This PDF file include three sets of Supporting Information (A-C).

A. Supplemental Figures S1–S20

B. Supplemental Discussion

- 1. GnRH and straying of hatchery fish
- 2. Reproduction
- 3. Immune system and bacterial diseases
- 4. Parasites and DNA damage repair
- 5. Growth
- 6. Athletic performance and metabolic efficiency of Japanese chum salmon References

C. Supplemental Note: The Japanese chum salmon hatchery propagation program

- 1. Release of juveniles
- 2. Returning chum salmon; stray and gene flow
- 3. Seed production
- 4. Migration of released juveniles References

Supplemental Tables are given in an Excel file.

D. Supplemental Tables S1–S10

A. Supplemental Figures S1–S20

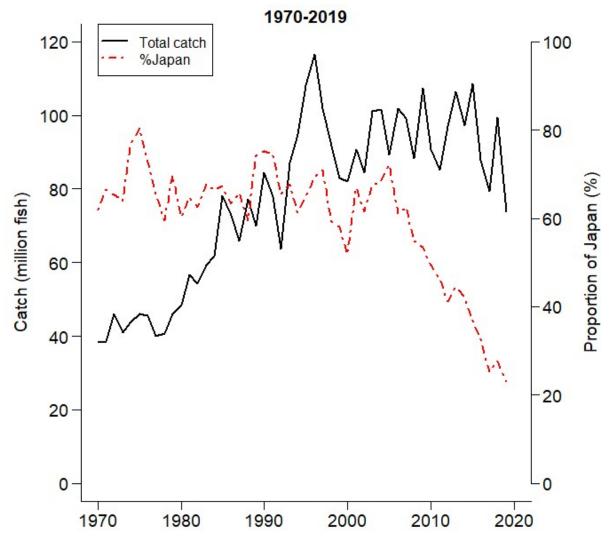


Figure S1 Changes in the total catch of chum salmon in the North Pacific and the proportion in Japan. Data from the North Pacific Anadromous Fish Commission, www.npafc.org, accessed July 2020 (NPAFC, 2020).

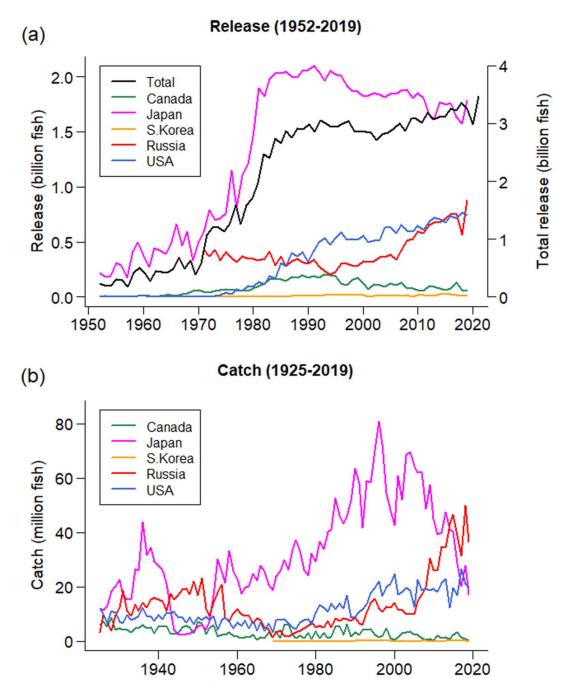


Figure S2 Changes in chum salmon releases and catches by country in the North Pacific. (a) Number of released juveniles (1952–2019). (b) Commercial catches (1925–2019). Updated from a previous study (Kitada, 2018). Data from the North Pacific Anadromous Fish Commission, www.npafc.org, accessed July 2020 (NPAFC, 2020).

