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FULL PAPER

Variation in growth performance of Ryukyu-ayu, *Plecoglossus altivelis ryukyuensis*, inferred from otolith analysis

Iki Murase[1] · **Tatsuya Kawakami**[2] · **Kei'ichiro Iguchi**[1]

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Abstract

Ryukyu-ayu (*Plecoglossus altivelis ryukyuensis*) is an amphidromous fish species that migrates between the sea and rivers over its one-year life span. Although growth performance during the early marine stage may affect growth in the later riverine stage of this species' life cycle, no studies have specifically examined this relationship in *P. a. ryukyuensis*. In the present study, we reconstructed the growth trajectories of *P. a. ryukyuensis* individuals collected from the Yakugachi River, Amami-Oshima Island, Japan in 2016 ($n = 47$) throughout their growth period in both the sea and river by using otolith analysis. Using this, we determined the age and body size of individuals at the time of their upstream migration, as well as their growth rates during the marine and riverine stages. Results showed that body size at upstream migration significantly affected body size at the riverine stage, indicating that juveniles with larger body size in the sea had better growth performance in the river. Individuals with higher growth rates during the marine stage tended to enter the river younger and at larger body sizes than those with lower marine growth rates. Our results demonstrated the close linkage between the growth performance in the sea and in rivers of *P. a. ryukyuensis*. This information will contribute to better understanding variations in growth patterns of this endangered species and potentially aid in its conservation.

Keywords Growth · *Plecoglossus altivelis ryukyuensis* · Amphidromy · Otolith analysis

Introduction

The Ryukyu-ayu fish, *Plecoglossus altivelis ryukyuensis*, is a subspecies of ayu (*P. a. altivelis*), the distributional area of which is limited to Amami-Oshima Island, southern Japan (Nishida 1988; Nishida et al. 1992). Similar to *P. a. altivelis*, *P. a. ryukyuensis* is an amphidromous species that migrates between coastal waters and rivers and has a one-year life span. Mature *P. a. ryukyuensis* spawn between mid-November and mid-March (Kishino and Shinomiya 2003). Newly hatched larvae drift downstream to the sea where they mainly feed on copepods (Oka et al. 1996; Aritomi et al. 2017) and grow for the remainder of the year. Juveniles then begin an upstream migration in late January at a body size of 35 mm (Kishino and Shinomiya 2003). After entry into rivers, juveniles predominantly feed on algae (Kume 2016) and spend the spring and summer in rivers where they undergo further growth (Kishino and Shinomiya 2004a). In rivers, some individuals establish their feeding territories, and territory size and stability are positively correlated with the body size of territorial individuals (Awata et al. 2012).

The *P. a. ryukyuensis* is now classified as "critically endangered (CR)" according to the national Red List of Japan (Ministry of the Environment 2017), and there is a need for detailed information on the ecology of this species to support its effective conservation. The importance of restoration of spawning site in rivers was proposed to enhance the reproductive success of *P. a. ryukyuensis* adults (Tsuruta et al. 2009). Additionally, the ecological information of *P. a. ryukyuensis* in both marine and riverine stages has been accumulated (e.g., Nishida and Uchimura 1987; Nishida et al. 1992; Oka et al. 1996; Kishino and Shinomiya 2003, 2004a, b, 2005, 2006; Awata et al. 2012; Kishino and Yonezawa 2013; Kume 2016; Aritomi et al. 2017), and the importance of estuary area as a nursery habitat for larvae and

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✉ Iki Murase
iki.murase@gmail.com

[1] Graduate School of Fisheries and Environmental Sciences, Nagasaki University, 1-14, Bunkyo-machi, Nagasaki, Nagasaki 852-8521, Japan

[2] International Coastal Research Center, Atmosphere and Ocean Research Institute, The University of Tokyo, 1-19-8 Akahama, Ohtsuchi, Iwate 028-1102, Japan

juveniles has been proposed (Kishino and Shinomiya 2005, 2006; Kishino et al. 2008; Aritomi et al. 2017).

