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Changes in the composition of stock origin and standard length of ayu *Plecoglossus altivelis altivelis* during the Tomozuri angling season in the Nagara River, central Japan

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Abstract Delay of upward migration from the coast is considered to be a cause of stunted growth in ayu *Plecoglossus altivelis altivelis* in the Nagara River. To evaluate the effect of stocked seeds (hatchery reared, originating from Lake Biwa and amphidromous stock) on the growth of native ayu in the river, monthly changes in origin composition and standard lengths of territorial ayu caught by Tomozuri angling were examined. Stock origin of 94 individuals caught in the middle reaches of the river from June to October 2010 were discriminated using the otolith Sr/Ca ratio and morphological characteristics. Percentage frequency of native ayu increased with time from 4.2 % in June to 73.3 % in September and 71.4 % in October. Hatchery-reared stocks were consistently caught throughout the sampling period (26.7–41.7 %), while the catch of both Lake Biwa and amphidromous stocks were limited to before August. Native ayu were significantly smaller than the others in July; however, no significant differences were found from August. Apparent growth of stocked seed stopped after August, while native ayu stopped in September. Thus, we suggest that large stocked ayu disturb the native ayu from making territories and stunt their growth in the early part of the season.

Keywords Feeding territory · Otolith Sr/Ca ratio · *Plecoglossus altivelis altivelis* · Stock origin

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Introduction

The ayu *Plecoglossus altivelis altivelis* is distributed over the Japanese Archipelago and partially along the Far East continental coast [1]. Most populations of ayu have an amphidromous and annual life history. They spawn in the middle to lower reaches of rivers in autumn, and the larvae and juveniles inhabit coastal marine waters during winter. The young migrate to rivers in spring and grow and mature in the middle reaches of rivers during summer to autumn. In the river, ayu mainly feed on benthic algae, and dominant individuals have a behavior of defending feeding territories [1, 2]. It is known that the territorial individuals grow faster than non-territorial ones [1–3]. In addition to amphidromous populations, a landlocked population inhabits Lake Biwa, an ancient lake in central Japan. They slightly differ from amphidromous ayu morphologically, genetically, and physiologically [4, 5]. Furthermore, they spawn about 1 month earlier than amphidromous ayu [6].

Because of their feeding and behavioral habits, territorial ayu are caught with a unique fishing method, Tomozuri angling. This method uses a live ayu as a decoy which is attached to hooks and territorial ayu attack the decoy as an intruder and in the process of attacking the intruder become hooked on the sets of hooks. The ayu is the most popular and important fish for Japanese inland fisheries, and fishery stocking programs are actively conducted throughout Japanese rivers [7].

The Nagara River (158 km length), a river which flows through the Gifu and Mie prefectures into Ise Bay, is famous for its commercial and recreational ayu fisheries. Although the native population migrates to the river from Ise Bay, large amounts of seed fish are also stocked every year by six fishery cooperative associations. The seeds used for stocking have three types of origin, i.e. hatchery-reared, the Lake Biwa population, and amphidromous populations caught in coastal waters [8]. Although body size is the most effective factor in territorial competitions [9], it is known that the Lake Biwa stock is more aggressive in territorial behavior than the others [10, 11]. The seeds are usually stocked earlier than the period of upward migration of native ayu [12]. Furthermore, body sizes of the seeds at stocking are much larger than native ayu that have migrated to the river [12]. Therefore, it is presumed that the origin of ayu caught by Tomozuri angling is not proportional to the relative composition of origins of the ayu in the river, and the ratios of the stock origins therefore change with time during the Tomozuri angling period, June to October. In previous studies in the Nagara River, Hara et al. [13, 14] discriminated the origin of ayu caught by Tomozuri angling by using allozyme markers and morphology and reported that the frequency of Lake Biwa-originated stock decreased in the period. However, body size differences among such stock origins and native populations were not clarified.

