水研機構研報, 第 53 号, 63 – 69, 令和 8 年
Bull. Jap. Fish. Edu. Agen. No. 53, 63-69, 2026

Current status of artificial seed production and selective breeding in Japanese yellowtail *Seriola quinqueradiata*: The progress achieved by Japan Fisheries Research and Education Agency

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Abstract: Japanese yellowtail *Seriola quinqueradiata* is one of the most important commercial fish species in Japan. The aquaculture of yellowtail has been practiced since the 1920s, using wild juveniles caught in the Pacific coast of southern Japan. However, the catch of juveniles is occasionally unstable, likely owing to various environmental factors. Additionally, the peak period of the harvest of yellowtail, which have been cultured for nearly two years from wild juveniles that could be obtained only in early spring, is from late fall to winter. Consequently, the quantity of yellowtail available in the market significantly decreases from May to September. Therefore, artificially produced seeds have begun to be utilized to overcome these problems. In this situation, a family lineage or strain with fast growth and disease resistance traits would be valuable. Therefore, the development of such a strain via selective breeding and production of artificial seed having beneficial traits have drawn significant interest in Japan. In the selective breeding program for yellowtail at Japan Fisheries Research and Education Agency (FRA), the seeds obtained via artificial spawning were raised to approximately 5 cm and then cultured until they reach the harvest sizes in commercial aquaculture farms. Subsequently, 2,000 adults (approximately 60 cm) were selected, and their body length and body weight data, and samples of fin chip were obtained for pedigree reconstruction from the individuals. Finally, based on the estimated breeding values using pedigree-based best linear unbiased prediction and inbreeding coefficients, 200 individuals were selected as broodstock candidates. Thus far, good correlations of breeding values between the first and second generations have been observed in both fish length and body weight, indicating that our breeding program is promising.

Key words: *Seriola quinqueradiata*, artificial seedling, selective breeding, BLUP

Accepted on Feb 26, 2026

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Introduction

Japanese yellowtail *Seriola quinqueradiata* is one of the most important commercial fish species in Japan. Hence, aquaculture of this species is prevalent in southwest Japan, particularly in Kagoshima, Oita, Ehime, Miyazaki, Kochi, and Nagasaki Prefectures. The Pacific sub-population of this species spawns in the Satsunan area, southern Kyushu, Japan, and the juveniles appear off the coast of Kagoshima Prefecture from March to April, following floating seaweeds. The wild juveniles of approximately 50-100 mm length are caught from April to May, cultured in cages until they reach 3-5 kg, and then shipped from late autumn of the following year.

Aquaculture of this species in Japan began in 1927 in the Adoike cove, which is separated from the Seto Inland Sea by the bank, in Kagawa Prefecture (Miyashita 2008). This practice is considered the first successful case of modern aquaculture of marine fish in the world. After the 1950s, the "cage culture method" had been developed in Japan, and promoted new entrants to aquaculture of this species. Therefore, the production of this species rapidly increased after 1964. Recently, the gross domestic production of this species, both through capture fishery and aquaculture, has been over two hundred thousand tons per year (Fishery and aquaculture statistics of Reiwa 6; Ministry of agriculture, Forestry and fisheries 2025). In 2023, the export value of fishery products showed that Japanese yellowtail positioned in the 3rd place (41.8 billion yen) after Japanese scallops and pearls, marking a steady increase from the previous year's value (36.3 billion yen) (FY2023 White Paper on Fisheries; Ministry of agriculture, Forestry and fisheries 2024).

However, in Japanese yellowtail aquaculture, the practice of using wild-caught juveniles as seedling is still common. Thus, the aquaculture production largely depends on the amount of wild-caught juveniles, which is occasionally unstable likely owing to various environmental factors. The poor harvest of wild juveniles in 2021 led to a drastic decrease in the aquaculture production in 2022, resulting in the short supply and price surge in the market. Additionally, the peak period of the harvest of yellowtail, which have been cultured for nearly two years from the wild juveniles that could be obtained only in early spring, is from late fall to winter. Thus, the quantity of yellowtail available in the market significantly decreases from May to September. Currently, although the aquaculture industry relies on approximately 20 million wild-caught juveniles each year, artificially reared seedlings account for only 5 % of the demand. Hence, promoting the use of artificial seedlings is a valid way to overcome the problems mentioned above, and is expected to further increase the productivity through selective breeding.

