Title:

Long-term translocation and hatchery release explain population genetic structure of a recreationally fished iconic species in Japan: A review and reanalysis

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Short running title: Effects of long-term translocation

Abstract

Ayu (*Plecoglossus altivelis altivelis*) occur widely in China, Korea, Japan, Taiwan and Vietnam. It is an important freshwater fisheries resource and popular recreational fishing species in Japan, lives for only one year and has a single breeding season. To supplement increased recreational fishing demand, huge numbers of wild-born landlocked juvenile have been translocated from Lake Biwa into most Japanese rivers for more than 50 generations. Hatchery-born juveniles have also been extensively released for more than 30 generations. Previous studies reported that landlocked and amphidromous forms of Ayu easily hybridise, but survival of landlocked larvae is very low in seawater, leading to a general consensus that the reproductive success of landlocked Ayu is very low, or even 0 in translocated rivers. However, there is limited information on the reproductive success of landlocked Ayu in translocated rivers and no study evaluated effects of translocation and hatchery release on genetic population structure in the distribution range. To provide an up-to-date review on the key management question, I performed a comprehensive literature search and analysed published microsatellite data sets collected from the distribution of this species in Japan. The analyses provide strong evidence for very high gene flow between populations, but population structure was retained in several regions, and several populations were nested. Genetic diversity was surprisingly homogeneous. Bayesian admixture analysis estimated hybrid proportion between both forms to be 37%, uncovered widespread hybridisation in Japanese rivers. Results are discussed in relation to the conservation and management of this species.

Keywards: amphidromous, Ayu, hatchery, hybrid proportion, landlocked, Lake Biwa

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). Hybridisation could also be beneficial to species adaptation in a changing environment, so a case-by-case evaluation is needed to elucidate its consequences (Hirashiki *et al.* 2021).

Translocation of organisms across geographic boundaries has been often used in conservation and management of wildlife; however, because it removes reproductive barriers, and can lead to hybridisation of introduced and resident populations (Seddon *et al.* 2014; Berger-Tal *et al.* 2020; 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 (Ricciardi & Simberloff 2009; Bucharova 2017). In endangered fish recovery plans, translocations are prevalent, but consequences are often poorly documented (Healy *et al.* 2020). Quantitative evaluation on empirical cases is needed to determine their conservation efficacy and future management efforts (Minckley 1995; George *et al.* 2009; Healy et al. 2020).

Herein, a case study on Ayu (*Plecoglossus altivelis altivelis*), which occur widely in coastal areas of China, Korea, Japan, Taiwan (Kwan *et al.* 2012), and Vietnam (Tran *et al.* 2017). Ayu is 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 well studied fish species in the country. Landlocked Ayu have been extensively translocated from Lake Biwa into amphidromous populations in rivers throughout Japan since 1970s. In addition, hatchery-born amphidromous fish (including amphidromous and landlocked form hybrids) have been extensively released. Below I briefly review the knowledge of the species and describe the aim of the present study.

Fisheries, translocation of landlocked juveniles, and hatchery supplementation

Ayu 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). The fishing season for ayu begins on June 1st and closes at the end of October in this river, which is a typical fishing period in Japan.

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 local 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 *et al.* 1991; Seki & Taniguchi 1998; Tago 2004; Takeshima *et al.* 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 (hatchery fish) are produced in Tochigi Prefecture, followed by Miyazaki, Tokushima, Yamaguchi, and Wakayama prefectures, implying that landlocked juveniles and hatchery fish have been translocated to non-natal rivers.

Life history

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 1958; 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 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. Taniguchi *et al.* 1983; Seki *et al.* 1994; Iguchi *et al.* 1997; Otake *et al.* 2002; Iwata *et al.* 2007). 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 *et al.* 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 (Seki *et al.*1994; Kaewsangk *et al.* 2000; Taniguchi *et al.* 2002), but a lower proportion in the spawning season. Studies using allozyme and mtDNA markers have concluded that released landlocked Ayu have little effect on the genetic characteristics of native populations (Pastene *et al.* 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 (Tabata & Azuma 1986; Iguchi & Yamaguchi 1994), though salinity tolerance of hybrids between both forms has not been tested. 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 *et al.* 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. Thus, translocations and hatchery releases of Ayu have regarded as put-and-take fisheries (e.g. Pawson MG 1982; Ross & Loomis 2001; Lorenzen et al. 2012; Beard & Mann 2020), which provide intra-generational benefits to Ayu commercial and recreational fisheries during early summer and fall (Tsuboi *et al.* 2019). However, inter-generational genetic effects of translocations and hatchery releases and its risk assessment have received little attention (see, e.g. Waples & Drake 2004; Araki & Schmid 2010; Laikre *et al.* 2010; Lorenzen et al. 2012; Christie *et al.* 2014; Taranger *et al.* 2015; Glover *et al.* 2017; Kitada 2018, references therein).

