3 in 1993) collected by 10 min. horizontal surface tows of a conical net (1.3 m mouth diameter) at the station approximately 2,500 m downstream from the backwater of the San-numata River (North Dam Construction Office, personal communication). Therefore, we decided the location of three stations as in the present study. The water temperatures of the river and reservoir fluctuated within 13.8-21.0°C and 17.6-23.2°C during mid-November 2000 to late March 2001 (Kawakami and Tachihara 2010), and 21.0°C and 25.6°C in May 2001, respectively.

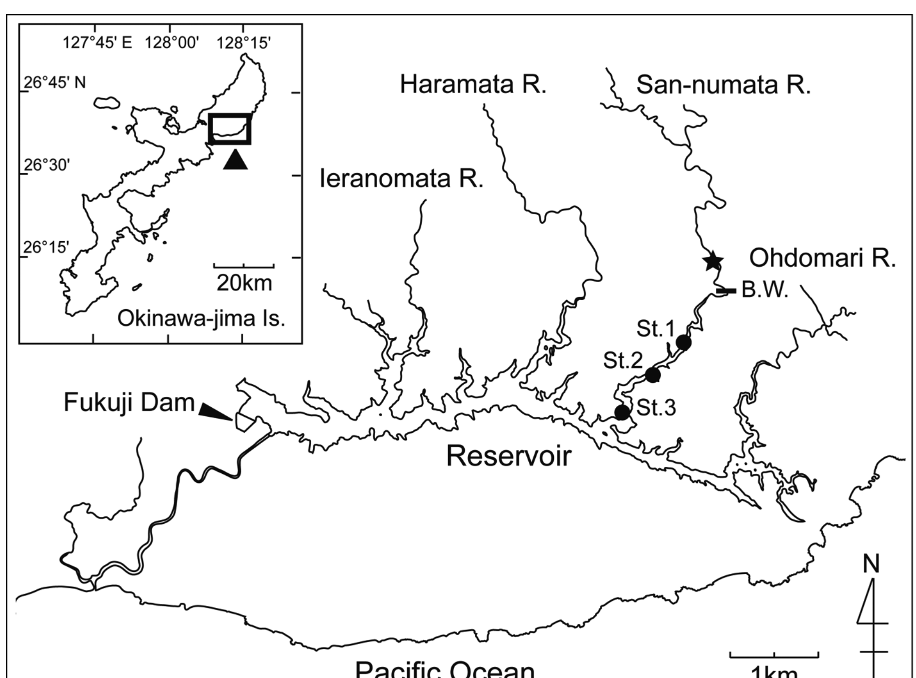

Figure 1. - Sampling stations for the collection of Ryukyu-ayu, *Plecoglossus altivelis ryukyuensis*, larvae and juveniles in the Fukuji Reservoir. Solid circles indicate stations where the larvae were collected. B.W. indicates the backwater at the highest water level. A black star indicates spawning ground of Ryukyu-ayu during November 2000 to March 2001.

Table I. - Monthly changes in the abundance (inds/tow) of Ryukyu-ayu, *Plecoglossus altivelis ryukyuensis*, larvae collected at St.1 to St.3 from November 2000 to April 2001. Numbers in parenthesis were number of collected larvae at each sampling and n indicate number of net tows.

	Month					
Station No.	November	December	January	February	March	April
1	6 (0-12, n = 2)	26 (17-44, n = 3)	13 (2-21, n = 3)	14 (7-29, n = 3)	2 (0-4, n = 3)	0 (n = 1)
2	0 (n = 1)	3 (0-8, n = 3)	2 (0-3, n = 3)	9 (5-15, n = 3)	4 (3-5, n = 2)	No data
3	0 (n = 1)	0 (n = 3)	0 (n = 3)	2 (1-4, n = 3)	1 (0-1, n = 2)	No data

A conical plankton net (45 cm mouth diameter, 90 cm long, 335 μm mesh size) was towed horizontally along the water surface by a motorboat for 5 min just after sunset. The collection was generally performed three times a month (early, middle, and toward the end of the month) at each station except for November 2000 (middle and end at St. 1 and at the end at Sts. 2 and 3) and March 2001 (three times at St. 1 and early and middle at Sts. 2 and 3) (Tab. I). An additional tow was conducted at St. 1 in late April 2001. It was reported that *P. a. ryukyuensis* larvae smaller than 10 mm perform diurnal vertical migration by moving up to the surface during the night, while they mainly inhabit bottom layers during the day (Kishino and Shinomiya, 2006). Therefore, although we could not obtain information regarding the vertical distributions of larvae in this study, our results were assumed to be representative of the horizontal distribution of larvae.