To conserve this amphidromous species, we need to reveal the linkage of growth history between marine stage (i.e., larvae and juveniles) and riverine stage (i.e., young and adults). Some amphidromous gobies showed higher growth rates after migrating to rivers from sea (Shen et al. 1998; Shen and Tzeng 2002). Also, in non-amphidromous species, changing nursery habitat allows low growth individuals to have better growth (Kraus and Secor 2004; Fuji et al. 2014). For example, seabass *Lateolabrax japonicus* juveniles with low growth rate migrate to a river and show higher growth rate than juveniles that remain in the lower estuary and surf zone (Fuji et al. 2014). In this case, favorable temperature and high prey abundance in a river may allow low growth individuals to grow rapidly (Fuji et al. 2014). Considering these previous studies, we can evaluate the conservation effect of marine nursery habitat by examining whether the growth in rivers is affected by the growth at the marine stage or not.

The aim of this study was to reveal the relationship between the growth performance in sea and that in rivers of *P. a. ryukyuensis*. We predicted that high growth rate at the marine stage positively affects the growth performance in rivers via the body size at upstream migration, because individuals with large body size have an advantage of territorial stability in rivers over small-sized individuals (Awata et al. 2012). To achieve the purpose, we examined the growth rates of *P. a. ryukyuensis* during their growth period in both the sea and a river through a combination of otolith increment and chemical analyses. The otolith Sr:Ca ratio is one of the most frequently used indicators of movement between the sea and rivers in fishes (e.g., Otake and Uchida 1998) because of the differences in the concentration ratio of strontium to calcium between seawater and freshwater. Additionally, rings are added daily to otoliths of *P. a ryukyuensis* (Kishino and Shinomiya 2003), as well as those of *P. a. altivelis* (Tsukamoto and Kajihara 1987), which allows the growth trajectory of this species to be estimated. The timing of upstream migration and growth rates during the marine and riverine growth periods were determined by counting daily rings within otoliths and analyzing otolith Sr:Ca ratios.

Materials and methods

Field collection. On 28 December 2016, a total of 50 wild adult *Plecoglossus altivelis ryukyuensis* were collected from the middle courses of the Yakugachi River using a cast net (Fig. 1). Since the bodies of the adults were supplied to Amami City to produce seed stock for conservation use, only heads were stored for analyses in this study. To estimate the standard length (SL) of our samples, we examined the relationships between three head length measurements and SL using data from another Yakugachi River sampling effort conducted in 1994 (n = 20; K. Iguchi, unpublished data). Specifically, we examined the relationships between log transformed snout length and SL ($r = 0.70$, $P < 0.001$), log transformed the length of the upper jaw and SL ($r = 0.37$, $P = 0.10$), and log transformed the length between the anterior margin of the eye and the margin of the upper jaw and SL ($r = 0.37$, $P = 0.11$). The relationship between log transformed snout length and SL showed the highest correlation coefficient, so we used the following equation based on this relationship to estimate the SL of our samples:

$$\log_{10}\mathrm{SL} = 0.5469 \times \log_{10}(\text{snout length}) + 1.4982.$$

The length (to the nearest 0.01 mm) was measured using a digital caliper. The estimated SL ranged from 129.5 to 157.2 mm (mean ± SD = 145.2 mm ± 6.6, n = 50).