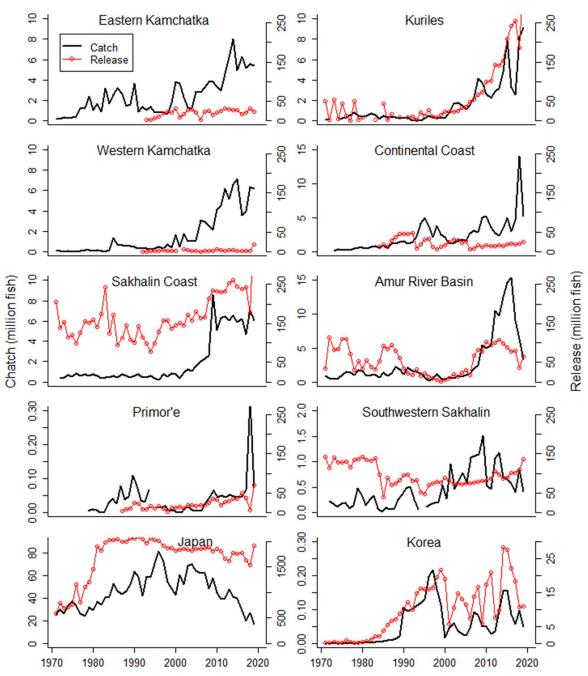


Figure S3 Changes in the number of caught and released chum salmon in Russian areas, Japan and Korea (1971–2019). Data from the North Pacific Anadromous Fish Commission, www.npafc.org, accessed July 2020 (NPAFC, 2020).

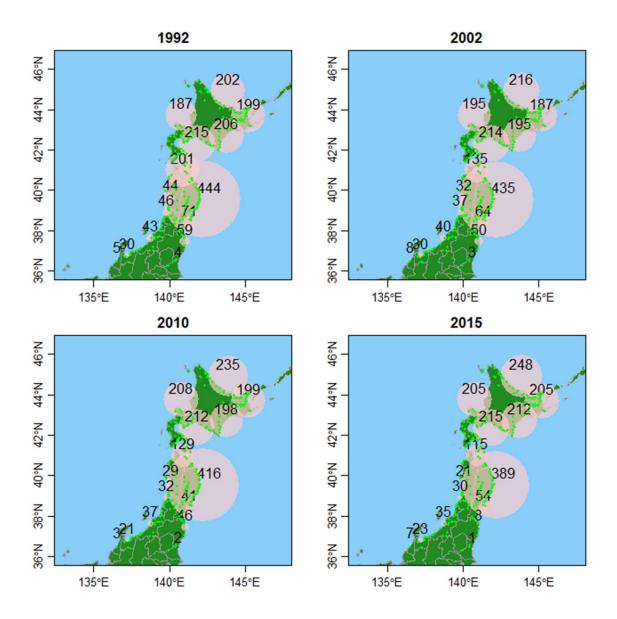


Figure S4 Changes in the number of released chum salmon in Japan during recent decades. Numbers of released chum salmon (in millions) are shown for 1992, 2002, 2010 and 2015. Circle sizes are proportional to the number of salmon. The four release years are 4 years before the catch years reported in Figure 2a. Green dots show hatcheries. Data are given in Table S2.

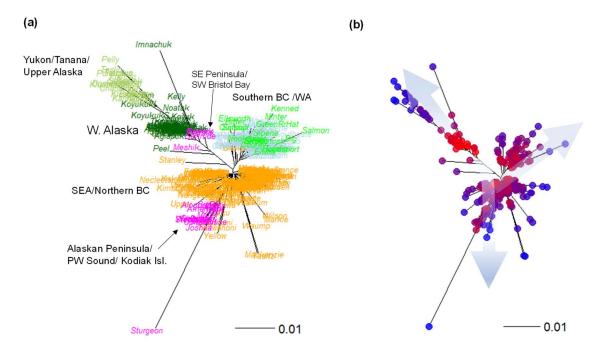


Figure S5 Chum salmon population structure in North America. (a) Unrooted NJ tree based on pairwise F_{ST} values estimated from 14 microsatellite loci of 319 populations (n = 45,623) (Beacham, Sato, Urawa, Le, & Wetklo, 2008). The tree indicates that chum salmon originated in western Alaska, where *He* values are the highest, and then expanded to the Yukon (Canada), the Alaskan Peninsula, SEA/Northern BC and southern BC/WA. (b) Unrooted NJ tree overlaid with *He* values. The color of each population reflects the magnitude of *He* values. Arrows show inferred directions of population expansion. Sampling locations and *He* values are given in Supporting Information Table S4. See Figure 1 in Beacham et al. (2009) for a map of sampling locations.

