Title:

The effects of long-term translocation on population genetic structure of Ayu in Japan: Management implications for a recreationally fished iconic species

Shuichi Kitada

Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan

Short running title: Genetic effects of translocation

Abstract

1. Ayu (*Plecoglossus altivelis altivelis*), an important freshwater fisheries resource and popular recreational fishing species, lives for only one year and has a single breeding season. To supplement increased recreational fishing demand, huge numbers of wild-born landlocked juvenile Ayu have been translocated from Lake Biwa into most Japanese rivers for more than 50 generations. Hatchery-born amphidromous fish (including amphidromous and landlocked form hybrids) have also been released for many generations.

2. Hatchery-born fish have low survival and maladapted behaviour in the wild. Landlocked and amphidromous forms of Ayu easily hybridise, but survival of progeny of landlocked forms is very low in seawater. Repeat backcrossing may cause introgression of landlocked forms into amphidromous populations, but this has not been previously identified.

3. Study objectives using genetic data from Ayu from 118 locations throughout the distribution of this species in Japan are to describe contemporary population structure, genetic diversity, and admixture proportions of Ayu forms in populations, and to evaluate how human-induced translocation has affected population genetic structure.

4. The analyses of published genotypes of 12 microsatellite markers provide strong evidence for very high gene flow between populations, but population structure has been retained in several regions, and several populations are nested. Genetic diversity is surprisingly homogeneous. Hybridisation between landlocked and amphidromous forms has occurred in all populations, with a mean hybrid proportion (± standard deviation) of 37 ± 10%, ranging 15%–60%.

5. Results are discussed in relation to the conservation and management of this species. Recommendations are made to reduce translocation and hatchery releases, by establishing rivers and/or areas in every prefecture where translocation does not occur. Release of juveniles is of value for short-term management objectives, but management of spawning escapements, and improving the spawning and nursery habitat are important for this species' long-term sustainability.

KEYWORDS: admixture, amphidromous, hatchery, hybrid proportion, Lake Biwa, no-release rivers/areas

1 | INTRODUCTION

Anthropogenic forces often break reproductive barriers, providing an opportunity for hybridisation between closely related species that might not otherwise exist under natural conditions (Grabenstein & Taylor, 2018). A geographic area where natural hybridisation occurs is referred to as a hybrid zone, while an area where two divergent populations or closely related species occur and potentially hybridise is called a contact zone (Barton & Hewitt, 1985; Hewitt, 1988; Johannesson et al., 2020). Hybrid zones work as selective filters, enabling determination of the evolutionary roles of reproductive barriers at various stages of speciation (Barton & Hewitt, 1985; Hewitt, 1988; Martinsen et al., 2001). Repeat backcrossing and recombination of genotypes in hybrid zones results in genetic changes beyond the first hybrid generation, allowing neutral and adaptive alleles to spread among populations (Barton, 1979; Barton & Hewitt, 1985; Hewitt, 1988; Johannesson et al., 2020).

Translocation of organisms across geographic boundaries has been often used in conservation; however, because it removes reproductive barriers, and can lead to hybridisation of introduced and resident populations (Berger-Tal, Blumstein & Swaisgood, 2020; Seddon et al., 2014; Taylor et al., 2021). Translocation can disturb the ecological and evolutionary processes of a species (Conant, 1988; Moritz, 1995), and unintended, negative outcomes of hybridisation can occur because of altered offspring fitness (Bucharova, 2017; Ricciardi & Simberloff, 2009). However, hybridisation could be beneficial to species adaptation in a changing environment, so a case-by-case evaluation is needed to elucidate its consequences (Hirashiki, Kareiva & Marvier, 2021).

Herein, a case study on Ayu (*Plecoglossus altivelis altivelis*), an iconic fish species targeted by recreational fishers in Japanese rivers, is presented. Ayu—an essentially amphidromous species, except for some land-locked populations (landlocked form) (Nishida, 1985)—can be found throughout Japan, and is one of the most extensively studied fish species in the country. Landlocked Ayu have been translocated from Lake Biwa into amphidromous populations in rivers throughout Japan for many years. Below we briefly summarize the knowledge and genetic studies of the species and describe the aim of the present study.

1.1 | Fisheries, translocation of landlocked juveniles, and hatchery supplementation

Ayu occur widely in coastal areas of China, Korea, Japan, Taiwan (Kwan et al., 2012), and Vietnam (Tran, Iida & Maeda, 2017). It is an important fisheries resource in Japanese freshwaters, and a most popular recreational fishing species. Government statistics (e-Stat, https://www.e-stat.go.jp/) indicate that Ayu is caught in almost all prefectures in Japan, with characteristically high catches along the central and southern Pacific coast (Figure S1a). The commercial catch increased markedly to reach a maximum in 1991 but declined thereafter (Figure S1b). While catch statistics exclude recreational catch, this is likely to be substantial, with recreational catch estimated at ~ 5.3×10^6 fish (260 t) over 485×10^3 days (day × fishers) in Nakagawa River, Tochigi Prefecture, alone (Kitada & Tezuka, 2002)—a tonnage similar to the commercial catch in the same year in one of the top production areas in Japan (Figure S1a).

Translocation of wild-born landlocked Ayu juveniles from Lake Biwa into Tama River, Tokyo, began in 1912, and this process rapidly expanded throughout Japan in the 1950s and 1970s to supplement increased recreational fisher demand (Takamura, 2009). The catch of wild-born juveniles of the landlocked form in Lake Biwa increased markedly and reached a maximum in 1991, but this also declined thereafter (Figure S1b). In the 1990s, many fisheries cooperatives gradually changed to using hatchery-born amphidromous juveniles and/or hybrids of the two forms instead of relying on landlocked juveniles for two main reasons: an outbreak of cold-water disease in Lake Biwa (Izumi & Wakabayashi, 1997), and because landlocked forms translocated into rivers were not expected to reproduce successfully (e.g.,

Pastene, Numachi & Tsukamoto, 1991; Seki & Taniguchi, 1998; Tago, 2004; Takeshima, Iguchi & Nishida, 2009).