Application of conventional selective breeding methods has been attempted in common carp *Cyprinus carpio* (Moav and Wohlfarth 1976), coho salmon *Oncorhynchus kisutch* (Hershberger *et al*. 1990), rainbow trout *O. mykiss* (Kincaid *et al*. 1977; Hörstgen-Schwark 1993), and red seabream *Pagrus major* (Murata *et al*. 1996). In addition, parentage analyses using DNA markers, such as microsatellite DNA and single nucleotide polymorphisms (SNPs), have been advanced, and the breeding values now could be determined using the pedigree-based best linear unbiased prediction (BLUP) method, which allows the selection of individuals with the most beneficial phenotype. In particular, the parentage analysis using SNP markers results in higher parental assignment rates than those using microsatellite DNA markers (Hauser *et al*. 2011; Trọng *et al*. 2013). The former method has been applied to various aquatic species, such as yellowtail kingfish *S. lalandi* (Premachandra *et al*. 2019), Atlantic salmon *Salmo salar* (Holman *et al*. 2017), rainbow trout (Liu *et al*. 2016), European abalone *Haliotis tuberculate* (Harney *et al*. 2018), and Australian greenlip abalone *H. laevigata* (Arbon *et al*. 2021). In the case of Japanese yellowtail, the SNP markers have recently been developed for a parentage analysis (Uchino *et al*. 2020), in addition to the conventional microsatellite DNA markers (Shimada *et al*. 2019).

In this study, we raised offspring (first generation) produced by artificial mating of wild individuals of yellowtail. The first broodstock candidates were then selected based on the breeding values using pedigree-based BLUP for adult size. Subsequently, offspring (second generation) were obtained from the first broodstock and raised to adult size. Finally, we assessed the effects of our selective breeding program by comparing the growth differences between the first and second generations in terms of adult size.

Materials and Methods

Samples

For the first generation (G_1), 26 family lineages were obtained from 26 (13 sires and 13 dams) wild fish (G_0) via insemination in April 2019 by a 1 × 2 factorial mating in Goto Field Station, Fisheries Technology Institute (FTI) (Nagasaki, Japan). The fertilized eggs were divided into two subsamples, and were grow in Goto Field Station and Kamiura Field Station (Oita, Japan), with 26 and 14 family lineages allocated to each

station, respectively. After they grew to adult sizes, we selected 20 individuals, taking the breeding values and inbreeding coefficient into account, for the parents of the next generation (see the next sub-section for details).

For the second generation (G_2), 48 and 84 family lineages were obtained from the 18 (14 sires and 4 dams) and 20 (14 sires and 6 dams) adult G_1 fish, by performing a 1 × 5 factorial mating in Goto Field Station and a full factorial mating in Kamiura Field Station, respectively, via insemination in April 2022. The first (48 family lineages) and second (84 family lineages) fertilized eggs resulting from these mating plans were allocated to Goto and Kamiura Field Station, respectively.

The obtained seedlings were raised to approximately 5 cm in indoor rearing tanks for approximately 70 days during which fish were sorted twice to uniform sizes to avoid cannibalism (Fig.1A). These seedlings were then transferred to two commercial aquaculture farms, designated here as A and B in the Kyushu region (Fig.1B), and cultured to harvest sizes. The G_1 seedlings were transported from Kamiura Field Station and G_2 seedlings from Goto Field Station, to both aquaculture farms. After approximately 18 months, ca 1,000 adult fish were randomly selected from each aquaculture farm, and fish length (total length or fork length) and body weight were measured, and samples of fin chip were obtained from the individuals for pedigree reconstruction (Fig.2A, 2B).