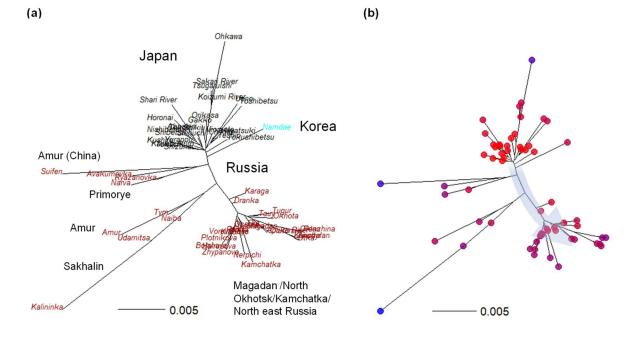


Figure S6 Chum salmon population structure in Asia. (a) Unrooted NJ tree based on pairwise F_{ST} values estimated from 14 microsatellite loci of 62 populations (n = 5,732) (Beacham, Sato, Urawa, Le, & Wetklo, 2008) showing three regional groups: Japan/Korea, southern Russia (Amur/Primorye/Sakhalin) and northern Russia (Magadan /Northern Sea of Okhotsk/Kamchatka/Northeast Russia). (b) Unrooted NJ tree overlaid with *He* values. The color of each population reflects the magnitude of *He* values. The arrows show inferred directions of population expansion. Sampling locations and *He* values are given in Supporting Information Table S4. See Figure 1 in Beacham et al. (2009) for a map of sampling locations.

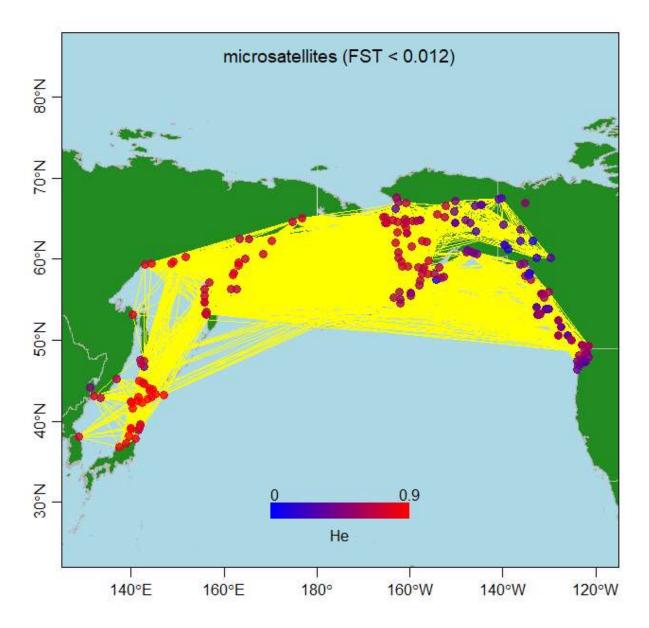


Figure S7 Visualization of genetic diversity and population connectivity based on 14 microsatellite markers. Populations connected by yellow lines are those with pairwise $F_{ST} < 0.012$. The color of each sampling point is proportional to the level of *He*. Data are given in Table S4.

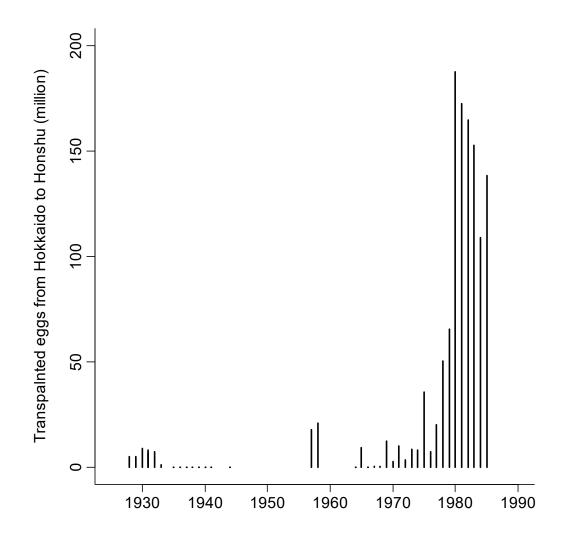


Figure S8 Number of fertilized eggs of chum salmon transplanted from hatcheries in Hokkaido to rivers in Honshu, 1928–1985. Data are given in Table S5.