In recent years, three types of juveniles have been released: hatchery-born amphidromous fish (including amphidromous and landlocked form hybrids), which have been released in the largest quantity, followed by wild-born landlocked juveniles from Lake Biwa, and wild-born amphidromous fish collected in coastal waters (Figure S2a). Most wild-born juveniles are caught in Shiga Prefecture (the landlocked Ayu collected in Lake Biwa (Figure S2b)), and most hatchery-reared juveniles are produced in Tochigi Prefecture, followed by Miyazaki, Tokushima, Yamaguchi, and Wakayama prefectures.

1.2 | Life history and population genetic structure

Ayu live for one year, and have a single breeding season (Nishida, 1986). The amphidromous form of Ayu spawns in the lower reaches of rivers during autumn. Hatched larvae immediately migrate downstream into brackish waters, then enter the sea where they overwinter. From spring to early summer, juveniles migrate upstream. In autumn, they mature and again descend downstream to spawn, then die (Nishida, 1986). Ayu in the Lake Biwa landlocked population—the largest of landlocked populations—spawn in autumn in the lower reaches of rivers that flow into the lake, after which larvae migrate downstream into the lake and overwinter. This form is differentiated from amphidromous populations in ecology, morphology, and molecular characteristics (e.g., Kawanabe, 1858; Nishida, 1985; Nishida, 1986; Tsukamoto & Kajihara, 1987). A previous study using allozymes inferred that genetic distance between the landlocked form in Lake Biwa and an amphidromous population was correspond to a divergence time approximately 100 k years before present (Nishida, 1985).

The population genetic structure of Ayu has been studied extensively in Japan. Initially (in the 1980s), molecular markers included allozymes (Nishida, 1985; Seki, Taniguchi & Jeon, 1988; Taniguchi, Seki & Inada, 1983), but with technological developments this expanded from the late 1990s to include mitochondrial DNA (mtDNA) (Iguchi, Tanimura & Nishida, 1997; Iguchi & Nishida, 2000; Iguchi, Ohkawa & Nishida, 2002; Ikeda & Taniguchi, 2002; Takeshima, Iguchi & Nishida, 2005) and microsatellite markers (Ikeda, Takagi & Taniguchi, 2005; Takagi, Shoji & Taniguchi, 1999; Takeshima, Iguchi & Nishida, 2009; Yamaguchi et al., 2016; Yamamoto et al., 2007) to estimate genetic characteristics of populations at regional scales. Microsatellite markers have been recently used to describe this species' population structure throughout its distribution based on a comprehensive sampling of Japanese rivers (Takeshima et al., 2016a). No population genetics study has used single nucleotide polymorphisms (SNPs).

1.3 | The reproductive success of translocated Ayu

In the early 1990s, experiments demonstrated that amphidromous and landlocked forms of Ayu interbred naturally in outdoor ponds, leading to concerns that the fitness of hybrid progeny would be reduced (Iguchi & Ito, 1994). Hybridisation in transplanted populations has remained a concern for Ayu management and conservation (e.g., Iguchi, Tanimura & Nishida, 1997; Iwata et al., 2007; Otake, Yamada & Uchida, 2002; Seki et al., 1994; Taniguchi, Seki & Inada, 1983). Strontium/calcium ratios of otoliths suggest that spawning of translocated Ayu from Lake Biwa represents about 20% of the total spawning of Ayu in natural spawning grounds, with a 35% hybridisation probability (Otake, Yamada & Uchida, 2002). Genetic stock identification using allozyme markers identified translocated landlocked Ayu to have mixed in populations, with a high proportion encountered during the high fishing season (Kaewsangk et al., 2000; Seki et al.,1994; Taniguchi et al., 2002), but a lower proportion in the spawning season. Studies using allozyme and mtDNA markers have concluded that released fish have little effect on the genetic characteristics of native populations (Pastene,

Numachi & Tsukamoto, 1991; Seki & Taniguchi, 1998).

The mixing proportion of the landlocked form in drifting larvae sampled in September was first estimated using single site of the mtDNA control region as the gene marker at ~25% in Sho River, Toyama Prefecture (Iwata et al., 2007). The study also estimated that mixing proportions in drifting larvae sampled in October/December and marine juveniles sampled in February/April. The proportion of larvae of the landlocked form in samples was < ~5% and/or close to 0, with 95% confidence intervals including 0 (Iwata et al., 2007), indicating that Ayu juveniles released from Lake Biwa reproduced in rivers into which they were stocked, but that the survival rate of progeny was extremely low or 0 in seawater. Experimental tests on salinity tolerance confirm the survival rates of landlocked-form larvae to be lower than those of the amphidromous form in seawater (Iguchi & Yamaguchi, 1994; Tabata & Azuma, 1986). These results have led to a general consensus among Japanese scientists and hatchery managers that the reproductive success of landlocked Ayu is very low, or even 0 in translocated rivers (Takeshima, Iguchi & Nishida, 2009). With the exception of Iwata et al. (2007), however, there is no direct evidence for the reproductive success of landlocked Ayu. No study has identified introgression of landlocked Ayu into amphidromous populations in Japan using neutral markers in the nuclear genome. Additionally, previous studies have focused on reporting the reproductive success of the landlocked form in translocated rivers, but the genetic effects of hatchery releases have received little attention.

1.4 | Study aims

Extensive translocation of landlocked Ayu has continued for at least 50 generations (Figure S2), with hatchery releases using captive-reared parents for more than 30 generations (Iguchi, Watanabe & Nishida, 1999; Ikeda, Takagi & Taniguchi, 2005). Hatchery fish include the amphidromous form, and hybrids of the amphidromous and landlocked forms. Repeat hybridisation over many generations in translocated rivers may cause introgression of genes from the landlocked form into amphidromous populations, leading to reduced fitness of stocked populations because of lowered survival of progeny of the landlocked form in seawater (Iguchi & Yamaguchi, 1994; Tabata & Azuma, 1986). Therefore, inferring hybridisation between the two forms is central to future management and conservation of this iconic species. However, there is limited information on the reproductive success of landlocked Ayu in translocated rivers.

