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GPI Isozymes and Birth Dates of Larval Ayu, *Plecoglossus altivelis* in the Surf Zone

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The ayu, *Plecoglossus altivelis* Temminck et Schlegel is an amphidromous and annual fish, which is distributed throughout the Japanese Archipelago. The fish spawns in the lower reaches of rivers in autumn. The larvae run into the sea soon after hatching, where they live throughout the larval and juvenile stages until next spring when they swim up the rivers.

In Lake Biwa, there is a landlocked form diversified genetically from the amphidromous form (Taniguchi et al., 1983; Nishida, 1985). This landlocked form has been transplanted to a lot of rivers since 1913 (Azuma, 1980) to be harvested during the fishing season from June to November. The landlocked form transplanted is provided with the peak of the spawning season (early September), about one and half month earlier than the amphidromous form (late October), in the rivers of Kochi Prefecture (Seki and Taniguchi, 1988).

It is considered that the landlocked form does not make hybrids with the amphidromous form and does not contribute to the production of the next generation in the sea area, because of the persistence of the difference in *Gpi-1*^{100} allele frequency between the two forms and of the constancy of the allele frequency within the amphidromous form throughout Japan (Taniguchi and Seki, 1983; Seki and Taniguchi, 1985).

On the other hand, Senta and Kinoshita (1985) reported that in the surf zones of Tosa Bay, the ayu larvae begin to occur in October. Considering that it takes about 7–10 days to hatch after fertilization at 20°C w.t. (Ito et al., 1971), there is a possibility that the larvae collected in October are the offspring of the landlocked form. Tsukamoto and Kajihara (1987) found that the number of otolith ring in reared specimens of ayu showed a good correlation with the age in days after hatching.

In this paper, we tried to get some information about the survival of the landlocked form from the samples of larvae collected in the surf zone using the biochemical genetic marker and the character of otolith ring.

Materials and methods

Semimonthly collections of larval and juvenile fishes with a small seine were made in the surf zone of the sandy beach of Tanesaki facing Tosa Bay (see Kinoshita, 1986) from October 13, 1985 to May 26, 1986. The seine used was the same as that described in Kinoshita et al. (1988). The specimens were iced in a cooler until sorting in the laboratory. The sorted ayu were measured for standard length prior to analysis.

Electrophoresis. A total of 649 larvae and juveniles was stored in temperature less than −20°C. Glucosephosphate isomerase (GPI) isozyme was detected from the drip of the whole body of the larvae and juveniles. Horizontal starch gel electrophoresis was carried out by the procedures of Taniguchi and Okada (1980). The buffer solution for electrode and gel, and the solutions for staining and destaining were prepared according to Taniguchi et al. (1983).

Otolith. A total of 168 larvae and juveniles was preserved in 10% formalin and immediately transferred into 80% ethanol. A pair of sagittae was removed and fixed on a microscope slide with epoxy resin, setting the convex face up. Increments were counted outside 15 μm radius with a light microscope at 400–600 magnification, and the average of five counts in each otolith was computed. We estimated their birth dates from these ages and the sampling dates.

Results

1. Seasonal occurrence. A total of 8,751 larval and juvenile ayu was collected during the study period. They occurred from October 30, 1985 to May 10, 1986, being most abundant in late November. Temperature and salinities at waters where any number of them were collected ranged from 13.0 to 23.8°C and from 20.4 to 36.7‰, respectively. Their sizes ranged from 7.5 to 45.4 mm SL (Fig. 1).

2. GPI electrophoresis. Table 1 shows *Gpi-1* genotypic proportions and allele frequencies of samples. Frequencies of 100 allele at *Gpi-1* ranged from 0.551±0.040 (SE) to 0.664±0.040,

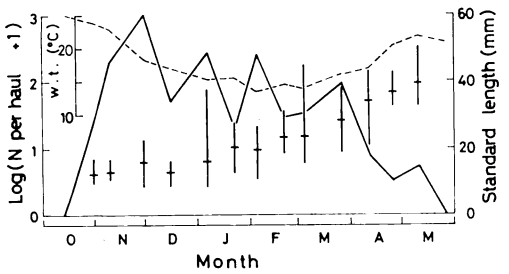

Fig. 1. Seasonal occurrence of larval and juvenile ayu in the surf zone of the sandy beach of Tanesaki facing Tosa Bay from October 1985 to May 1986. Horizontal and vertical bars in the figure indicate means and ranges of standard length, respectively. Water temperature is shown by a broken line.