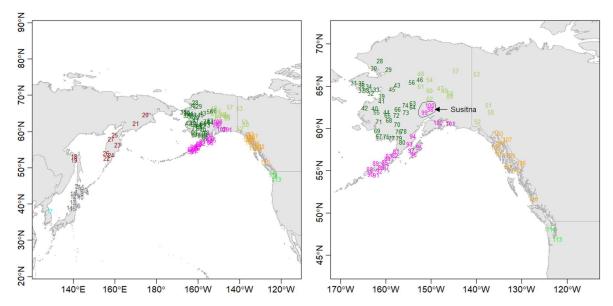


Figure S9 Sampling locations for chum salmon SNP genotyping. The entire area and North America are shown. Samples were collected in 1989–2006 from 114 populations across the Pacific Rim (n = 10,458). Data from Seeb et al. (2011) and are given in Supporting Information Table S6.

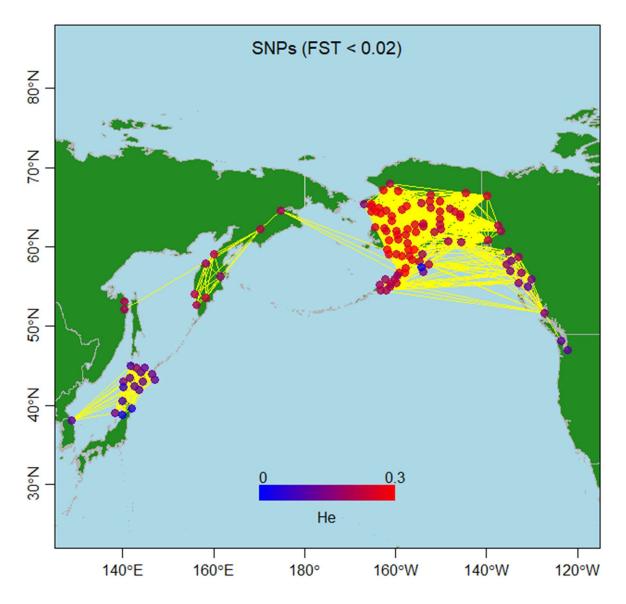


Figure S10 Visualization of genetic diversity and population connectivity based on 53 microsatellite markers. Populations connected by yellow lines are those with pairwise $F_{ST} < 0.02$. The color of each sampling point is proportional to the level of *He*. Data are given in Table S6.

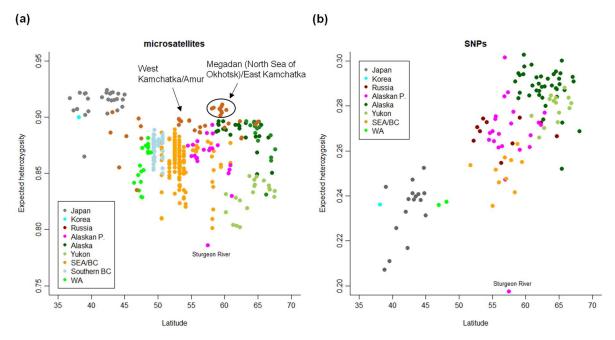


Figure S11 Latitude vs expected heterozygosity (*He*) for (a) 14 microsatellites and (b) 53 SNPs ($r = 0.77, t = 12.7, df = 112, p < 2.2 \times 10^{-16}$). Data are from (a) Beacham et al. (2009) and (b) Seeb et al. (2011) and *He* values are given in Tables S4 and S6.