To assist with conserving and managing Ayu, the current status of populations is assessed from a population genetics perspective, and introgression of the landlocked form into amphidromous populations is inferred throughout its Japanese distribution. Hybridisation and hybrid individuals in contact zones can be identified by Bayesian clustering methods using highly polymorphic molecular markers such as microsatellites (Beaumont et al., 2001; Manel, Gaggiotti & Waples, 2005; Pierpaoli et al., 2003; Vähä & Primmer, 2006). Using existing (published) microsatellite data, the population structure, gene flow, geographic distribution of genetic diversity, and admixture proportions of putative ancestry populations throughout Japan, are analysed. Magnitudes of introgression of landlocked Ayu throughout Japan are inferred from hybrid proportions.

2 | MATERIALS AND METHODS

2.1 | Microsatellite data

Data are sourced from a publicly available (Dryad, https://datadryad.org/stash) dataset of microsatellite genotypes for Ayu (Takeshima et al., 2016a), comprising genotype data from 12 microsatellite loci (Takeshima et al., 2016b). The overall independence of the 12 microsatellite loci was confirmed, and all loci were selectively neutral (Takeshima et al., 2016a). Deviations from Hardy–Weinberg equilibrium were observed at the *Pag-070* locus

but excluding this locus did not change results (Takeshima et al., 2016a). These data are the most suitable to address the objectives of this current study; their quality have been checked and they cover the distribution of this species throughout Japan.

Ayu samples were collected from 1999 to 2007 from 118 locations (n = 4746) (Table S1). Samples of the amphidromous form comprised upstream migrants and/or marine juveniles collected from 98 rivers/streams and or coastal waters, and individuals of the landlocked form collected from 20 rivers flowing into Lake Biwa (Takeshima et al., 2016a).

2.2 | Population structure and genetic diversity

Sample site coordinates were retrieved from original studies, but approximate coordinates for river mouths of sampling sites from Lake Biwa and Toyama Bay were obtained from Google Maps. Sample locations were plotted on a map using the 'sf' package in R. Genotype data were converted into Genepop format (Rousset, 2008) for implementation in the R package FinePop2_ver.0.2 at CRAN. Expected heterozygosity (*He*) for each population was automatically estimated in the 'read.GENEPOP' function. Genome-wide (averaged over loci) global F_{ST} values of Weir & Cockerham (1984) were computed using the 'globalFST' function. Pairwise F_{ST} values from the G_{ST} moment estimator (Nei & Chesser, 1983) were computed using the 'pop_pairwiseFST' function. Sampling locations were connected with pairwise F_{ST} values smaller than a given criterion by yellow lines to visualise gene flow. Wright's island model assumes $F_{\mathrm{ST}} \approx \frac{1}{4N_e m+1}$, where N_e is the effective population size and m is the average migration rate between populations (Slatkin, 1987). For example, a criterion of $F_{\mathrm{ST}} = 0.005$ refers to $4N_e m \approx 199$ migrants per generation (see Waples & Gaggiotti, 2006; Whitlock & Mccauley, 1999). An unrooted neighbour-joining (NJ) tree (Saitou & Nei, 1987) was created for the pairwise F_{ST} distance matrix using the 'nj' function in the R package 'ape.' The performance of the F_{ST} analysis applied here is evaluated by simulations and empirical data analysis (Kitada, Nakamichi & Kishino, 2021).

Sample points were visualised using a colour gradient of populations *He* values. The colour of population i was rendered as RGB $(1 - H_{e0,i}, 0, H_{e0,i})$, where $H_{e0,i} = (H_{e,i} - \min H_e)/(\max H_e - \min H_e)$. This conversion represents the standardised magnitude of an *He* value at the sampling point, with a continuous colour gradient ranging from blue (lowest) to red (highest) for *He* values. Populations were grouped into nine geographic areas: Hokkaido, northern Sea of Japan, western Sea of Japan, northern Pacific, central Pacific, southern Pacific, Kyushu (facing the East China Sea), and Lake Biwa. Differences in average *He* values between all pairs of the nine groups were tested by one-way ANOVA using the 'anova' function, with the p-values being adjusted for multiple comparisons with a 5% significance using the 'TukeyHSD' function in R.

2.3 | Admixture proportions

STRUCTURE (Pritchard, Stephens & Donnelly, 2000) and NEWHYBRIDS (Anderson & Thompson, 2002; Anderson, 2008) have been applied to detect hybrids in populations (Vähä & Primmer, 2006). STRUCTURE assigns probabilities for individuals to have K putative ancestry populations (individual q-values), while NEWHYBRIDS estimates the probability of individuals belonging to distinct hybrid or purebred classes. Both methods produced mostly consistent results when applying empirical data with 14 microsatellite markers (Steyer et al., 2018). Here, the Ayu dataset of 12 microsatellites was analysed using STRUCTURE. Genotype data in Genepop format were converted into STRUCTURE format using PGDSpider ver. 2.1.1.5 (Lischer & Excoffier, 2012). STRUCTURE 2.3.4 was run with no prior information about sampling locations (Pritchard, Stephens & Donnelly, 2000), assuming admixture and corelated allele frequencies between populations (Falush, Stephens &

Pritchard, 2003). A burn-in of 50,000 sweeps followed by 100,000 MCMC sweeps was used for the number of original populations K = 1–5 with 10 runs for each K. Longer runs with 500,000 MCMC sweeps following a burn-in period of 100,000 sweeps were initially tested, but very similar results were obtained, and shorter sweep length were used. The most likely K was estimated based on ln($P|D$) for each K. A threshold q-value of 0.1 (Devitt, Baird & Moritz, 2011; Fogelqvist et al., 2015; Vähä & Primmer, 2006) and/or 0.2 (Geraldes et al., 2014; Karamanlidis et al., 2021; Larison et al., 2021) has been used often to identify hybrids of two species and/or distinct populations; a 0.20 threshold q-value is used herein, where individuals with landlocked ancestral genes ($0.8 > q > 0.2$) are classified as hybrids, those with $q \geq 0.8$ as the purebred landlocked form, and those for which $q \leq 0.2$ as the purebred amphidromous form (Vähä & Primmer, 2006).