Sr:Ca ratio analysis. Otolith specimens removed from the collected fish heads were coated with Pt–Pd in an ion-sputtering machine (E-1030; Hitachi, Tokyo, Japan) before

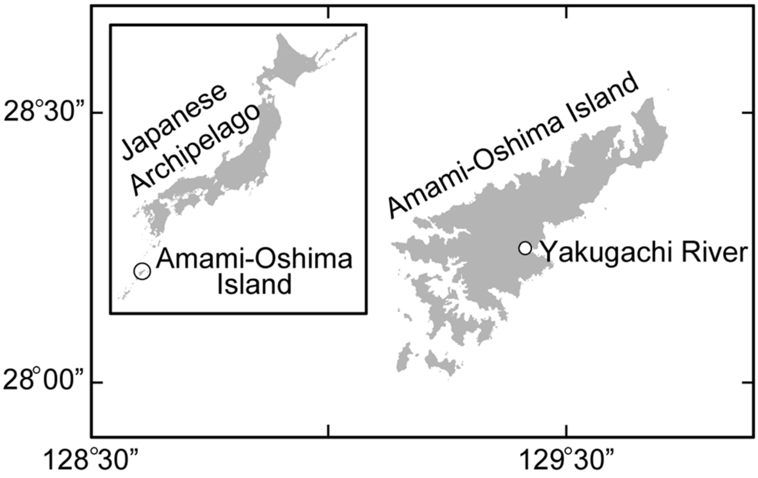


Fig. 1 Location of the Yakugachi River on Amami-Oshima Island, where samples were collected

analysis. Sr and Ca concentrations were measured in all specimens using a wavelength-dispersive X-ray electron microprobe (JXA-8230; JEOL, Tokyo, Japan). The analytical line was defined as the longest axis from the core to the posterior edge of the otolith. The electron beam was focused on a 5 µm diameter point with measurements spaced at 5 µm intervals, and counting time was 2 s. The accelerating voltage and beam current were 15 kV and 50 nA, respectively. Strontium titanate ($SrTiO_3$) and wollastonite ($CaSiO_3$) were used as standards. The otolith Sr:Ca ratio was determined as a weight percent ratio.

Otolith measurements and aging. The number of daily rings of otoliths and their incremental width were counted and measured along the electron probe microanalyzer (EPMA) line under a binocular microscope connected to a digital camera and a computer system with image analysis software (Ratoc System Engineering, Tokyo, Japan). The first ring around the core was assumed to be formed at hatch (day 0). The daily Sr:Ca ratio was determined by combining the results of EPMA and incremental analysis. The age at upstream migration was defined as the end point of a rapid decrease in Sr:Ca ratio. Three individuals were excluded from further analyses because they did not show any significant decrease in their Sr:Ca ratio, so their date at upstream migration could not be identified. In most individuals, the edge of the sagittal otolith was translucent, and it was difficult to identify daily growth increments. Therefore, we did not include hatching dates estimated from this section of otoliths in analyses to avoid problems due to unreliable estimates.

SLs at age *i* were back-calculated using the biological intercept method (Campana 1990, eq (4)):

$$\mathrm{SL}_i = \mathrm{SL}_{catch} + \left(\mathrm{OR}_i - \mathrm{OR}_{catch}\right) \left(\mathrm{SL}_{catch} - \mathrm{SL}_{hatch}\right)\left(\mathrm{OR}_{catch} - \mathrm{OR}_{hatch}\right)^{-1},$$

where OR is otolith radius. We set the body size at hatch (SL_{hatch}) as 5.8 mm in notochord length following Kishino and Shinomiya (2004a). The otolith radius at hatch (OR_{hatch}) of each individual was defined by measuring the distance from the core to the first ring. Mean growth rate at the marine stage (GR_{sea}) was calculated as:

$$\mathrm{GR}_{sea} = \left(\mathrm{SL}_{ups} - 5.8\right)/\mathrm{age}_{ups},$$

where SL_{ups} and age_{ups} are SL and age at upstream migration, respectively. Mean growth rate at the riverine stage (GR_{river}) was defined as the growth rate during the riverine period until an age of 152 days, because the lowest number of daily rings that could be recognized was 152 in all 47 individuals. The GR_{river} was calculated as:

$$\mathrm{GR}_{river} = \left(\mathrm{SL}_{152} - \mathrm{SL}_{ups}\right)/\left(152 - \mathrm{age}_{ups}\right),$$

where SL_{152} is SL at age 152 days.