Collections of ascending larvae and juveniles were performed on the same days as the larval collections in the reservoir from late January to late May 2001 (once in January, April and May, three times in February and March). Ascending larvae and juveniles were collected in the backwater of the San-numata River during the day using a hand-net while snorkelling. Due to the drop in water level of the reservoir through the period, locations of ascending individuals collected were slightly different among months.

Samples collected by net tows were fixed in 5% formalin. *P. a. ryukyuensis* larvae were sorted and preserved in 95% ethanol within a day of collection. Body length (BL: notochord length in preflexion and flexion larvae or standard length in postflexion larvae) was measured to the nearest 0.1 mm with a profile projector (V12-B; Nikon, Tokyo, Japan) using preserved samples. Damaged larvae were excluded from the analysis. These larvae were also used for diet analysis by Kawakami and Tachihara (2005). Ascending larvae and juveniles were fixed in 5% formalin, and BL was measured to the nearest 0.1 mm with a digital caliper. The developmental stage of larvae and juveniles was determined based on the description by Tachihara and Kawaguchi (2003).

Statistical analysis

To compare the number of larvae collected among stations and sampling months, Friedman tests followed by Dunn's multiple comparison tests were performed using PRISM 4.0c (GraphPad Software Inc., San Diego, CA, USA). November 2000 and April 2001 were not included in this analysis because the larval collections at Sts. 2 and 3 were performed once in November 2000 and not performed in April 2001 (Tab. I). Larval BL was compared among stations and months using a Kruskal-Wallis test followed by Dunn's multiple comparison tests using PRISM 4.0c since some data sets were not normally distributed (Shapiro-Wilk test using JMP 5.0.1J, SAS Institute Japan, Tokyo, Japan; $p < 0.01$). Because of the small sample sizes from Sts. 2 and 3, a Kruskal-Wallis test was only performed among months for St. 1 and among stations in February.

Estimates of age and hatching date

The age (days) of the larvae was estimated based on BL and growth rates determined by Tachihara and Kawaguchi (2003). Accordingly, age (days) could be estimated by the following formula:

(1) $y = (BL - 5.7)/0.41$ (for 5.7-13.8 mm BL larvae)
(2) $y = 20 + (BL - 13.9)/0.01$ (for 13.9-14.0 mm BL larvae)
(3) $y = 40 + (BL - 14.1)/0.45$ (for 14.1-36.5 mm BL larvae)
(4) $y = 90 + (BL - 36.6)/0.03$ (for 36.6-37.1 mm BL larvae)
(5) $y = 110 + (BL - 37.2)/0.35$ (for 37.2-54.6 mm BL larvae)
where y is the estimated age (days) and BL is body length.

Postlarvae smaller than 7.8 mm were regarded as 5-day larvae because *P. a. ryukyuensis* larvae completely consume their yolk within 5 days (Tachihara and Kawaguchi, 2003) and the estimated body size at 5 days calculated by formula (1) is 7.8 mm. Hatching date was back-calculated based on the estimated age and collection date.

RESULTS

Larval abundances

In total, 232 larvae were collected in the reservoir using 40 net tows. Of these, most larvae (76.3%) were collected at St. 1 and a smaller number was collected at St.2 (20.7%) and St.3 (3.0%). Larval abundance differed significantly among stations (Friedman test, $p < 0.05$; Dunn's multiple comparison test: St. 1 vs. St. 3, $p < 0.05$) and generally tended to decrease as the distance from the backwater of the San-numata River increased (Tab. I). Although no significant differences in the number of larvae collected among months were detected from December to March ($p > 0.05$), the first occurrence of larvae was delayed at Sts. 2 and 3. The larvae occurred from late November to the middle of March at St. 1 with a peak abundance in December (Tab. I; 26 individuals/tow). The larvae at St. 2, however, were first collected in mid-December with a peak abundance in February (9 individuals/tow) and at St. 3 from early February (2 individuals/tow).