2.4 | Isolation-by-distance

Isolation-by-distance relationships between populations are examined. Great circle geographic distances between all pairs of populations were computed using the 'distm' function in the geosphere R package, and the linear relationship between geographic distances and genetic distances (as measured by pairwise F_{ST}) was estimated using the 'lm' function in R. The landlocked Ayu have been translocated form Lake Biwa into almost all rivers and major seed producers have sold hatchery-born juveniles to other prefectures (Figure S2), waypoints (Ramachandran et al., 2005; Kitada, Naka Michi & Kishino, 2017), which account for natural migration paths were not assumed. A Mantel correlation test was performed using the 'mantel' function in vegan R package.

3 | RESULTS

Sampling locations were plotted on a map (Figure S3). The global F_{ST} (± standard error) was 0.0162 (± 0.0074) for all populations, but after excluding populations from Lake Biwa, this dropped to 0.0058 ± 0.0038. The mean of pairwise F_{ST} was 0.0088 ± 0.0099 (SD) for all population pairs. Using the criterion of pairwise $F_{ST} < 0.005$ ($4N_e m \approx 199$), substantial gene flow between populations was visualised, with very high gene flow between populations and geographic areas (Figure 1a). The unrooted NJ tree (Figure 1b, Figure S4) indicates clear differences between amphidromous and landlocked forms, with a sample from Yodo River (#98) included in the landlocked cluster. Tone River (#67) was located slightly away from the amphidromous cluster in a direction towards the landlocked cluster. Hokkaido and the northern Sea of Japan group fall into one cluster, and Kyushu and the southern Pacific form into another. Populations from the western Sea of Japan, northern Pacific, central Pacific, and Seto Inland Sea were located relatively close to each other in the centre of the tree and retained regional characteristics, with a number of nested populations.

The mean ± standard deviation (SD) of *He* values was 0.66 ± 0.013, with a very narrow range between 0.62 and 0.68. No clear pattern in geographic distribution of *He* was apparent (Figure 2a). Excluding Lake Biwa (Figure 2b), *He* values were homogeneous; average *He* values in the northern and western Sea of Japan, and northern and central Pacific were significantly higher than those for Lake Biwa, while those in Hokkaido, the southern Pacific, Kyushu, and the Seto Inland Sea did not differ from those in Lake Biwa.

STRUCTURE suggests the most likely number of putative ancestral populations (K) to be 3 for all replicate runs (Figure S5). A bar plot of individual q-values for K = 3 (Figure 3a) depicts Ayu in Japan to have three common putative ancestral origins, with similar admixture patterns within amphidromous and landlocked populations. Blue and green colours refer to amphidromous ancestries, and orange to the landlocked one. The amphidromous population in Yodo River has a similar admixture pattern to that of Lake Biwa. Figure 3b depicts the geographic distribution of admixture patterns.

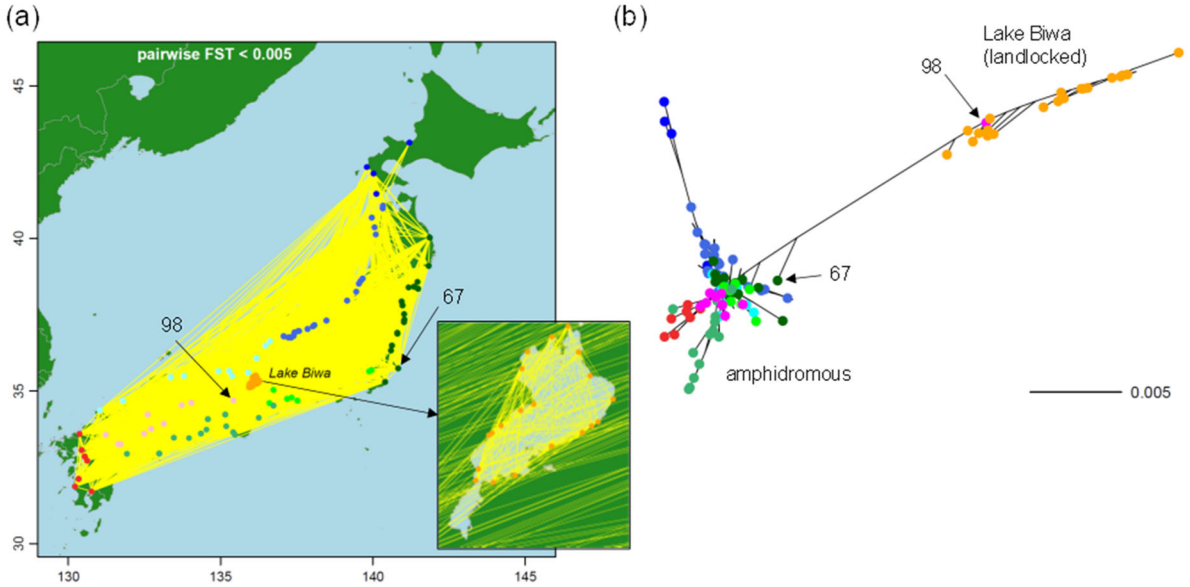

FIGURE 1 Ayu population structure throughout Japan. (a) Map visualising geneflow between populations using 12 microsatellite genotypes from 118 populations (n = 4746). Yellow lines connect population pairs with pairwise F_{ST} < 0.005. (b) Unrooted neighbour-joining (NJ) tree based on the pairwise F_{ST} matrix. Numbers: 67, Tone River, and 98, Yodo River (see Table S1 and Figure S3 for sampling information)