Pearson's correlation coefficient was used to check the relationships between growth rates at the marine stage and age at upstream migration, SL at upstream migration, growth rates at the riverine stage, and SL at catch ($P < 0.05$). Mann–Whitney *U*-test was used to compare the growth rate between sea and rivers ($P < 0.05$).

Results

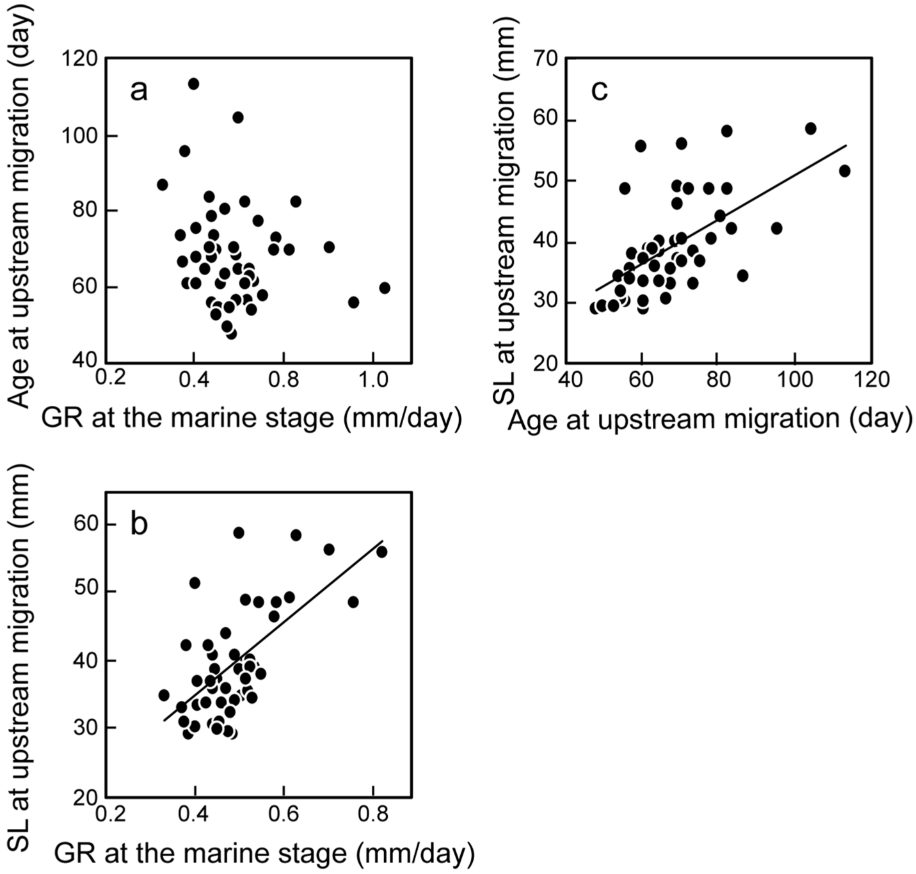
The calculated SL at upstream migration ranged from 29.2 to 58.7 mm (mean ± SD = 39.5 mm ± 8.1, n = 47) and the range of estimated age at upstream migration was 48–114-day age (mean ± SD = 68.6-day age ± 13.6, n = 47). The SL range of age 152 days was from 64.3 to 141.1 mm (mean ± SD = 93.2 mm ± 16.8, n = 47). Mean growth rate ± SD during the marine stage (0.50 mm/day ± 0.10) was lower than those at the riverine stage (0.65 mm/day ± 0.14, $P < 0.001$). The relationship between growth rate at the marine stage and age at upstream migration tended to be negative (Fig. 2a), although this trend was not statistically significant ($r = -0.22$, n = 47, $P = 0.13$). SL at upstream migration increased with growth rate at the marine stage (Fig. 2b, $r = 0.64$, n = 47, $P < 0.001$) and with age at upstream migration (Fig. 2c, $r = 0.59$, n = 47, $P < 0.001$). The SL at upstream migration positively affected the SL at catch (Fig. 3, $r = 0.32$, n = 47, $P = 0.02$).