Recently, stunted growth of ayu in many rivers is a concern, and the delay of migration of ayu to the river seems to be a main cause in the Nagara River and Sho River [15–17]. In addition, disadvantages of small native individuals in territorial competition against larger stocked fish was suggested as one of the other causes in our previous study [12]. Thus, we investigated if the frequency of native ayu in Tomozuri fish is low in the early period of the season and whether it increases with time as well as if the body size of native ayu is small in the early period and if the difference decreases with time. Furthermore, in the stocking seeds, we examined if Lake Biwa-originated stock predominate in the early period and if the frequency increasingly shifts to hatchery-reared stock with time.

Materials and methods

Samples

A total of 94 ayu were caught by Tomozuri angling from June 2 to October 14 in 2010 in the middle reaches of the Nagara River, Gifu Prefecture (Fig. 1). The number of monthly samples from June to October was 24, 25, 23, 15, and 7 individuals, respectively. Standard samples were extracted from released stocks around the sampling area and used for the stock origin discrimination. A total of 26 individuals were randomly sampled in the fish for hatchery-reared stock (HR) when released on April 26 and May 19, 2010, 41 individuals for Lake Biwa-originated stock (LB) on April 21, and 36 for amphidromous seed-originated stock (AM) on May 19, 2010, respectively. HR was produced by the Fish Farm Center of Gifu Prefecture using an amphidromous population that had been caught in the previous year in the Kiso River flowing into Ise Bay (Fig. 1). LB were fish caught in mouths of some rivers flowing into Lake Biwa and reared in a culture farm. AM were fish caught along the coast of Kii Peninsula from February to March 2010, and acclimated to freshwater and reared in a culture farm in Wakayama Prefecture. For native ayu, seven were caught by using a dipping net in the lower reach of the Nagara River on April 9, 2010, as an index of native population before stocking. For all samples, standard length (SL, mm) and number of scales above the lateral line (TRa) were measured according to Hubbs and Lagler [18].

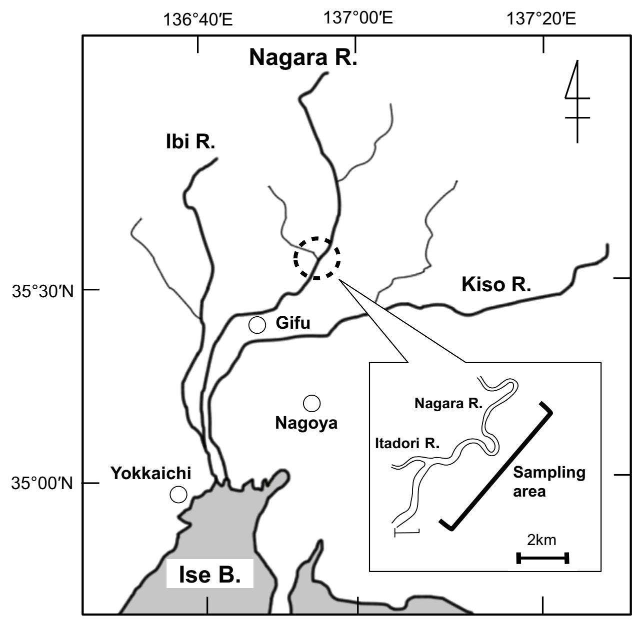

Fig. 1 Map showing the sampling site

Otolith examination and origin discrimination

Sagittal otoliths except abnormal ones were prepared for Sr/Ca analysis according to Otake and Uchida [19]. Otoliths were embedded in epoxy resin (SpeciFix-20, Struers) and individually mounted on a slide glass. Then they were ground with a diamond cup-wheel (Discoplan-TS, Struers) and polishing film (Imperial Lapping Film, 3 M) to expose the core. Finally, they were lapped by a polishing machine (RotoPol-35, Struers), with a polishing cloth (MD-Chém, Struers) and oxide polishing suspension (OP-S Suspension, Struers).