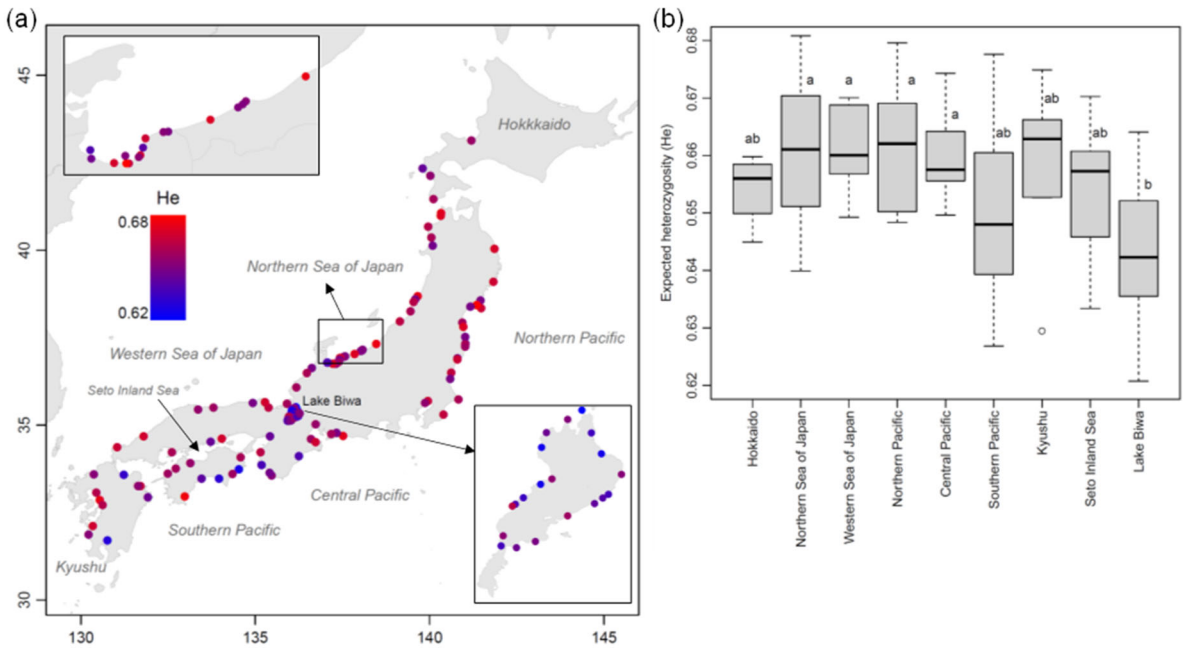

FIGURE 2 Expected heterozygosity (*He*) of Ayu throughout Japan. (a) Geographic distributions of population-specific *He* averaged over 12 microsatellite loci in 118 populations (n = 4746). The colour of each population reflects the magnitude of *He* values, with a continuous colour gradient ranging from blue to red for the lowest and highest *He* values, respectively (see text). (b) Geographical distribution of *He*. Differences between areas (p < 0.05) are indicated by different lowercase letters