Study aims

Extensive translocation of landlocked Ayu has continued for at least 50 generations (Figure S1b), with hatchery releases using captive-reared parents for more than 30 generations (Iguchi *et al.* 1999; Ikeda *et al.* 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 (Tabata & Azuma 1986; Iguchi & Yamaguchi 1994). 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 and no study inferred hybridisation in translocated rivers. Moreover, no study evaluated effects of translocation and hatchery release on genetic population structure in the distribution range.

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; Pierpaoli *et al.* 2003; Manel *et al.* 2005; 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. Using a Bayesian clustering method, magnitudes of introgression of landlocked Ayu are inferred from hybrid proportions. Results are discussed in relation to the future conservation and management of this species.

Materials and Methods

Genetic data screening

Published genetic data were screened using the Google Scholar search system with keyword searches of “Ayu”, “genetic diversity”, “landlocked”, “*Plecoglossus*” and “population structure”. Studies that were known to us were also added. The population genetic structure of Ayu has been studied extensively in Japan. Initially (in the 1980s), molecular markers

included allozymes (Taniguchi *et al.* 1983; Nishida 1985; Seki *et al.* 1988), but with technological developments this expanded from the late 1990s to include mitochondrial DNA (mtDNA) (Iguchi *et al.* 1997; Iguchi & Nishida 2000; Iguchi *et al.* 2002; Ikeda & Taniguchi 2002; Takeshima *et al.* 2005) and microsatellite markers (Takagi *et al.* 1999; Ikeda *et al.* 2005; Yamamoto *et al.* 2007; Takeshima *et al.* 2009; Yamaguchi *et al.* 2016) 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 polymorphism (SNPs).

The data screening found that it is the only publicly available one and covered the distribution range. In this study, the genotypes of Ayu at 12 microsatellite markers collected throughout Japan were analysed (118 populations, *n*=4,746) (Takeshima *et al.* 2016a,b). 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. The overall independence of the 12 microsatellite loci was confirmed, and all loci were selectively neutral, and 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. Two samples of Ryukyu Ayu (*P. a. ryukyuensis*) were excluded from the data in the subsequent analysis. In addition, previous results using the same 12 microsatellite markers for different samples of upstream migrants in the Yodo River system were analysed, which is the sole outlet to flow from Lake Biwa and the contact zone (Takeshima *et al.* 2009).

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_v0.2 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 $F_{\mathrm{ST}} = 0.005$ corresponds to $4N_e m \approx 199$ migrants per generation (see Whitlock & Mccauley 1999; Waples & Gaggiotti 2006). 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 *et al.* 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.

Admixture proportions

STRUCTURE (Pritchard *et al.* 2000) and NEWHYBRIDS (Anderson & Thompson 2002) 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 v2.1.1.5 (Lischer & Excoffier 2012). STRUCTURE 2.3.4 was run with no prior information about sampling locations (Pritchard *et al.* 2000), assuming admixture and corelated allele frequencies between populations (Falush *et al.* 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 (Vähä & Primmer 2006; Devitt *et al.* 2011; Fogelqvist *et al.* 2015) 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).

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 S2b), waypoints (Ramachandran *et al.* 2005; Kitada *et al.* 2017), which account for natural migration paths were not assumed. A Mantel correlation test was performed using the 'mantel' function in vegan R package.

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).