The number of rings formed outside over 15 µm from the otolith core was counted as daily growth increments [20]. These increments were counted and measured from the core to the posterior edge of the otolith with the Otolith Daily Ring Measurement System (Ratoc System Engineering Co., Ltd.).

Table 1 Standard samples used for stock origin discrimination

Origin	HR	LB	AM	Native
Number of individuals	26	41	36	7
Mean SL ± SD (mm)	83.51 ± 9.99	82.28 ± 6.23	75.18 ± 8.02	70.24 ± 7.18
Estimated range of hatching date in 2009	October 7–November 10	August 10–September 25	October 12–December 10	October 17–November 4

HR hatchery-reared stock, *LB* Lake Biwa-originated stock, *AM* amphidromous seed-originated stock

After the counting and measuring, otoliths were cleaned in an ultrasonic bath and rinsed with distilled water. For electron probe microanalyses (EPMA), otoliths were carbon coated using a high vacuum evaporator (JEC-530, JEOL Ltd.); strontium and calcium concentrations were analyzed using a wavelength dispersive X-ray electron probe microanalyzer (JXA-8900R and JXA-8530F, JEOL Ltd.) along the lines used for the daily growth increment counts and measurements. $SrTiO_3$ and $CaSiO_3$ were used as standards. The electron beam was focused on a point 5 μm in diameter, with measurements spaced at each 5 μm interval. The accelerating voltage and beam current were 15 kv and 2×10^{-8}A, respectively. Each counting time was 4.0 s. Each value presented was the average of three counts.

According to our previous work [12], we discriminated the origin of ayu caught in the Nagara River by morphological characteristics (TRa and abnormal otolith frequency), marine water dwelling period (otolith Sr/Ca aspects), and hatching date (otolith daily growth increments) by using the data from the standard samples mentioned above.

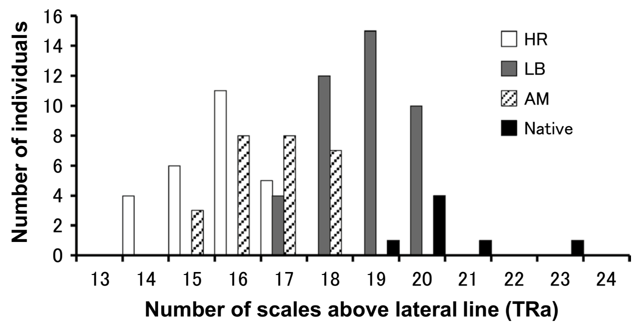

Fig. 2 The frequency distribution of number of scales above the lateral line (TRa) of standard samples used for the stock origin discrimination

Information of stocking amount

A total of 41,450 kg of ayu seeds were stocked in the Nagara River basin in the spring of 2010 (Nagaragawa, Nagaragawa-chuou, Gujo, Itadorigawa-jouryu, Miyamachou, and Tsubogawa Fishery Cooperatives, pers. comms., 2010). Of these total amounts, 32,770 kg were HR (79.1 %), 7,680 kg LB (18.5 %), and 1,000 kg AM (2.4 %), respectively. In the region around the sampling area of this study, 6,000 kg of HR (46.5 %), 5,900 kg of LB (45.7 %) and 1,000 kg of AM (7.8 %) were stocked by the Nagaragawa-chuou Fishery Cooperative, and their seed origin composition was significantly different from the seed origin composition for the basin (Chi square test, $p < 0.05$).

Statistical analysis

For standard length comparison, the Student's *t* test was used between two groups and one-way ANOVA, and Sheffe's post hoc test were conducted among more than two groups. The Chi square test was used to determine the significance of differences among the frequency of origins for each month. These analyses were conducted by using an add-in program for Microsoft Excel (Ekuseru-Toukei 2010 for Windows, Social Survey Research Information Co., Ltd.). Differences were considered as significant at the 5 % level.