Larvae in the reservoir

At St. 1, the BL of the larvae ranged from 5.1 to 23.0 mm with a mean of 6.8 mm, and these similar-sized larvae were also collected at St. 2 (5.7-22.2 mm, mean = 8.5 mm) and St. 3 (6.6-20.5 mm) (Fig. 2). However, yolk-sac larvae (5.7-7.4 mm BL; mean ± SD, 6.5 ± 0.45 mm BL) were only collected at St. 1. Additionally, larvae smaller than 10 mm BL accounted for 80.2% of the all larvae collected at St. 1, whereas 56.3% of the all larvae collected at St.2 were smaller than 10 mm BL.

Although the size of smallest larvae was almost constant at St. 1 (5.1-6.1 mm) and St. 2 (5.7-7.4 mm), the size of the largest larvae increased through the sampling period at both stations (Fig. 2). Larvae larger than 20 mm in BL started to appear in February. At St. 1, the larvae collected in February were significantly larger than those collected in other months (Kruskal-Wallis test, $p < 0.0001$; Dunn's multiple comparison test: Nov. vs. Feb., $p < 0.01$; Dec. vs. Feb. $p < 0.001$; Jan. vs. Feb. $p < 0.001$). Although only the larvae collected in February were compared, the size of the larvae was not significantly different among three stations ($p > 0.05$). The largest larva in the reservoir was collected at St.1 and was 23.0 mm in BL and estimated to be 60 days old. Notochord flexion was completed and the pelvic fin had not yet formed. Pigmentation was present on the head, the ventral side of the myomeres, and the caudal fin (almost the same as stage H as described by Tachihara and Kawaguchi, 2003).

The composition of estimated age (days) was similar among the stations, ranging from 0 to 60, 5 to 58, and 5 to 54 at St. 1, St. 2, and St. 3, respectively. However, the estimated hatching period of the larvae was shorter at Sts. 2 and 3 than at St. 1 (St. 1, 13 November to 9 March; St. 2, 8 December to 7 March; St. 3, 27 December to 25 February), and larvae