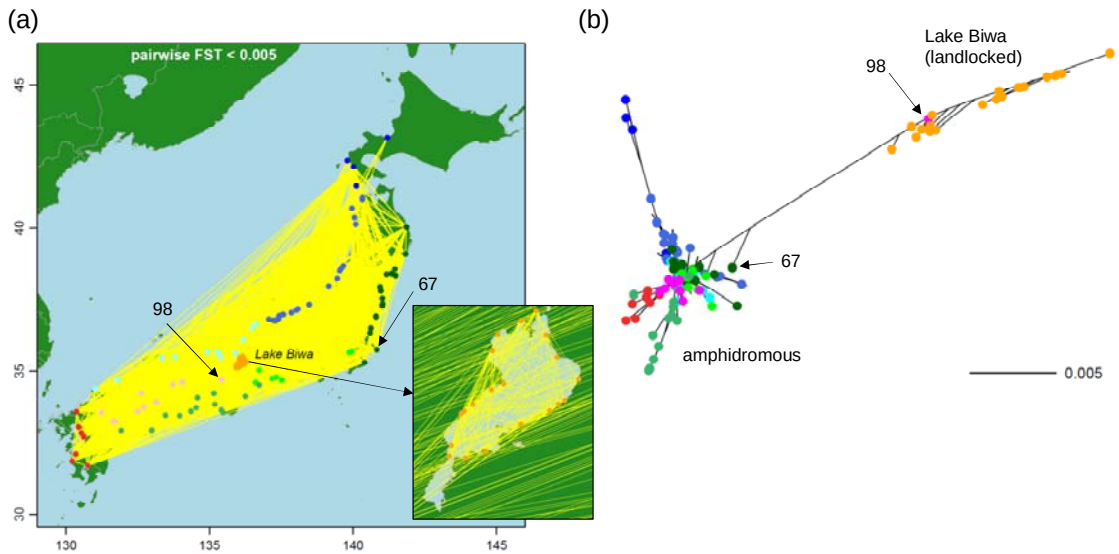

Figure 1 Ayu population structure throughout Japan. (a) Map visualising geneflow between populations using 12 microsatellite genotypes from 118 populations (n = 4746). Yellow lines connect population pairs with pairwise F_{ST} < 0.005. (b) Unrooted neighbour-joining (NJ) tree based on the pairwise F_{ST}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

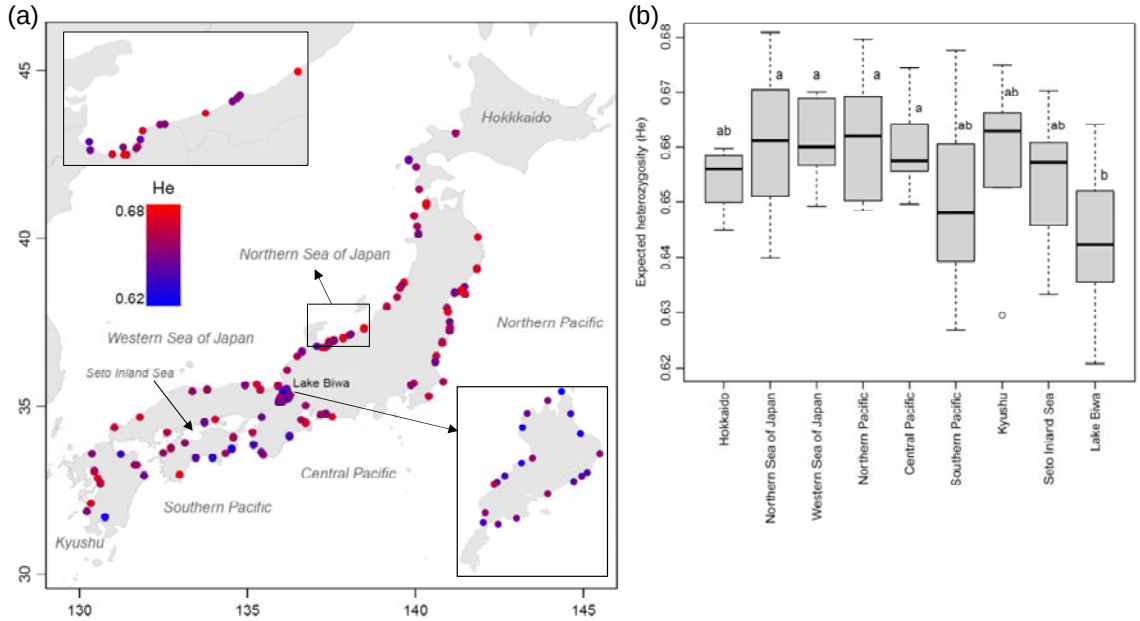

Figure 2 Expected heterozygosity (*He*) of Ayu throughout Japan. (a) Geographic distributions of population-specific *He* averaged over 12 microsatellite loci in 118 populations (n = 4746). The colour of each population reflects the magnitude of *He* values, with a continuous colour gradient ranging 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

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. Contrarily, the NJ tree (Figure S5) created using the F_{ST} matrix (Takeshima *et al.* 2009) located upstream migrants between Lake Biwa and amphidromous populations, being consistent with the original study.