Results

Characteristics of standard samples

The standard length of standard samples of AM was significantly smaller than that of HR and LB (Table 1). The range of estimated hatching dates of HR, AM, and natives overlapped with each other, though that of LB was earlier than the others (Table 1). For TRa, the range overlapped between HR and LB, HR and AM, LB and AM, and LB and natives, respectively, whereas it did not overlap between AM and natives, and HR and natives (Fig. 2). Abnormal sagittal otoliths on both the left and right sides were found in eight individuals only from HR standard samples (30.8 %).

Changes in the Sr/Ca ratio from the core to the edge of otoliths of standard samples showed different patterns depending on the origin (Fig. 3). The otolith Sr/Ca ratio of HR showed 0–3 $\times$ 10^{-3} around the core and temporarily increased to 3–5 $\times$ 10^{-3} at 200–300 μm from the core. The ratio subsequently decreased to 0–3 $\times$ 10^{-3} and kept the value until the edge. The ratio of LB kept 0–3 $\times$ 10^{-3} from

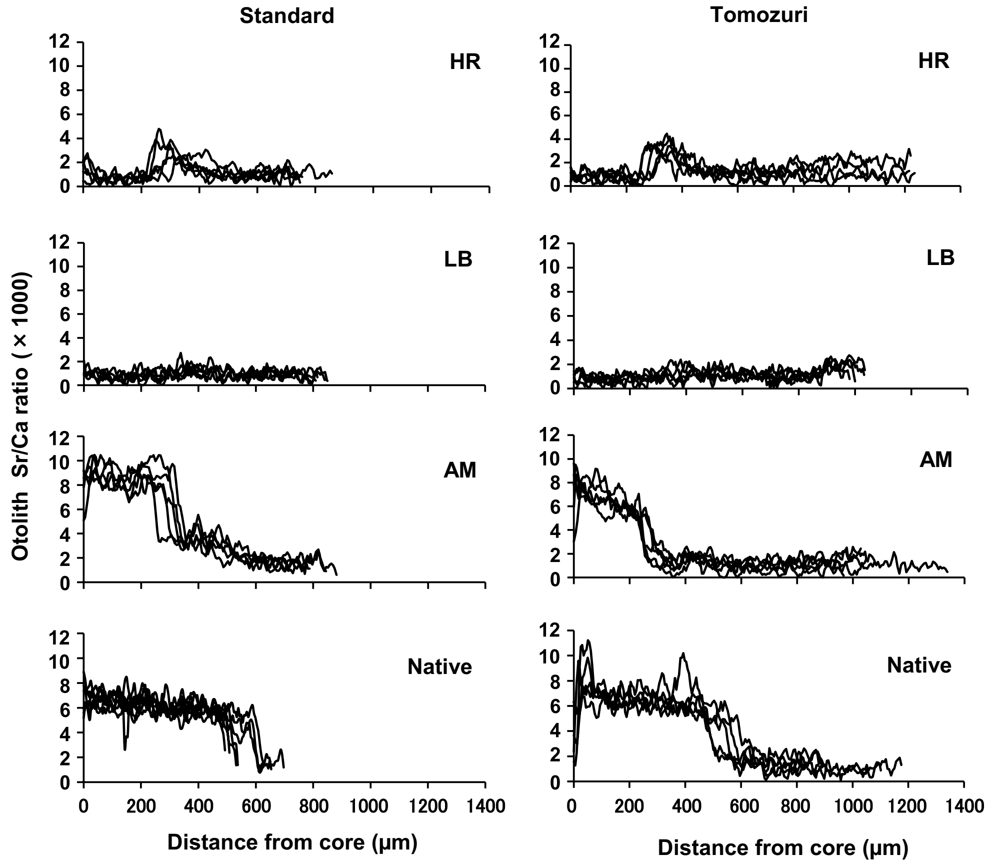

Fig. 3 Profiles of the otolith Sr/Ca ratios from the core to the edge of otolith

the core to the edge. In contrast, the ratio of AM showed 6–11 $\times$ 10^{-3} around the core and sharply decreased to 3 $\times$ 10^{-3} or less around a 300-μm in radius and kept 0–3 $\times$ 10^{-3} to the edge. In the case of native individuals, the Sr/Ca ratio decreased at 500–600 μm from the core later than AM.