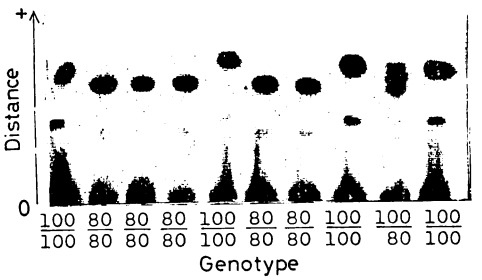

Fig. 2. Example of zymogram of electrophoretic gel banding patterns of GPI isozyme in larval ayu collected on October 30, 1985.

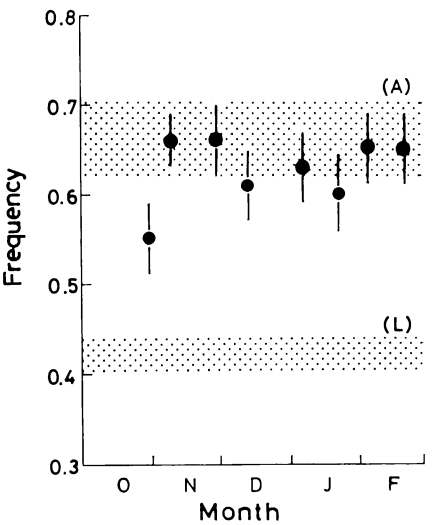

Fig. 3. Frequencies of 100 allele at *Gpi-1* of samples. Circles and vertical bars indicate means of *Gpi-1*^{100} allele frequencies and their standard errors, respectively. Shaded bands indicate the ranges of *Gpi-1*^{100} allele frequencies in adults of amphidromous (A) and landlocked (L) forms of ayu, as reported by Seki and Taniguchi (1985) and Nishida (1985).

Table 1. *Gpi-1* genotypic proportions, *Gpi-1*^{100} allele frequencies of larvae and juveniles of ayu collected with a small seine in the surf zone of Tosa Bay from October 1985 to February 1986.

Sample No.	Date	N	Genotype						*Gpi-1*^{100} allele frequency±SE
			100/100	100/80	100/60	80/80	80/60	60/60	
1	Oct. 30, 85	79	25	37	0	17	0	0	0.551±0.040
2	Nov. 9, 85	144	66	59	0	19	0	0	0.663±0.028
3	Nov. 30, 85	58	27	23	0	8	0	0	0.664±0.044
4	Dec. 15, 85	79	33	30	1	15	0	0	0.614±0.039
5	Jan. 6, 86	76	33	30	0	13	0	0	0.632±0.039
6	Jan. 22, 86	60	20	32	0	8	0	0	0.600±0.045
7	Feb. 4, 86	74	30	34	2	8	0	0	0.649±0.039
8	Feb. 20, 86	79	33	36	1	9	0	0	0.652±0.038

Table 2. Examination records of the estimated birth dates of larval and juvenile ayu collected in the surf zone of the sandy beach of Tanesaki from October 1985 to February 1986.

Date	No. fish examined	SL (mm)	Age (days)	Estimated birth dates	No. fish hatched out		
					before Oct. 10	during Oct. 10 to Oct. 19	during Oct. 20 to Oct. 29
Oct. 30, 85	23	10.3–13.6	8–24	Oct. 6–Oct. 22	1	19	3
Nov. 2, 85	63	7.5–18.4	3–27	Oct. 7–Oct. 30	1	15	45
Dec. 15, 85	30	10.3–16.3	7–20	Nov. 25–Dec. 8	0	0	0
Jan. 6, 86	27	13.2–19.1	11–32	Dec. 5–Dec. 26	0	0	0
Feb. 4, 86	25	14.3–26.4	17–61	Dec. 5–Jan. 18	0	0	0
Total	168	7.5–26.4	3–61	Oct. 6–Jan. 18	2	34	48

and the GPI zymogram of samples completely accorded with that of adult in Taniguchi et al. (1983) (Fig. 2).