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, *He* values were homogeneous (Figure 2b); 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.

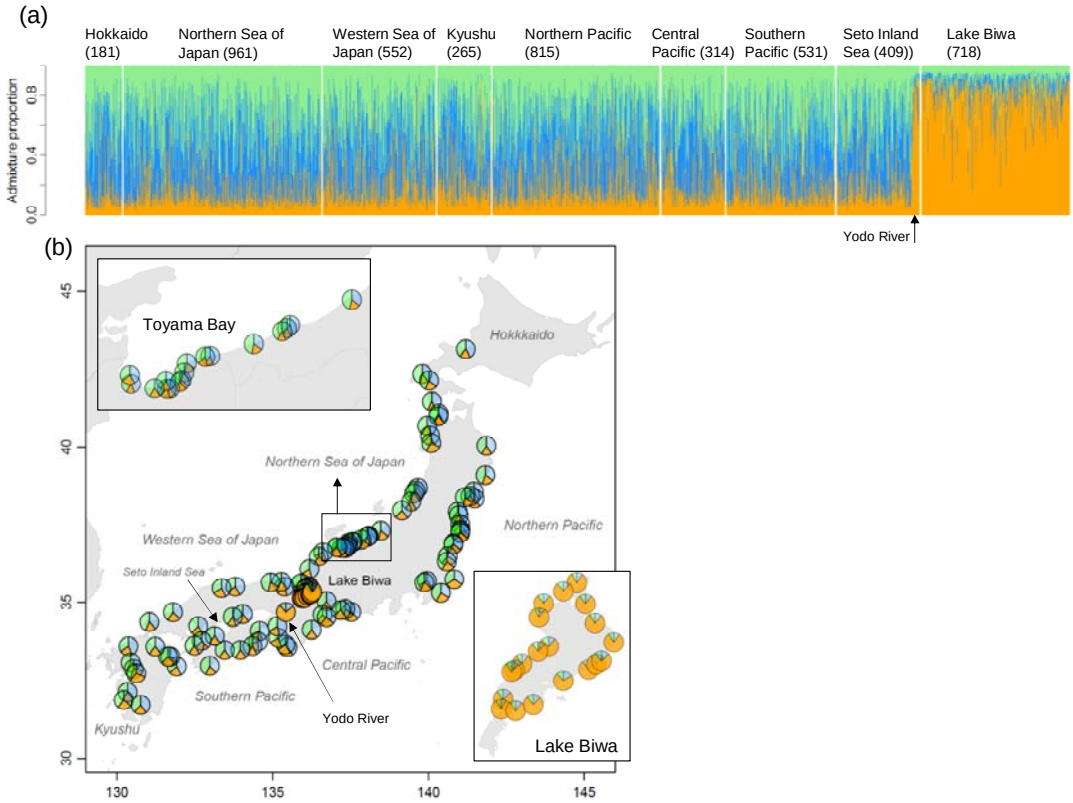

Figure 3 STRUCTURE analyses. (a) Coloured bars of individual *q*-values illustrate admixture proportions of inferred ancestries at $K = 3$ ($n = 4746$). Numbers in parentheses refer to sample sizes. (b) Geographic distribution of admixture proportions in 118 populations

STRUCTURE suggests the most likely *K* to be 3 for all replicate runs (Figure S6). 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 putative 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. 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 S7); 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.