Discrimination of origin

Both left and right otoliths were abnormal in 14 of 94 (14.9 %) individuals caught by Tomozuri angling. These fish were discriminated as HR because abnormal otoliths occurred at a high frequency in hatchery-reared ayu in our previous study [12], and such individuals were observed only in standard samples of HR in this study.

Forty individuals of the remaining 80 fish were not regarded as amphidromous fish based on the otolith Sr/Ca ratio, which remained at low values. Furthermore, 12 individuals were discriminated as HR because TRa values were less than 16, while 20 of them were designated as LB with TRa values more than 20. The origins of 8 individuals with 17 or 18 TRa could not be discriminated by TRa but by hatching date; 7 with hatching date of October were discriminated as HR and one with that of September to LB. Figure 3 show the representative patterns of otolith Sr/Ca ratio of individuals discriminated as HR and LB, respectively.

On the other hand, the remaining 40 individuals were thought to be amphidromous. Thirteen individuals were discriminated as AM from their otolith Sr/Ca ratios, which were high around the core and sharply decreased at 200–300 μm from the core and their TRa of 16–18. In the same way, 27 individuals were discriminated as native ayu from the otolith Sr/Ca ratio, which decreased to less than 3 $\times$ 10^{-3} at 500–600 μm from the core and TRa of 18–24.

Monthly changes in origin composition and standard length

The results of origin discrimination of fish caught by Tomozuri angling in each month are shown in Table 2. Native ayu were few in the early season (June and July), but increased toward September and were dominant in September and October. The frequency of native ayu was positively correlated with the month ($n = 5$; $r = 0.93$; $p < 0.05$). HR was dominant in the early season but decreased with time in

Table 2 Results of origin discrimination of fishes caught by Tomozuri angling in the Nagara River

Month	Number of individuals (%)				
	HR	LB	AM	Native	Total
June	10 (41.7)	6 (25.0)	7 (29.2)	1 (4.2)	24
July	10 (40.0)	8 (32.0)	2 (8.0)	5 (20.0)	25
Aug	7 (30.4)	7 (30.4)	4 (17.4)	5 (21.7)	23
Sep	4 (26.7)	0 (0)	0 (0)	11 (73.3)	15
Oct	2 (28.6)	0 (0)	0 (0)	5 (71.4)	7

Table 3 Results of multiple comparisons about origin composition in each month

	July	Aug	Sep	Oct
June	ns	ns	*	*
July	–	ns	*	ns
Aug	–	–	*	ns
Sep	–	–	–	ns

ns not significant

* Significant at 5 % level (with Bonferroni correction)

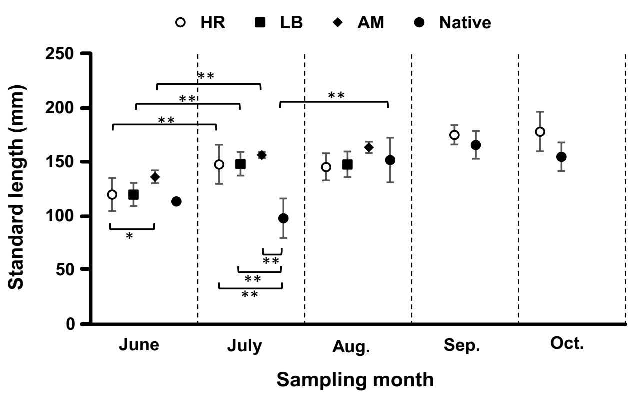

Fig. 4 Monthly changes in mean standard length of ayu caught by Tomozuri angling. *Vertical bars* represent mean ± SD, and *asterisks* represent a significant difference (**$p < 0.01$, *$p < 0.05$)

number. LB and AM were caught until August, while HR continued to be caught after September. The origin compositions between June and September, June and October, July and September, August and September were significantly different (Table 3).