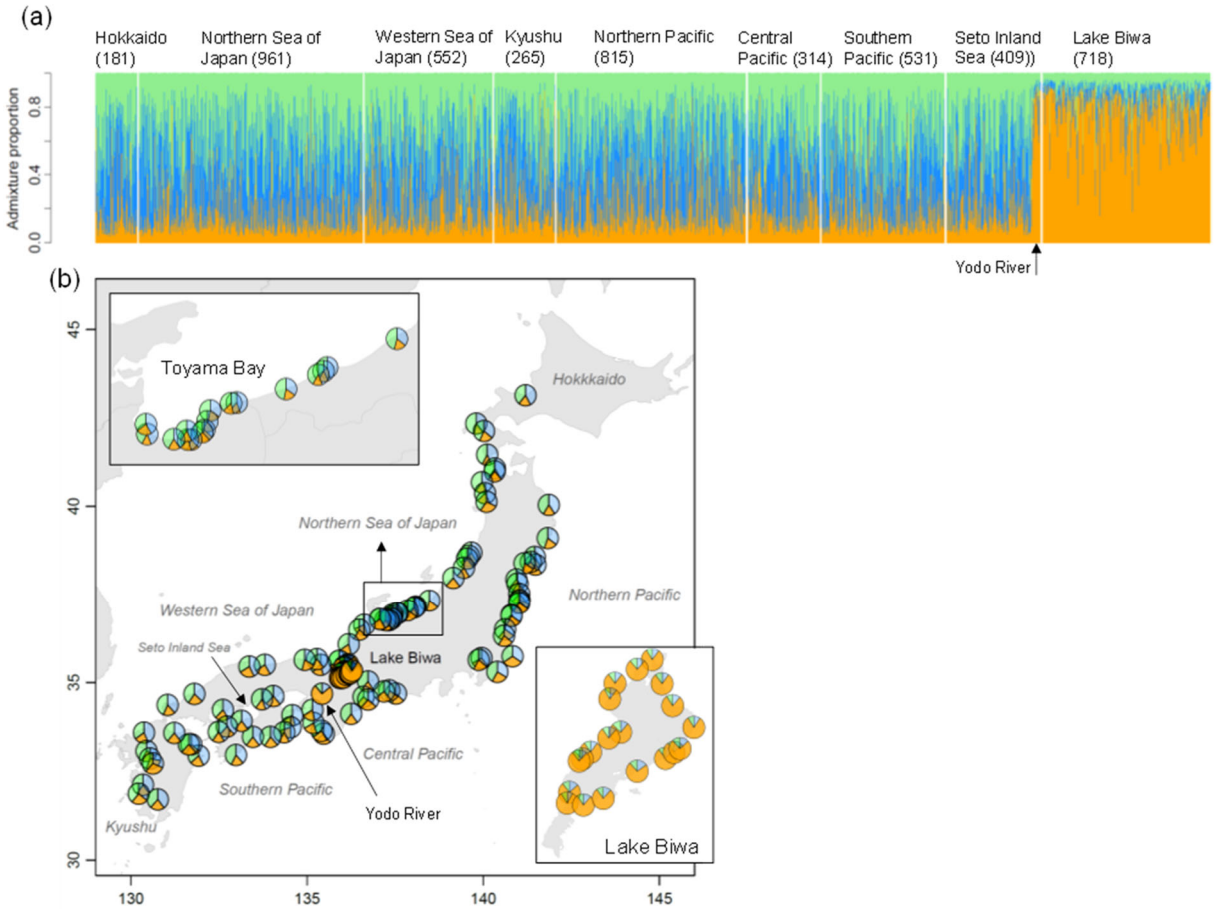

FIGURE 3 STRUCTURE analyses. (a) Coloured bars of individual *q*-values illustrate admixture proportions of inferred ancestries at $K = 3$ ($n = 4746$). Numbers in parentheses refer to sample sizes. (b) Geographic distribution of admixture proportions in 118 populations

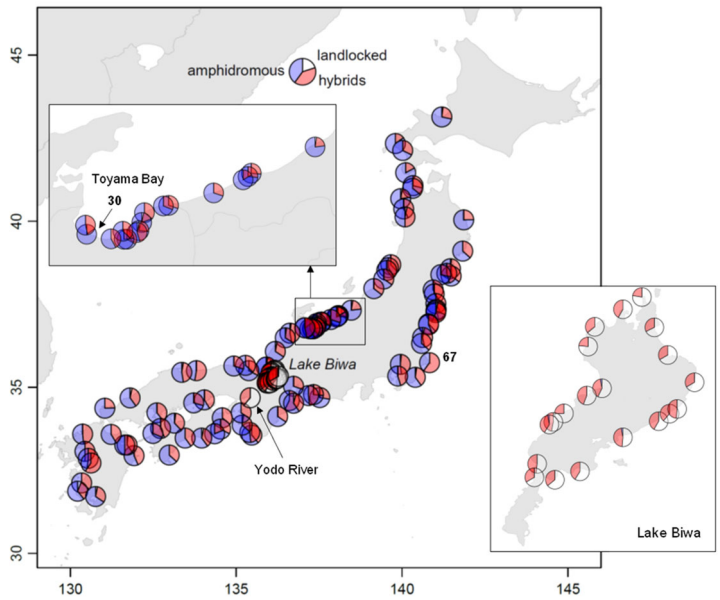

FIGURE 4 Inferred proportions of hybrid and purebred classes in 118 populations ($n = 4746$). Individuals with landlocked ancestral genes ($0.8 > q > 0.2$) ae classified as hybrids; those with $q \geq 0.8$ ae classified as purebred landlocked form, and $q \leq 0.2$ as purebred amphidromous form (Table S1). Numbers: 30, Sho River; 67, Tone River (see text)

Box plots of individual q-values of landlocked ancestry in 118 populations illustrate that amphidromous and landlocked populations to have similar patterns with outliers (Figure S6); the average of individual q-values for amphidromous populations is 0.21, and 0.79 for landlocked ones. The geographic distribution of proportions of hybrid, purebred landlocked, and amphidromous forms using the threshold q-value of 0.2 (Figure 4, Table S1) reveals hybrids to have expanded into all amphidromous populations with mean (± SD) proportion of 37% ± 10%. A maximum 60% of individuals were hybrid in Tone River (#67), and a minimum of 15% in Sho River (#30). The average proportion of purebred landlocked individuals was 1 ± 6%, and that of purebred amphidromous individuals 62 ± 12%. In Yodo River, the proportion of hybrids was 39%, and that of the purebred landlocked form 61.0%, with no purebred amphidromous individuals. A similar result was obtained for Lake Biwa, where the proportion of purebred landlocked individuals was 62 ± 8%, while that of hybrids was 38 ± 8%; similarly, no purebred amphidromous individuals were recognised.

The relationship between great circle geographic distances and genetic distances as measured by pairwise F_{ST} is depicted in Figure 5. The linear regression of F_{ST} on geographic distance produced an isolation-by-distance relationship within amphidromous populations (black dots). The Mantel correlation was significant (r = 0.32, p = 0.001), and R^2 was 0.10. Contrarily, the scatter plot (coloured dots) for Lake Biwa and amphidromous populations indicates that the linear relationship between geographic distance and genetic distance was disrupted. Many amphidromous populations in the within-amphidromous-population-comparison are overlapped by those in the between-Lake Biwa-comparison.