Figure 4 illustrates how mean Sr:Ca values of individuals were related to their age. Mean Sr:Ca ratios (calculated per every ten days) gradually decreased from values of approximately 7–8 to 5–6 throughout the marine growth period (approximately, 0–70 day), indicating gradual movement toward estuaries before upstream migration.

Discussion

We demonstrated the close linkage between growth performance in the sea and that in rivers of *Plecoglossus altivelis ryukyuensis*, in that the growth performance at the marine stage positively affected the body size at catch. However, the correlation coefficient of the relationship between SL at upstream migration and SL at catch was lower than that expected. This trend might be explained by the variation in timing of upstream migration, reflecting the seasonally changes in environmental conditions of rivers such as water temperature and prey availability. At the timing of upstream migration, relatively young individuals at larger body sizes entered rivers first, and then in subsequent months the age-size structure gradually shifted as older but smaller fishes migrated into the river (Kishino and Shinomiya 2003). This

Fig. 2 Relationships between a) growth rate (GR) at the marine stage and age at upstream migration (n = 47), **b**) GR at the marine stage and standard length (SL) at upstream migration (n = 47), and **c**) age at upstream migration and SL at upstream migration (n = 47). Regression line was plotted in the case that the relationship was statistically significant



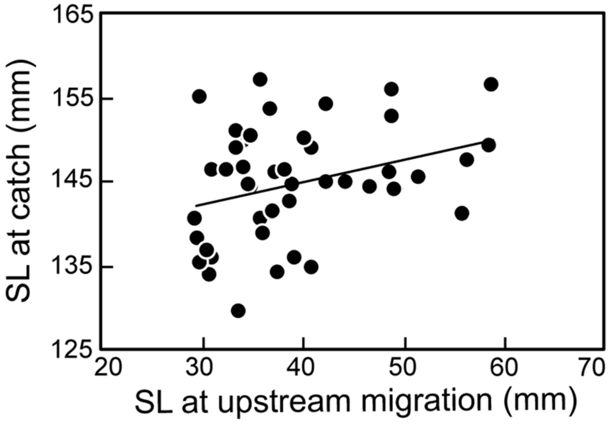


Fig. 3 Relationship between standard length (SL) at upstream migration and SL at catch (n = 47). Regression line was plotted because the relationship was statistically significant

monthly trend could not be examined in this study because we lacked adequate information on specific dates at upstream migration. But, we can generally state that individuals with relatively high growth rates tended to enter the river at a larger body size and younger age, and even longer marine period allows the individuals to have a larger body size at upstream migration.

Entering rivers earlier in the season at a larger size may permit high growth performance of *P. a. ryukyuensis* in rivers. Considering that the length of the upstream migration is about four months (Kishino and Shinomiya 2003, 2004a) and high water temperature (over 20 °C) negatively affects the survival rates of larvae (Kishino et al. 2008), migrating into a river at an earlier season (i.e., January and February) may allow individuals to grow at riverine water temperatures that are better suited to them. Additionally, recruiting into rivers earlier and at a larger size may allow individuals to gain more advantageous positions, such as by establishing feeding territories sooner than late-recruiting individuals (Aino et al. 2014).

Only about 10 % of *P. a. ryukyuensis* establish feeding territories in rivers (Awata et al. 2012), and larger-sized individuals likely have an advantage when acquiring and defending territory. Awata et al. (2012) reported that territory size and stability are positively correlated with the body size of territorial individuals. Also, a pairwise study of *P. a. altivelis* in a closed system suggested that larger-sized individuals have a competitive advantage in territorial acquisition over smaller-sized ones (Iguchi 1996). Further experiments in ponds that simulated natural streams revealed that growth rates of individuals are correlated with the length of the territorial period (Katano and Iguchi 1996).