Figure 4 shows changes in the mean standard length of samples caught by Tomozuri angling. In June, AM was significantly larger than HR. In July, native ayu were significantly smaller than the others, while there was no significant difference among ayu of stocked origins. In August to October, the standard length of natives was not significantly different from fish of stocked origins. Standard lengths of all stocked origins were significantly larger in July than in June. The standard length of native ayu was significantly larger in August than in July.

Discussion

Most samples caught by Tomozuri angling from June to July were stocked individuals. Hara [14] also reported that native ayu were few within the fish caught by Tomozuri angling during the early part of the season in the Nagara River. Two hypotheses are considered as the reason why native ayu were relatively few in the Tomozuri fish during the early season in the Nagara River. One is the absence or shortage of native ayu in the study area in June and July. If native ayu do not reach the study area from the sea in these months, we cannot catch them. Most native ayu are known to migrate to the Nagara River from late April to the middle of May in 2010 from counting information by Nagaragawa Estuary Barrage (Nagaragawa Estuary Barrage Operating and Maintenance Office Website: http://www.water.go.jp/chubu/nagara/ "Accessed on 24 June, 2014"). Because native ayu migrate upstream up to 2 km per day [10], native ayu need ca. 35 days to reach the study area from the Estuary Barrage. It is enough for native ayu to reach the study area in June and July. Another hypothesis is the disadvantage for native ayu in territorial competition against stocked individuals. From our previous work [12], standard lengths of native ayu when they migrated into the river were smaller than those of seeds at stocking in the Nagara River. Our present study also showed the same results (Table 1). It seems that the size difference remains in the early season and native ayu have a disadvantage for territorial competition.

After August, the ratio of native ayu in the Tomozuri fish increased while those of stocked seeds decreased. One reason stocked ayu decreased seems to be the removal by Tomozuri angling; another is the abandonment of territories caused by migration to spawning sites [21, 22], especially in Lake Biwa stocks since they matured earlier than the others [6]. Furthermore, in many cases, formerly non-territorial native ayu may newly acquire territory to replace the removed or migrated stock seeds [9].

Concurrent to the composition change, the significant difference in standard length among natives and stocked fishes disappeared. Standard lengths of native ayu increased from July to August, but sizes of stocked fish did not. Because large territorial individuals are removed selectively by Tomozuri angling, selective catching of large territorial stocks in the early season may allow native ayu to make territories and grow. This means that native ayu that were able to make a territory become competitive in comparison to stocked fish in the late season. This result is supported by our previous study [12]. However, after August, standard lengths of native ayu did not increase either. These results suggest that fishing pressure by Tomozuri angling may reduce the apparent growth of ayu in the Nagara River. In our previous study [12], individuals smaller than 120 mm SL in late October in the Nagara River were estimated to be native ayu.

Thus we suggest that the early stocking of large seeds hinders native ayu from making territories early in the season and is a factor in increasing the number of small individuals in the spawning season. To reduce the problem, timing of stocking, size, and amount of stocked seed and utilization of fishing grounds should be optimized so that the native population can attain sufficient growth before the spawning season and make territories early in the season. However, as in other rivers, the amount of native ayu that migrate to the Nagara River fluctuates every year (Nagaragawa Estuary Barrage Operating and Maintenance Office Website: http://www.water.go.jp/chubu/nagara/ "Accessed on 13 September, 2014"); the relative influence of released stocks on native ayu may vary by year. Therefore, further studies are required about the prediction and to understand the factors affecting native resources of ayu, including more information for the establishment of resource management such as the restriction of the fishing season, body size, and catch amount.

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