Finally, 100 individuals were selected (see the next sub-section) from approximately 1,000 individuals in the cages. The individuals were identified based on PIT tags (Biomark,

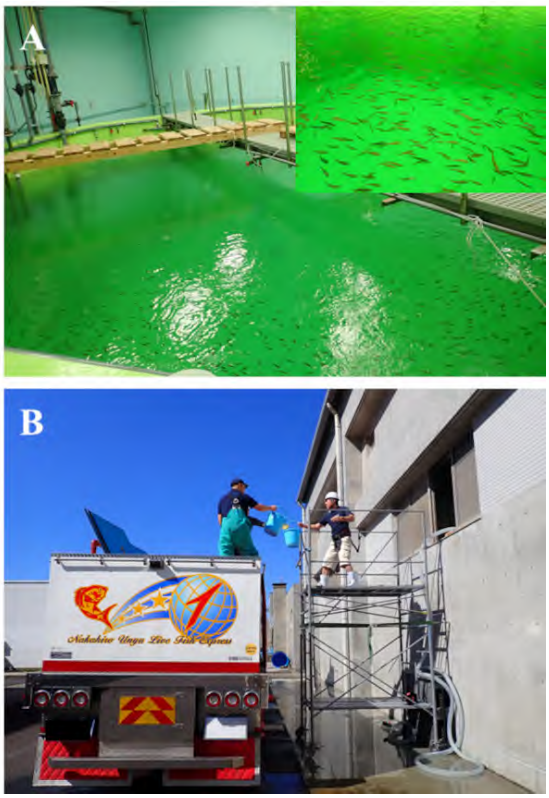

Fig.1 Mass production of artificial seedlings of Japanese yellowtail in Kamiura Field Station

A, artificial seedlings that are raised to approximately 5 cm in 120 kL indoor rearing tanks for approximately 60 days. B, transportation of artificial seedlings from Kamiura Field Station to aquaculture farms after approximately 70 days post hatching.

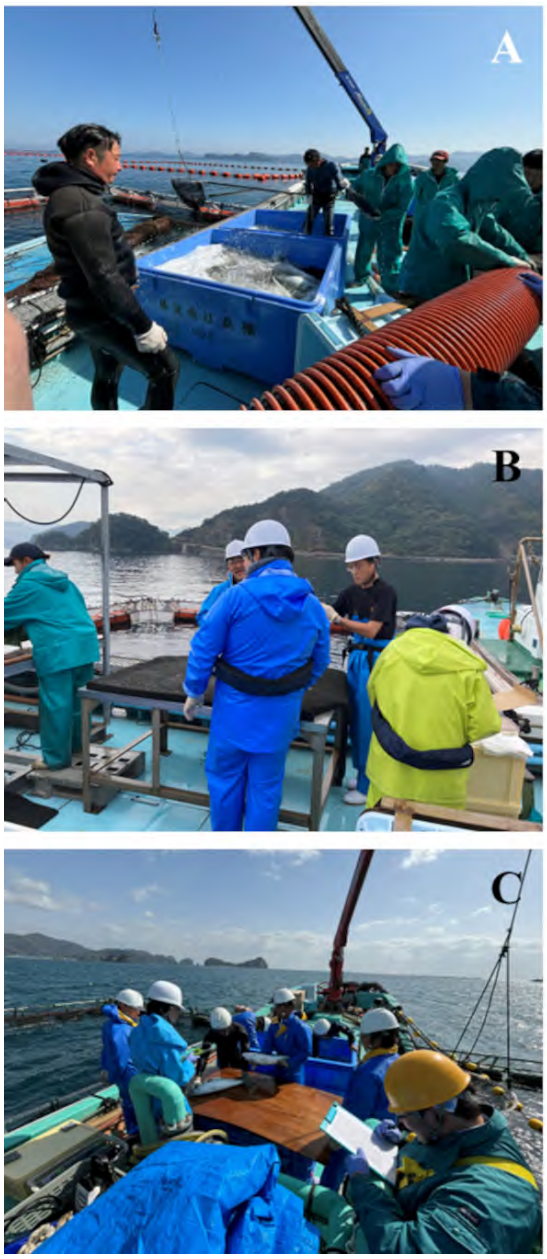

Fig.2 Phenotyping and selection of broodstock candidates of yellowtail in aquaculture farms

A, anesthetization of fish; B, measurement of body weight and fish length; C, identification of individuals for selection using PIT Tag data.