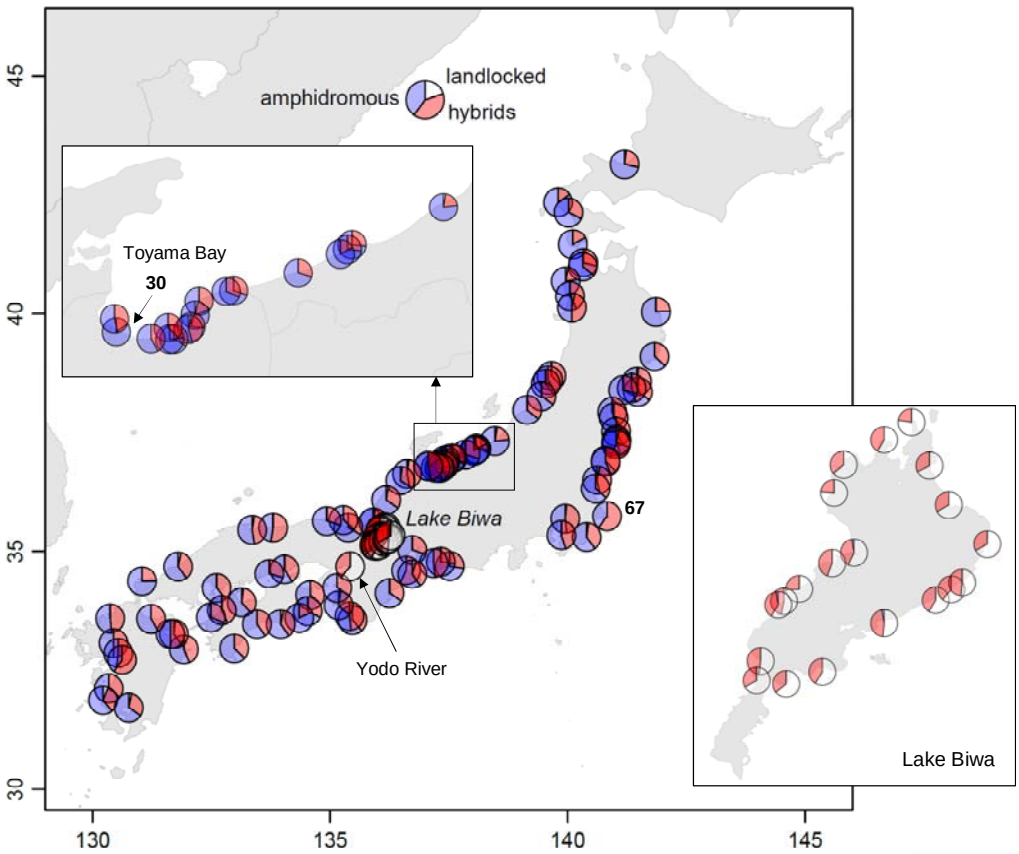

Figure 4 Inferred proportions of hybrid and purebred classes in 118 populations (n = 4746). Individuals with landlocked ancestral genes ($0.8 > q > 0.2$) ae classified as hybrids; those with $q \geq 0.8$ ae classified as purebred landlocked form, and $q \leq 0.2$ as purebred amphidromous form (Table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

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.