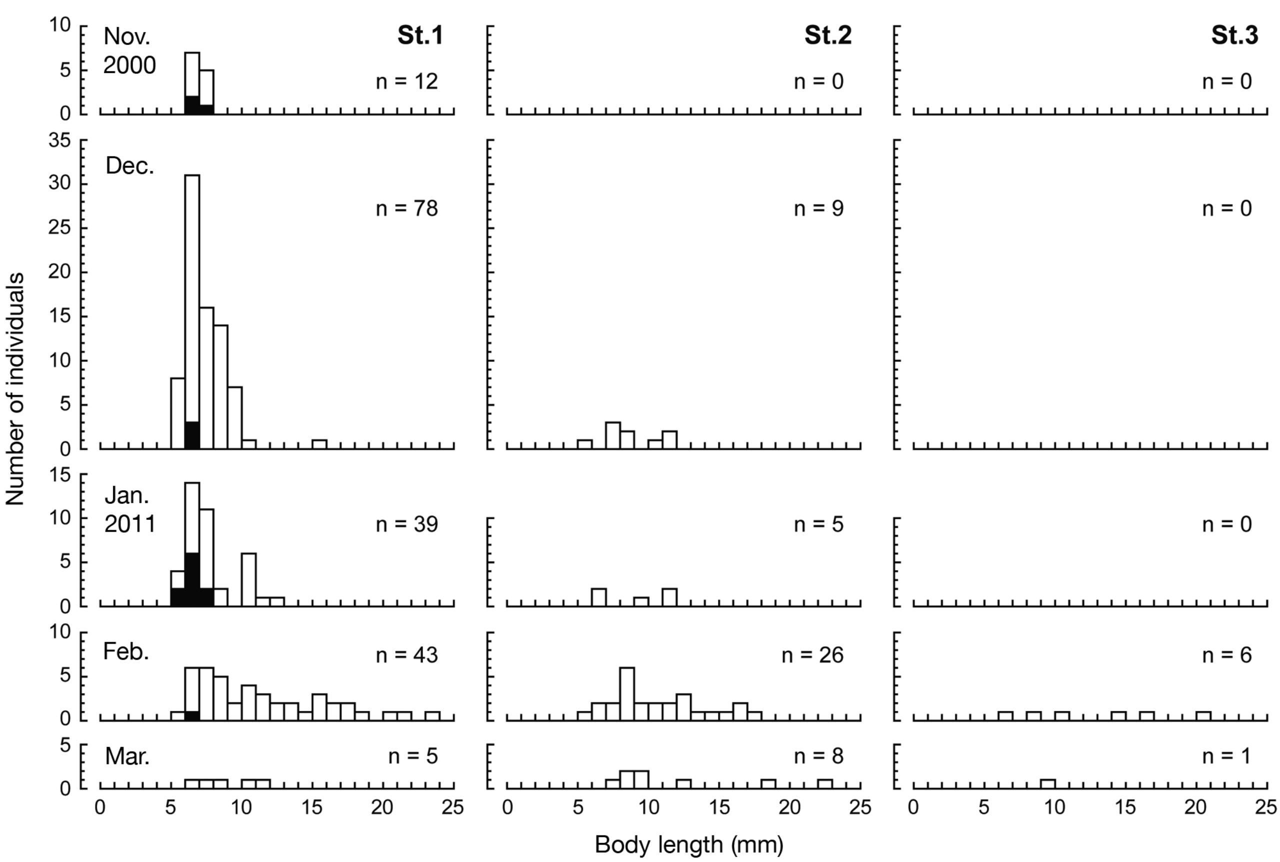

Figure 2. - Comparison of the size distribution of Ryukyu-ayu, *Plecoglossus altivelis ryukyuensis*, larvae collected at Sts. 1-3 from November 2000 to March 2001. The left, middle, and right panels indicate St. 1, St. 2, and St. 3, respectively. The solid and open bars represent yolk-sac larvae and postlarvae, respectively.

hatched in November were only collected at St. 1.

Ascending larvae and juveniles

Seven ascending larvae and juveniles were collected in the backwater of the San-numata River from late January to late April 2001. Their BL ranged from 24.2 to 55.9 mm (mean = 34.7 mm) and the estimated age (days) ranged from 63 to 163 (mean = 93 days). Estimated birth date was from 3 November to 28 January. The smallest ascending larva (24.2 mm, estimated to be 63 days) was collected in late January on the first day of the collection and its morphology almost corresponded to stage J as described by Tachihara and Kawaguchi (2003); all of its fins had formed, but it exhibited fewer fin rays than an adult. Pigmentation was present on the head, gill cover, ventral side of the myomeres, dorsal side of the tail, the centre of the ventral side, and the caudal fin.

DISCUSSION

In the present study, *P. a. ryukyuensis* larvae in a part of the reservoir and ascending individuals in the backwater of the San-numata River were collected and their dispersal pattern could be estimated. At St.1 where the closest station to the backwater, the larvae occurred from late November to mid-March with peak abundance in December, and they were estimated to hatch during mid-November to early March. This result corresponds well with the estimated spawning period of this population (Kawakami and Tachihara, 2010). At St. 1, yolk-sac larvae and the larvae estimated to be 0-4 days were exclusively collected and the most abundant size class (6.0-6.9 mm) was also similar to the size of drifting larvae of this land-locked population (5.7 mm; Kawakami and Tachihara, 2010). Given that *P. a. ryukyuensis* larvae complete yolk absorption in 5 days after hatching (Tachihara and Kawaguchi, 2003), the larvae hatched in the San-numata River may have drifted within a day after hatching and then dispersed throughout an area within approximately 1 km of the backwater in a few days.

However, in contrast to St.1, the larvae around 6.0-6.9 mm were scarcely collected at St.2 and few larvae were collected at St.3. So the land-locked *P. a. ryukyuensis* larvae seem to only disperse approximately 1.4 km away from the backwater. Additionally, the body size and estimated age of the largest larva collected in the reservoir caught at St.1 (23.0 mm, 60 days) was similar to that of the smallest ascending larva (24.2 mm, 63 days). These results suggested that land-locked *P. a. ryukyuensis* larvae may mostly stay close to the backwater during their entire larval period, without being dispersed over long-distances before commencing their upstream migration after at least 2 months.