ID, USA) that had been implanted in their bodies (Fig.2C), and used as the broodstock.

Parentage analysis

We extracted total DNA from all fin chip samples using the InnuPure C96 and Smart DNA prep (a) kits (Analytik Jena, Jena, Germany). Uchino *et al*. (2020) designed and synthesized the SNP probes for 96 loci using SNP Type Assay (Fluidigm, CA, USA). We amplified these SNP markers using a 2-step PCR protocol and genotyped using the Biomark HD system and BioMark Genotyping Analysis Software v4.5.1 (Fluidigm). The obtained genotype data allowed us to perform parental assignments using an exclusion method (Sekino and Kakehi 2012), and then we reconstructed the pedigree of the samples. We estimated heritability and breeding values for the growth traits of the reconstructed pedigree. We applied the BLUP method to the data under the animal model using the breedR package of R (Muñoz and Sanchez 2019). Based on the breeding values and inbreeding coefficients, we selected 100 individuals mentioned above as the candidates of the parents for the next generation.

Results

The phenotypic data of our Japanese yellowtail breeding population are summarized in Fig.3. The mean body weight and total length (minimum-maximum value) of G_1 fish in aquaculture farms A and B were 4.5 ± 0.2 kg (4.0-5.7 kg) and 69.6 ± 1.5 cm (65.0-75.5 cm) (n = 1,000), and 3.7 ± 0.2 kg (3.1-4.8 kg) and 65.8 ± 1.5 cm (60.5-71.3 cm) (n = 1,000), respectively. The average values of total length and body weight in aquaculture farm A were larger than those in farm B. The body weight and fork length of G_2 fish in these aquaculture farms were 3.2 ± 0.3 kg (2.4-4.1 kg) and 57.6 ± 1.6 cm (50.2-61.8 cm) (n=1,012), and 3.1±0.3 kg (2.2-4.0 kg) and 61.1 ± 1.6 cm (53.5-65.5 cm) (n=1,040), respectively.

All 14 family lineages of G_1 were detected in both aquaculture farms by the parentage analysis. The parents of 968 individuals from farm A and those of 1,039 individuals from farm B were assigned, and 42 family lineages of G_2 were found in both aquaculture farms.

We then estimated the heritability and breeding values for growth traits. The heritability estimates in G_1 and G_2 for body