Only larvae collected in October (No. 1 in Table 1) had the *Gpi-1*^{100} allele frequency less than 0.60. This value (0.551 ± 0.040) lay between those of the amphidromous and landlocked forms (Fig. 3), and the value was distinctly different from those of both forms ($p<0.05$, t-test). However, *Gpi-1*^{100} allele frequencies of Nos. 2–8 except Nos. 4 and 6 ranged within those of the amphidromous form (Table 1, Fig. 3).

3. Otolith ring. Otolith ring counts ranged from 3 to 61. The birth dates of the samples ranged from October 6, 1985 to January 18, 1986 (Table 2). A total of 20 and 16 larvae having birth dates before October 20 were confounded with collections on October 30 and November 2, respectively.

Discussion

The *Gpi-1*^{100} allele frequency of the larvae collected on October 30 fell between the frequency ranges of the landlocked and amphidromous forms (Fig. 2). Furthermore, from the result of the otolith ring analysis, 36 larvae out of the 86 larvae collected on October 30 and November 2 are considered to have hatched out before October 20 (Table 2). Their parents must have spawned them from late September to early October because it takes about 7–10 days to hatch out at 20°C w.t. These results suggest that the sample No. 1 (Table 1) is a simple mixture of the larvae of both amphidromous and landlocked forms, or hybrids between the two forms. However, our data is not enough in number to discuss mixture or hybrids. According to Seki and Taniguchi (1988), the spawning periods of the two forms hardly overlap each other in natural rivers in Kochi Prefecture, thus there is little chance of the production of hybrids.

On the other hand, the *Gpi-1*^{100} allele frequencies of the sample Nos. 4 and 6 collected on December 15 and January 22 tended to be slightly lower than the frequency range of the amphidromous form (Fig. 3), but these two samples are not supposed to include the landlocked form because they were estimated to have hatched out from November 25 to December 8 (Table 2). Thus, these low allele frequencies were probably due to simple sampling errors. Other larvae collected from November to February are identified as the amphidromous form on the basis of the allele frequencies of *Gpi-1*^{100} and the otolith ring analysis.

It is not known in what part of the sea the larvae of the landlocked form are distributed, but if they inhabit the surf zone like the larvae of the amphidromous form, they ought to occur there from September to early October. Nevertheless, in the surf zones of Tosa Bay, we have never caught any ayu larvae during this period from 1981 to 1987. The offspring of landlocked ayu may almost die out soon after flowing into the sea due to too high water temperature (e.g. 24.5–29.0°C in the surf zone of Tosa Bay in September and early October from 1981 to 1987). Tabata and Azuma (1986) actually indicated that the reared larvae of the landlocked ayu hardly survived at 23°C sea water temperature (survival rate, 0–9%), but half of those of the amphidromous ayu survived under the same condition. However, it is also possible that they are distributed in deeper waters with a temperature low enough for them to survive.

We found the offspring of the transplanted landlocked form of ayu in the sea. Do they survive to the stage of swimming upriver, i.e. to the juvenile and young stages? It is very difficult to settle this question by utilizing an isozyme marker since the larvae of the landlocked form surviving in the surf zone will decrease much in number due to natural mortality. Furthermore, they are extremely smaller than those of the amphidromous form in quantity, as suggested by Seki and Taniguchi (1985) and Nishida (1986).

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砕波帯におけるアユ仔魚の GPI アイソザイムと孵化日

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土佐湾の砕波帯において，1985 年 10 月から 1986 年 5 月にかけて採集されたアユ仔稚魚から GPI アイソザイムを検出し，放流された陸封型アユに由来する仔魚の砕波帯における出現状況を追跡した．サンプルの $Gpi\text{-}I^{100}$ 遺伝子頻度は，陸封型および両側回遊型との中間的な数値を示した 10 月下旬の標本以外はほぼ両側回遊型を表した．各個体の耳石からそれらの孵化日を推定すると，10 月上旬までに産出された個体が 10 月下旬および 11 月上旬のサンプル中に計 36 尾出現した．これらは，陸封型の仔魚の混入もしくは両型の交雑によるものであると思われるがその判別はできなかった．しかしながら，これら陸封型アユに由来する仔魚は土佐湾砕波帯においては海産アユ仔魚に比べて量的に極めて少ないものと推測された．

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