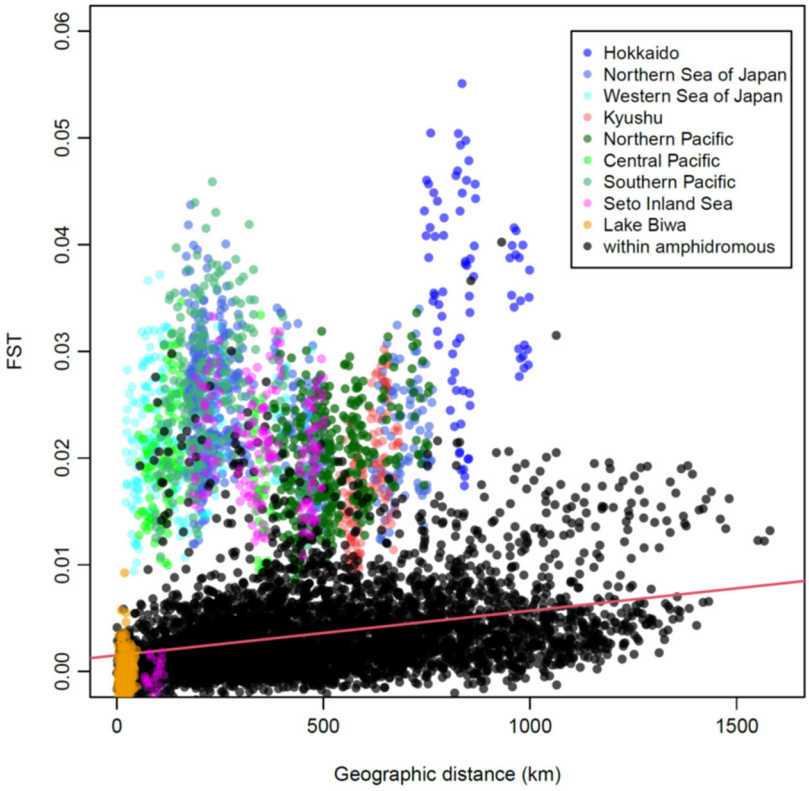

FIGURE 5 Scatterplot of geographic distance and pairwise F_{ST}. Black dots depict within-amphidromous-populations comparison, with a regression line [$\hat{F}_{ST} = 1.518 \times 10^{-3} + 4.184 \times 10^{-6} \times$ (geographic distance)] in red, with R^2 0.01; the Mantel correlation between F_{ST} and geographic distance is 0.32 (p = 0.001). Orange dots depict the samples from within the Lake Biwa population; other colours are for between Lake Biwa and amphidromous populations comparison

4 | DISCUSSION

Analysis of published microsatellite data for Ayu throughout its distribution in Japan presents strong evidence for very high gene flow between populations and regions, and for population structure to be retained in several regions, with some nested populations apparent (Figures 1, S4). Genetic diversity in terms of *He* was homogeneous (Figure 2), and admixtures of landlocked and amphidromous ancestral populations occurred throughout Japan (Figure 3). Analyses also reveal that hybrid individuals have expanded their distribution throughout Japan, with a relatively high mean hybrid proportion (37%) in populations (with mean values for the purebred amphidromous form (62%), and purebred landlocked (1%) in amphidromous populations (Figure 4, Table S1)). In the Lake Biwa population, the estimated purebred landlocked form accounts for 62% of individuals and the hybrid proportion 38%, with no purebred amphidromous individuals. The average hybrid proportion in Lake Biwa was almost the same as that of amphidromous populations. A very similar result to Lake Biwa was found in Yodo River. The linear regression of pairwise F_{ST} on geographic distance produced an isolation-by-distance relationship within amphidromous populations, but many amphidromous populations were overlapped by those in the between-Lake Biwa-comparison. Contrarily, an isolation-by-distance relationship was disrupted in the comparison of Lake Biwa and amphidromous populations.

4.1 | Population structure and effects of translocations and hatchery releases

Analysed sample data was obtained from upstream migrant and/or marine juvenile Ayu (Takeshima et al., 2016a). With the exception of the sample from Yodo River, no amphidromous population was included in the cluster of Lake Biwa, and the proportions of the purebred landlocked form were 0 in most amphidromous populations (Figures 1, 4, Table S1). This indicates that the probability of samples containing released landlocked juveniles is very low.

The population structure of Ayu was maintained in Hokkaido, the northern Sea of Japan, Kyushu, and the southern Pacific (Figure 1a), despite very high gene flow between populations and areas. Juvenile Ayu spend months at sea over winter (Nishida, 1986), and drift in sea currents and/or move to natal and adjacent river mouths (Iguchi, Konishi & Takeshima, 2006). Juveniles commence their upstream migration in spring at ~ 60 mm (Tsukamoto & Kajihara, 1987); those individuals that stray, that do not return to natal rivers, contribute to gene exchange among rivers. Natural recruitment of amphidromous Ayu depends primarily on the coastal environment, particularly that of estuaries (Tago, 2002; Takahashi et al., 1998). Long-range dispersal to offshore waters might reduce survival of marine juveniles, resulting in environmental-induced natal river fidelity (Iguchi, Konishi & Takeshima, 2006). Temporally stable and differentiated structures with high gene flow were reported for Atlantic cod (*Gadus morhua*) populations (F_{ST} = 0.0023; Knutsen et al., 2003), Atlantic herring (*Clupea harengus*) in Swedish waters (F_{ST} = 0.002–0.003; Larsson et al., 2010), and Pacific herring (*C. pallasii*) in British Columbia (F_{ST} = 0.003; Beacham et al., 2008). Results for Ayu population structure in some regions, despite the high gene flow (F_{ST} = 0.0058), are comparable to these findings for cod and herring. The isolation-by-distance relationship within amphidromous populations (Figure 5) agrees with the maintenance of population structure in several regions.

Genetic differentiation was very low in the western Sea of Japan, northern and central Pacific, and Seto Inland Sea, with many populations nested among clusters (Figure 1b). The coefficient of variation (CV) of *He* values was surprisingly low at only 2%, indicating the homogeneous distribution of *He* in Japan (Figure 2). The nested population structure, very high gene flow, and homogeneous distribution of genetic diversity, suggest that

translocated landlocked juveniles and released hatchery reared juveniles have reproduced with native amphidromous fish in Japanese rivers. The isolation-by-distance relationship (Figure 5) supports these results.

4.2 | Hybridisation between landlocked and amphidromous form

The sample from Yodo River (#98) was included in the landlocked cluster, with the proportion of hybrid and purebred landlocked individuals being very similar to that of Lake Biwa, in that there were no purebred amphidromous individuals. Upstream migration of Ayu along Yodo River, the sole outlet to flow from Lake Biwa, was interrupted by the construction of the Amagase Dam in 1954. Well-developed larvae have been supplied from Lake Biwa into this contact zone every year, and they might survive in coastal waters and return to migrate up Yodo River (Takeshima, Iguchi & Nishida, 2009). Results agree with Takeshima et al.'s speculation, in that sample #98 collected from Yodo River was supplied from Lake Biwa. Hybrid proportions (Figure 4) in Lake Biwa should reflect the demographic history of the landlocked form, which might have been most recently, naturally invaded by amphidromous individuals about 100 k years before present (Nishida, 1985).