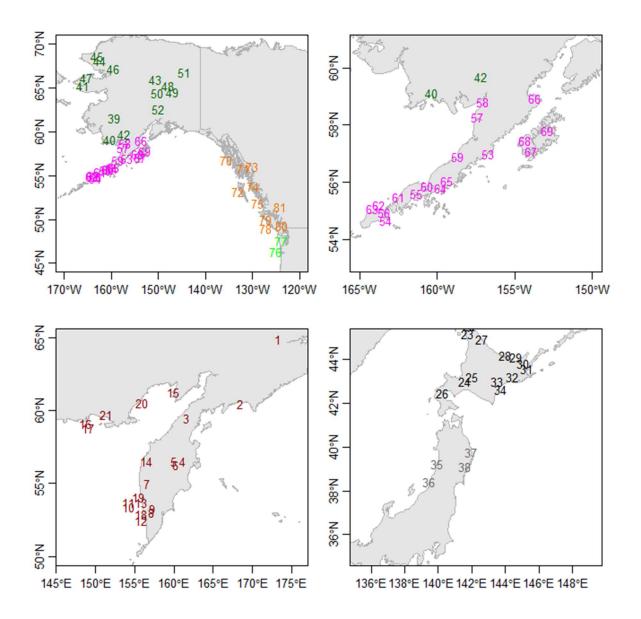


Figure S12 Sampling locations for determination of chum salmon allozyme allele frequencies. Samples were collected from 81 locations from 1976 to 1994. Data are from Winans et al. (1994) and Seeb et al. (1995) and are given in Table S7.

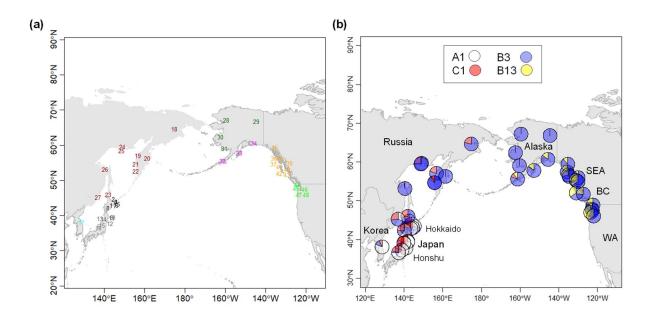


Figure S13 mtDNA D-loop haplotyping of 48 chum salmon populations. (a) Locations of samples collected from 1990 to 2000 (n = 2,162). (b) Distribution of mtDNA D-loop major haplotype frequencies. Data are from Sato et al. (2004) and are given in Table S8.

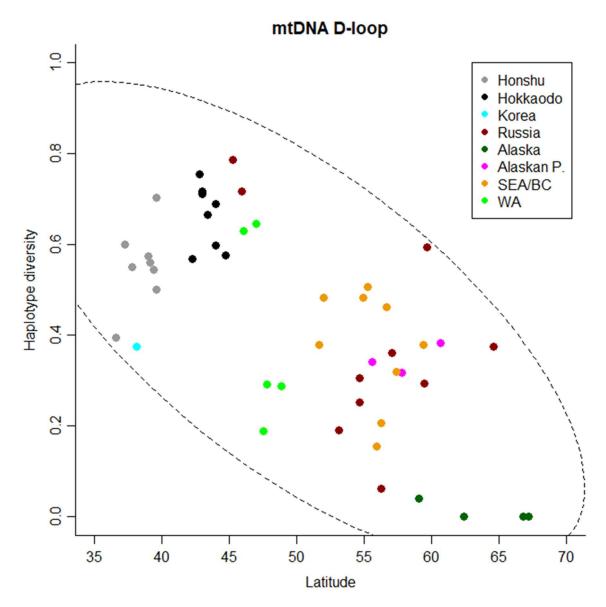


Figure S14 Latitudinal cline in haplotype diversity of the mtDNA D-loop region ($r = -0.68, t = -6.3, df = 46, p = 9.3 \times 10^{-8}$). Each point corresponds to a sampling location in Figure S13. The dotted circle shows 95% confidence ellipse.