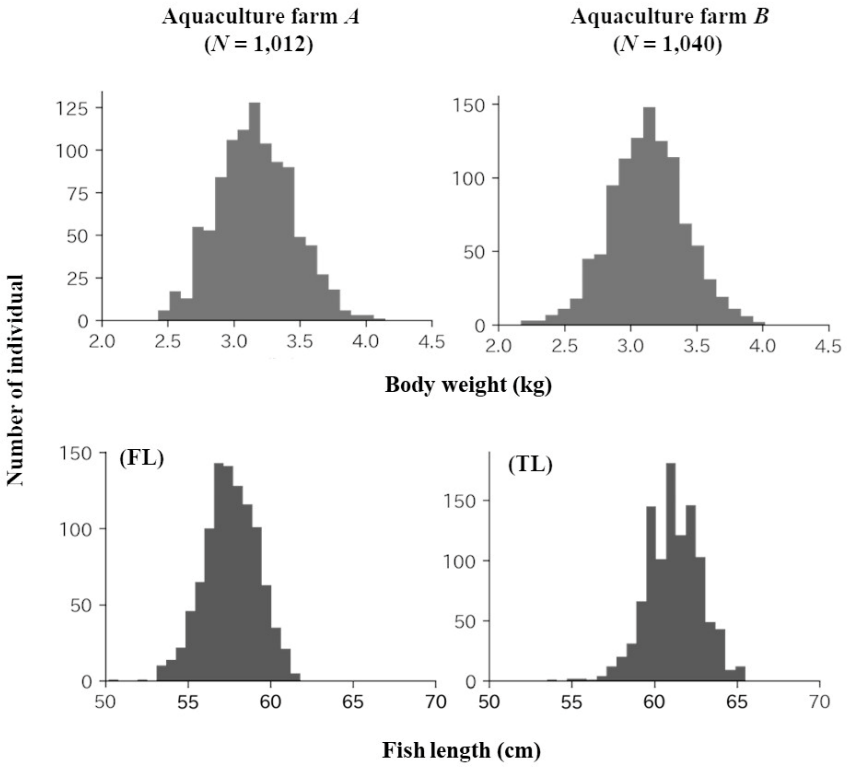

Fig.3 Distributions of body weight (kg) and fish length (cm) in randomly selected 2,000 adults (G_2) in aquaculture farm A and B

N, individual number; TL, total length; FL, fork length.

weight and fish length are shown in Table 1. Despite that we analyzed the data obtained from two independent aquaculture farms, the average breeding values of each family lineage exhibited strong correlations between the two aquaculture farms both in the G_1 and G_2 generations. This suggests that the environmental effects were similar between the two farms.

We further examined if our breeding program is progressing well. Unexpectedly, the average values of the body weight and fish length of G_2 were smaller than those of G_1 in both aquaculture farms though we had selected G_1 individuals with high breeding values as the parents of G_2 generation. This is probably because the individuals of G_2 were raised in much lower water temperatures in both farms than those for G_1. Nevertheless, we observed strong correlations between the average breeding values of a sire and a dam in G_1 and the average ones of their offsprings in G_2 for fish length (farm A, $r = 0.89$; farm B, $r = 0.74$) and body weight (farm A, $r = 0.77$; farm B, $r = 0.82$) (Fig.4). These observations are consistent with what are expected under the animal model; the high breeding values of our selected parents were inherited to their offsprings.

Table 1 The heritability estimates for body weight and fish length in both aquaculture farms

Generation		Aquaculture farm A	Aquaculture farm B
G_1	BW	0.320	0.402
	TL	0.606	0.684
G_2	BW	0.504	0.323
	FL	0.680	0.384

BW, body weight; TL, total length; FL, fork length

Discussion

In this study, we evaluated the efficiency of our selective breeding program for Japanese yellowtail by comparing the differences in growth between two generations, G_1 and G_2 in terms of adult size. Although the mean body weights of G_2 in the two aquaculture farms were lower than those of G_1, positive