Tone River (#67), located a short distance from the amphidromous cluster in the NJ tree (Figure 1b), had the highest hybrid proportion (60%) of all 118 amphidromous populations. Each year throughout the 1970s more than 1×10^6 landlocked Ayu juveniles were translocated from Lake Biwa into a tributary of Tone River (Takamura, 2009). The substantial catch in this area (Figure S1) suggests an appropriate estuarine environment for juvenile survival, which might enhance hybrid fitness. Marine juveniles collected in coastal waters in late March and early April (samples 37, 38) comprised 33% and 37% hybrids, respectively (Table S1), indicating that juvenile hybrids do survive at sea. In Sho River (#30), where the estimated proportion of landlocked form individuals was close to 0 (with 95% confidence intervals including 0 (Iwata et al., 2007)), the hybrid proportion was the lowest (15%) and the purebred amphidromous form was 85%. The single-site marker using the mtDNA control region could detect a maternal but not paternal effect on reproduction of the landlocked form (Iwata et al., 2007).

Pre-translocation population structure is unknown because no genetic data exist, and translocation and hatchery releases have been performed throughout Japanese rivers. Therefore, a BACI design (before-after-control impact, Smith, 2002) is not possible. However, analyses consistently explain present-day population structure. Our results suggested substantial backcrossing of landlocked and amphidromous forms throughout Japanese rivers, while purebred landlocked fish were not survived (Figure 4). Crossing experiments of hatchery and wild salmon demonstrated that most of the genes have an additive effect and showed intermediate gene expression (Christie et al., 2016). The hybrid Ayu produced from hatchery-reared and wild fish had intermediate susceptibility to bacterial cold-water disease (Nagai & Sakamoto, 2006). However, the genetic background of gene transcription looked to be largely nonadditive (Gibson et al., 2004; Hedgecock et al., 2007; Roberge et al., 2008). Hybrid individuals can have nonadditive inheritance of quantitative traits, such as outbreeding depression (Gilk et al., 2004) and heterosis (Lippman & Zamir, 2007). The effect of hybridisation on genes controlling fitness traits of Ayu is unknown, but nation-wide hybridisation between landlocked and amphidromous forms (Figure 4) should reduce fitness of populations in Japanese rivers.

4.3 | Implications for conservation and management

Translocation of landlocked Ayu may reduce fitness of amphidromous populations because of their lower fitness. Therefore, an important action to ensure the sustainability of this freshwater species would be to avoid the use of translocated landlocked Ayu juveniles to stock

rivers.

In hatcheries, parent fish have been captive-reared for generations, mainly because of difficulties experienced collecting sufficient mature wild fish for synchronous spawning each year (Iguchi & Mogi, 2007). Consequently, genetic diversity of hatchery-born juveniles has decreased over generational (4–31) time (Iguchi, Watanabe & Nishida, 1999; Ikeda, Takagi & Taniguchi, 2005; Tsuboi et al., 2019). Continued release of captive-reared juveniles could reduce the effective population size of the entire population (hatchery + wild) and replace wild genes (Ryman & Laikre, 1991). Moreover, evidence exists for reduced reproductive success of hatchery-reared salmonids when they breed in the wild (Araki, Cooper & Blouin, 2007; Fleming et al., 2000; McGinnity et al., 2003; Christie, Ford & Blouin, 2014). Field experiments (Tsuboi et al., 2019) found that hatchery-reared Ayu born from captive-reared parents for more than seven generations had low survival, maladapted behaviour of migration, and poor vulnerability to fishing when they were released in the wild. Apparently, reducing hatchery releases is crucial to reduce genetic effects of hatchery releases (Waples & Drake, 2004). As recommended by Takeshima and colleagues (2016a), translocation of hatchery reared juveniles should also be limited within regions.

Because the migration range of marine juveniles is expected to be limited, individual rivers might be inappropriate management units. Rivers or areas where there was no release of landlocked or hatchery-reared Ayu should be established within each prefecture to limit gene flow and dilute any genetic effects of translocations and hatchery releases through backcrosses with wild individuals (Anderson, 1949; McGinnity et al., 2003; Hindar et al., 2006; Tymchuk et al., 2006; Roberge et al., 2008; Kitada et al., 2019). Healthy stocks of wild fish are necessary for no-release rivers and/or areas to succeed as a management option. Fortunately, wild fish genes remain dominant in Ayu (Figures 4). Suitable spawning and nursery habitat is essential for reproduction of marine and freshwater species. A rigorous monitoring of red sea bream (*Pagrus major*) demonstrated that both increased seaweed communities in nursery habitat and reduced fishing effort were primarily responsible for wild population recovery, while the effects of hatchery released fishes decreased because of the exponentially reduced survival rates of juveniles produced from captive-reared parents (Kitada et al., 2019). Releases of hatchery reared juveniles may be useful for short-term management objectives, but management of spawning escapement, and improving spawning and nursery habitat are the keys for the long-term sustainability of fishery resources in Japan (Kitada, 2020). Management began by the Shubutogawa Fishers Cooperatives, Hokkaido, where Ayu juveniles had been translocated from Honshu since 1984; this practice was stopped in 2013 to avoid genetic risks to the conservation of this species at its northern margin. Efforts to create and/or restore spawning grounds in this river have continued in collaboration with fishers and locals (https://www.shubutogawa.com/).

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CONFLIFT OF INTEREST

The author has no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

All source data used are in the public sector, and links to their online sources are specified in the text or in Supporting information.

ORCID

Shuichi Kitada http://orcid.org/0000-0001-5838-0374

SUPPORTING INFORMATION

Additional Supporting Information may be found in the Supporting Information section at the end of this article.

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