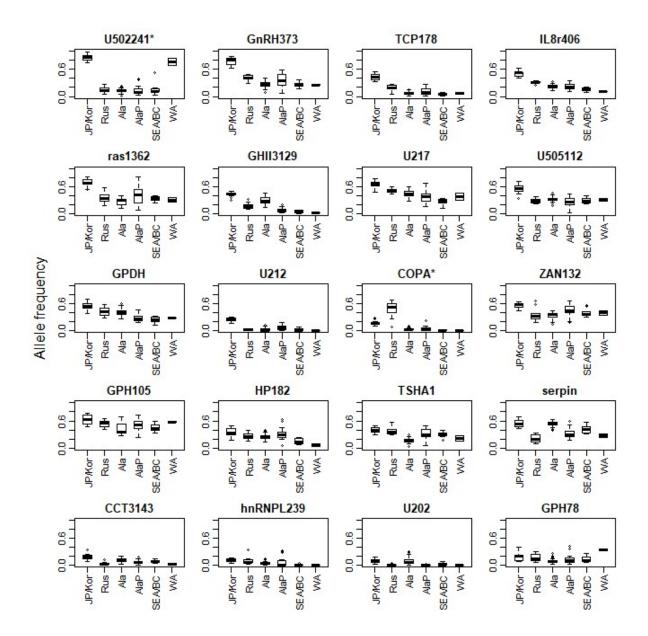


Figure S15-1 Boxplots of allele frequencies in six geographical areas. All markers shown are ordered according to the magnitude of the eigenvectors of PC1.

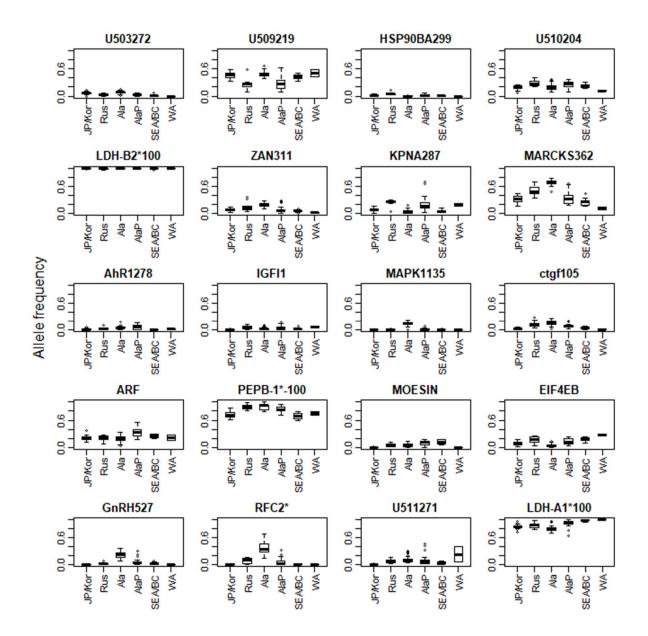


Figure S15-2 Boxplots of allele frequencies in six geographical areas. All markers shown are ordered according to the magnitude of the eigenvectors of PC1.

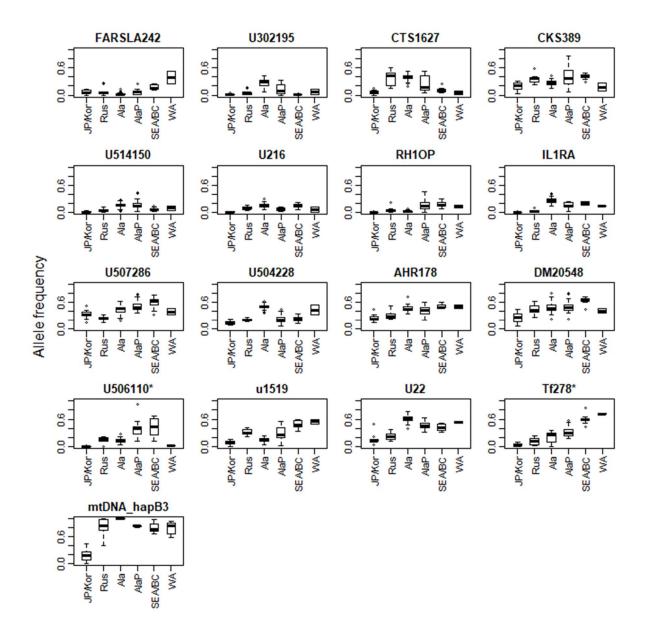


Figure S15-3 Boxplots of allele frequencies in six geographical areas. All markers shown are ordered according to the magnitude of the eigenvectors of PC1.