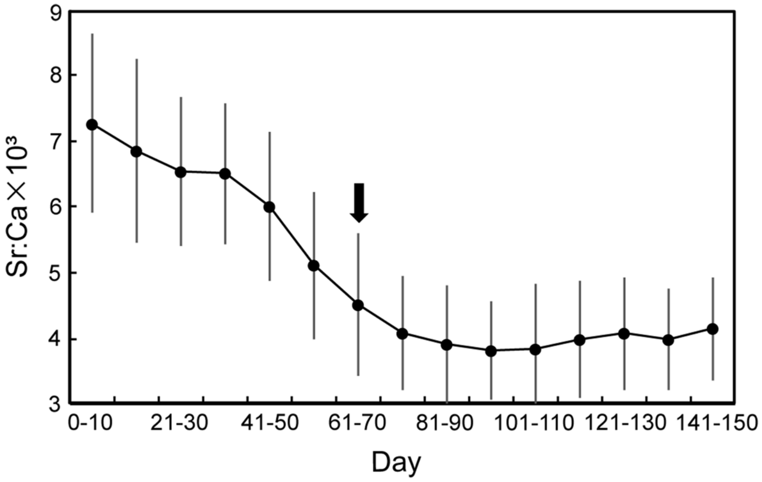


Fig. 4 Temporal changes of Sr:Ca ratios (averaged per ten day age intervals for clarity) during the marine and early riverine stage (0–150 days). Arrow indicates the mean age at upstream migration (mean ± SD = 68.6 days ± 13.6, n = 47). Error bars represent standard deviation (SD)

The body-size advantage of non-territorial (i.e., schooling, solitary, and floating) individuals, however, has not been examined yet for *P. a. ryukyuensis*. Non-territorial individuals mainly feed on drifting materials (Awata et al. 2012). Nakano (1995) showed that there were size-structured linear dominance hierarchies in a drift forager, the red-spotted masu salmon (*Oncorhynchus masou ishikawai*), in stream pools. The large, dominant individuals tended to occupy focal points where they had priority to access drifting materials, and they had higher foraging rates and therefore higher growth rates than their smaller subordinates (Nakano 1995). Considering this, the body size at the timing of entry into rivers may thus also play an important role affecting later growth in non-territorial *P. a. ryukyuensis*.

The mean growth rates in riverine stage were higher than those at the marine stage of *P. a. ryukyuensis*. A similar trend is observed in amphidromous gobies *Sicyopterus japonicus* (Shen and Tzeng 2002) and *Stenogobius genivittatus* (Shen et al. 1998). Especially, like *P. a. ryukyuensis*, *S. japonicus* changes its feeding habitat from planktivorous to herbivorous on migrating from sea to rivers. The transition in feeding habitat temporarily reduces the growth rate of *S. japonicus*, but the changes in feeding habitat and functional morphology may contribute to increase the growth rate in rivers (Shen and Tzeng 2002). Although we could not examine the effect of environmental conditions on the growth rate of *P. a. ryukyuensis*, the body size at recruitment into rivers is affected by temperature and prey availability in the sea of *S. japonicus* (Shen and Tzeng 2008), indicating the necessity to examine the effect for understanding the variation in growth performance.

Previous studies have suggested the importance of conserving the estuary area to avoid the extinction of this endangered species (Kishino and Shinomiya 2005, 2006; Kishino et al. 2008; Aritomi et al. 2017). Our results support the suggestion by showing that the growth performance in sea also affects the body size in rivers. Also, our results suggest that the final body size may be affected by environmental factors in rivers, indicating the importance of conserving nursery sites in rivers as well as spawning sites. The impact of environmental changes on the growth of this species remains an open question. To conserve *P. a. ryukyuensis*, it is essential to continuously monitor environmental changes at the island (Amami-Oshima) where it is found, and make more effort to study the links between its environment and its life history.

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