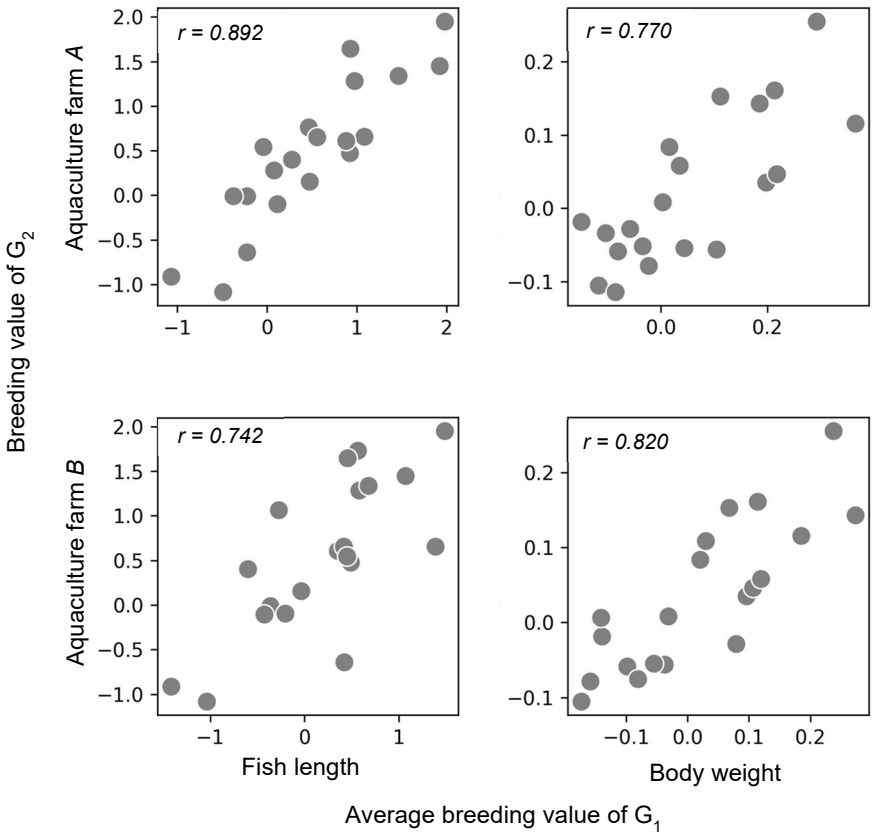

Fig.4 Testing the fit to an additive genetic model

The x-axis represents the average breeding values of sires and dams in the G_1 generation. The y-axis represents the average breeding values of their offspring in the G_2 generation. Each dot represents a sibling. The four panels correspond to combinations of aquaculture farms (A or B) and phenotypes.

correlations were observed between the breeding values of the two generations, indicating that family lineages with high breeding values in G_2 were originated from the family lineages of G_1 with high breeding values. This also implies that the fast-growing family lineage had been appropriately selected using pedigree-based BLUP, thereby making our breeding program promising. Similarly, Morishima (2019) reported that the mean body weight (5 kg) of the group selected by pedigree-based BLUP was higher than the maximum body weight (4 kg) of the group consisting of wild seedlings at the same period. Additionally, selective breeding of this species based on pedigree-based BLUP has also been promoted by other private enterprises in Japan. The developing family lineage at our institute will reach the third generation by 2025 through two selections. In contrast, Shimada *et al*. (2019) reported that the bias in family composition in the G_1 artificial seedlings increased after size selection. As the bias of family composition in the artificial seedlings provided to each aquaculture farm might result in reduced genetic diversity and survival rate in the broodstock in the future, it is necessary to closely monitor the status of the selectively bred next generation. Recently, genome sequencing of various species has been conducted using next-generation sequencing, allowing breeding practices based on genomic selection. Genomic selection is a breeding method introduced by Meuwissen *et al*. (2001), which utilizes genome-wide markers to predict the breeding value (GEBV) of individuals within a breeding population. A modified implementation of the BLUP approach (GBLUP) has already been used in various practical genomic selection applications. In dairy cattle breeding, genomic selection has largely replaced the traditional methods based on progeny testing, which often yielded unparalleled results. Maize and wheat are at the forefront of crop genomic selection (de Koning 2016). In aquatic species such as *S. salar*, *O. mykiss*, yellow croaker *Larimichthys crocea*, Pacific abalone *H. discus hannai*, and Japanese scallop *Patinopecten yessoensis*, the possibility of genomic selection for body size, parasite and disease resistance, fatty acid composition and nutritional quality-related traits has been reported (Ødegård *et al*. 2014; Tsai *et al*. 2015, 2016; Dong *et al*. 2016; Vallejo *et al*. 2016; Hosoya *et al*. 2017; Liu *et al*. 2024). To perform GBLUP in yellowtail, we have been accumulating genome-wide SNP data based on GRAS-Di, which will be beneficial for improving selection strategies and preventing anticipated risks in the future. In summary, this study revealed that our breeding program is promising for selection of yellowtail that grow faster relative to conventional use of wild seedlings. The development of strains via selective breeding and the stable supply of their artificial seedlings will greatly contribute to the expansion of aquaculture production and export to the global market.

Acknowledgments

We thank Dr. A. Ozaki, Mr. K. Yoshida, Dr. K. Okuzawa, Dr. Y. Shimada, Ms. J. Tabata, Mr. T. Noda, Mr. R. Shinoda, and Mr. Y. Nakagawa of Japan Fisheries Research and Education Agency for their valuable suggestions and supports. We thank all members of the HYOSHOKU Co., Ltd. and KEITEN Co., Ltd. for cultivating to harvest size to obtain the main results of this paper.

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