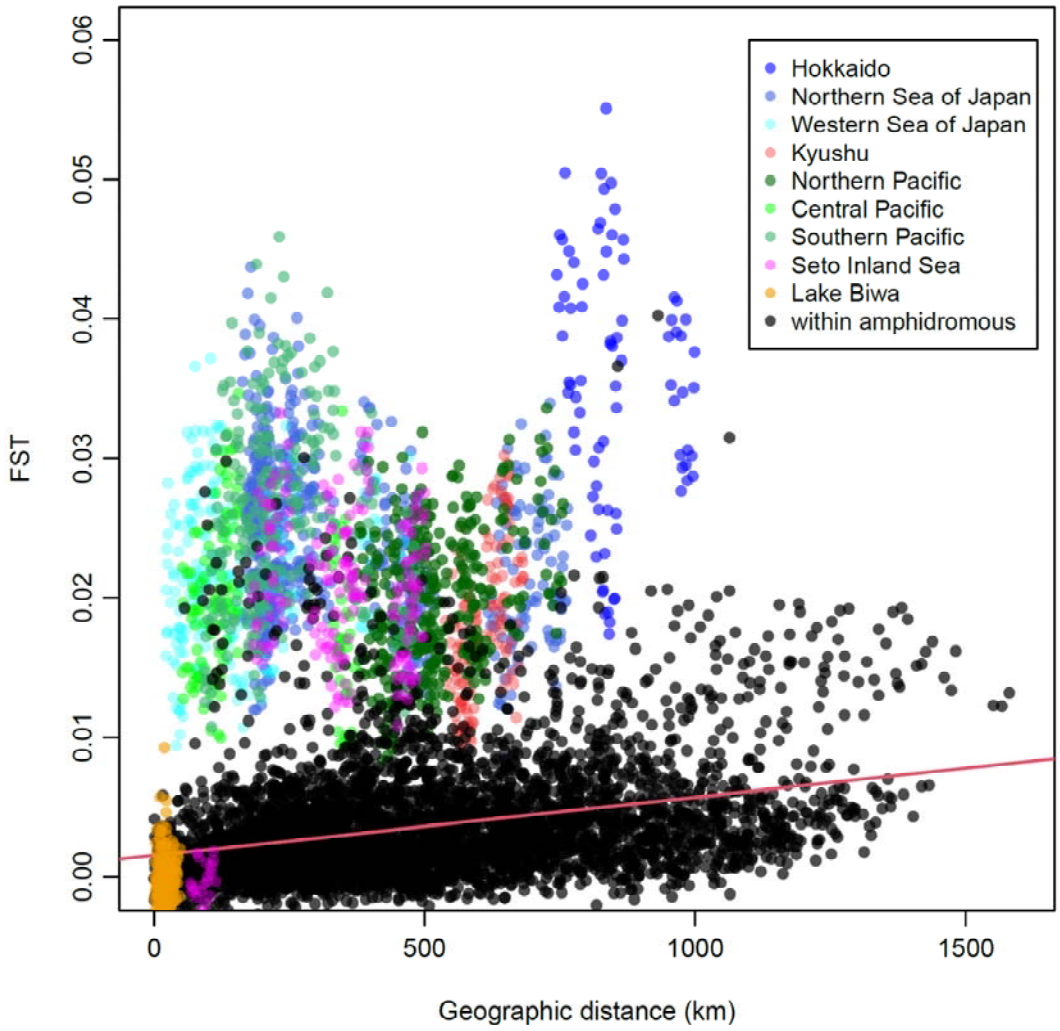

Figure 5 Scatterplot of geographic distance and pairwise F_{ST}. Black dots depict within-amphidromous-populations comparison, with a regression line [$\hat{F}_{ST} = 1.518 \times 10^{-3} + 4.184 \times 10^{-6} \times$ (geographic distance)] in red, with R^2 0.01; the Mantel correlation between F_{ST} 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

Discussion

Reanalysis 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 surprisingly 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. 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.

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 *et al.* 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 (Takahashi *et al.* 1998; Tago 2002). Long-range dispersal to offshore waters might reduce survival of marine juveniles, resulting in environmental-induced natal river fidelity (Iguchi *et al.* 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 (Figures 1b, S4). 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 hatchery reared juveniles have reproduced with native amphidromous fish in Japanese rivers. The isolation-by-distance relationship (Figure 5) supports these results.

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 *et al.* 2009). Results agree with Takeshima et al.'s speculation, in that

sample #98 collected from Yodo River was supplied from Lake Biwa. However, the unrooted NJ tree based on different samples (Figure S5) located samples of upstream migrants between Lake Biwa and amphidromous populations, suggesting hybridization occurred in the Yodo River system. Contrarily, 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). mtDNA plays a key role in aerobic ATP production and the ability for endurance exercise training (reviewed by Stefàno *et al.* 2019), and the mtDNA control region functions in dNTP metabolism (Nicholls & Minczuk 2014) and oxygen consumption (Kong *et al.* 2020). Non neutral mtDNA control region might affect their mixing proportion estimates.

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, because pure landlocked Ayu show very low survival in seawater (Tabata & Azuma 1986; Iguchi & Yamaguchi 1994).

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 *et al.* 1999; Ikeda *et al.* 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 (Fleming *et al.* 2000; McGinnity *et al.* 2003; Araki *et al.* 2007; Christie *et al.* 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. 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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Conflict 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.

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Supporting Information

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

Table S1 Ayu sampling locations and dates, and expected heterozygosities and hybrid proportions
Figure S1 Ayu catch and release statistics in Japan
Figure S2 Juvenile release and production statistics
Figure S3 Ayu sampling locations throughout Japan
Figure S4 Unrooted neighbour-joining (NJ) trees for Ayu in Japan
Figure S5 Unrooted neighbour-joining (NJ) trees for Ayu in the Yodo River system
Figure S6 Results from STRUCTURE analysis for optimal *K*
Figure S7 Distribution of individual *q*-values of landlocked ancestry in 118 populations