Combining the results in this study with previous studies (Kawakami and Tachihara, 2005, 2010), we could depict the specific life cycle of the land-locked *P. a. ryukyuensis* as follows: November to March, spawning season with a spawning peak in late November to early December; November, beginning of larval growth phase in the reservoir close to the backwater and the larvae feed on zooplankton; January, beginning of upstream migration to the river. This larval migration pattern is highly similar to that previously reported for the amphidromous population on Amami-oshima Island (Kishino and Shinomiya, 2003, 2004, 2005, 2006). In addition, the mean body size and estimated age of ascending individuals in the present study (34.7 mm and 93 days) were almost the same as that of amphidromous *P. a. ryukyuensis* in the Yakugachi River on Amami-oshima Island (35.2 mm and 83.7 days; Kishino and Shinomiya, 2003). This may imply the same migration behaviour is expressed in both the land-locked form and amphidromous forms regardless of the salinity of their environment.

Based on the estimated age of larvae, speed of dispersal away from the backwater can be calculated. The youngest larvae in Sts. 2 and 3 were estimated as 5 days after hatching. Assuming that the larvae took at least 5 days to reach Sts. 2 and 3, the movement speed can be estimated as approximately 0.3 cm/s and 0.5 cm/s, respectively, which is much slower than the cruising speed of *P. a. altivelis* larvae smaller than 10 mm (1-5 cm/s; Tsukamoto *et al*., 1975). Therefore, it is unlikely that the larvae actively swam away from the river mouth.

Amphidromous *P. a. altivelis* and *P. a. ryukyuensis* larvae smaller than 10 mm are thought to reduce the probability of dispersal and remain in the estuary or brackish water using behavioural mechanisms, such as aggregation in the bottom layer and diurnal movements of sinking to the bottom during the day (Takahashi *et al*., 1998; Kishino and Shinomiya, 2006). Thus, it is reasonably expected that the land-locked *P. a. ryukyuensis* larvae also inhabit bottom layers during the day and tend to stay in the area near the river. Considering the freshwater environment, the larval behaviour that prevents the long-distance dispersal is probably not a response to the saline environment or tidal currents like would be possible in the ocean. Instead it can be hypothesized that such behaviour of *P. a. ryukyuensis* larvae is triggered by intrinsic factors such as changes in specific gravity (Kitajima *et al*., 1998) and swimming ability (Tsukamoto and Kajihara, 1984) with growth, as well as phototaxis as suggested by Takahashi (2004) and Kishino and Shinomiya (2005).

With the exception of larval behaviour, passive transport by currents is thought to determine the distribution of larvae (Azuma, 1970; Tachihara and Kimura, 1991; Azuma *et al*., 2003). The larvae estimated to hatch in November were only collected at St.1, although the larvae hatched during December to March were also found in Sts. 2 and 3. Current speed and direction was not measured in the present study, but it can be assumed that the larvae collected in Sts. 2 and

3 were dispersed through the reservoir by currents caused by inflows from the river due to rainfall, water releases from the dam and a decline of water level of the reservoir after December.

Differences in the survival of larvae caused by water temperature, presence of predators and prey availability could also influence larval distribution. However, water temperature was unlikely to have affected the larval distribution in the present study, because the water temperature was assumed to be stable in the reservoir. Additionally, predator fish for *P. a. ryukyuensis* larvae are scarce in the reservoir (Tachihara, unpubl. data). The relative abundance of zooplankton (copepods, cladocerans and dinoflagellates) that is preyed upon by *P. a. ryukyuensis* larvae (Kawakami and Tachihara, 2005) might play some role in larval distribution. That was significantly higher in the upper part of the sampling area (including St. 1 and St. 2) compared to the lower part (including St. 3) from October 1999 to March 2000 (Kawakami and Tachihara, unpubl. data).

This study demonstrates the retention of land-locked *P. a. ryukyuensis* larvae in an area close to the river mouth in the Fukuji Reservoir. The characteristics of this population with regard to larval dispersal pattern were similar to amphidromous populations on Amami-oshima Island. However, we could not completely determine the distribution and dispersal pattern of the land-locked population. To reveal the dispersal mechanism of *P. a. ryukyuensis*, behavioural studies and hydrological research, in addition to more detailed temporal and spatial sampling, are required. Because changes in environmental factors such as tides and salinity are absent in the reservoir, studying the ecology of this land-locked population will lead to an enhanced understanding of the ecology and effective conservation of wild populations of *P. a. ryukyuensis*. Furthermore, comparable studies on other amphidromous fishes such as *Rhinogobius* spp. and *Sicyopterus japonicus* will contribute to understanding the evolution of amphidromy.

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