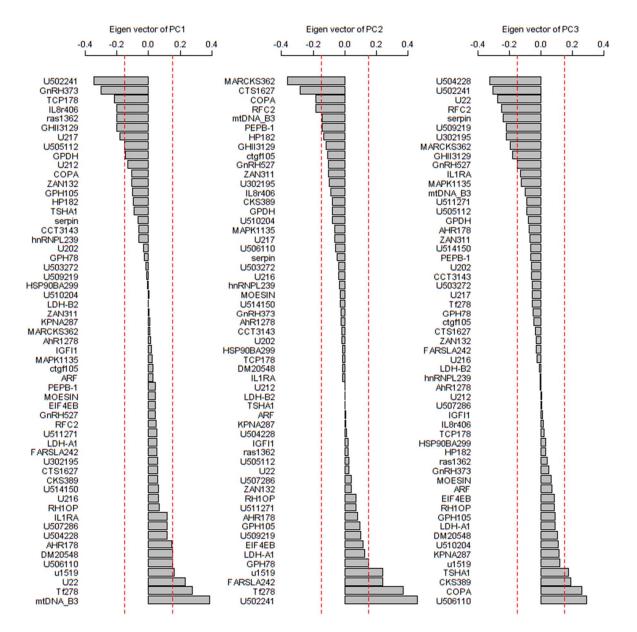


Figure S16 Eigenvectors of genes from a principal component analysis. PC1, PC2 and PC3 respectively explained 47%, 27% and 14% of the variance. The cumulative proportion explained by these components was 88%. Red dashed lines show eigen vectors of 0.15 and -0.15. Data are given in Table S9.

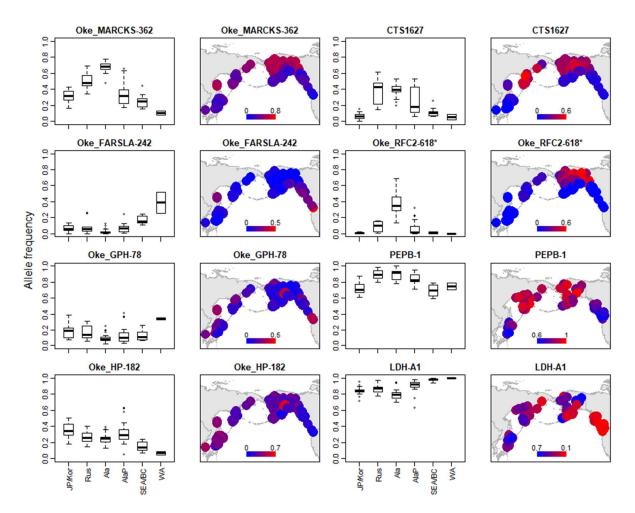


Figure S17 Geographical distribution of the allele frequencies of the top eight genes constituting PC2, all of which followed a latitudinal gradient, in American and Russian chum salmon populations. Unknown SNPs and the top eight genes contributing to PC1 were excluded. The plots are ordered according to the absolute eigenvectors of PC2, as shown in Figure S16 and Table S10.

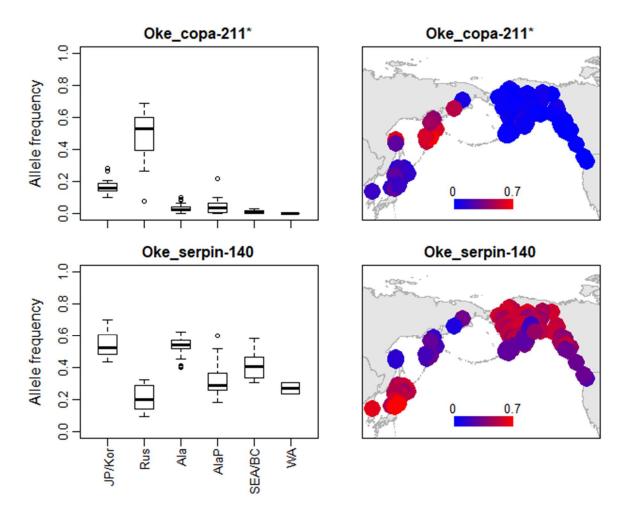


Figure S18 Geographical distribution of the allele frequencies of the top two genes contributing to PC3, which revealed the heterogeneity of Russian and Japanese populations. Four unknown SNPs and *Oke_RFC2-618**, one of the top eight genes contributing to PC2, were excluded. The plots are ordered according to the absolute eigenvectors of PC3, as shown in Figure S16 and Table S10.

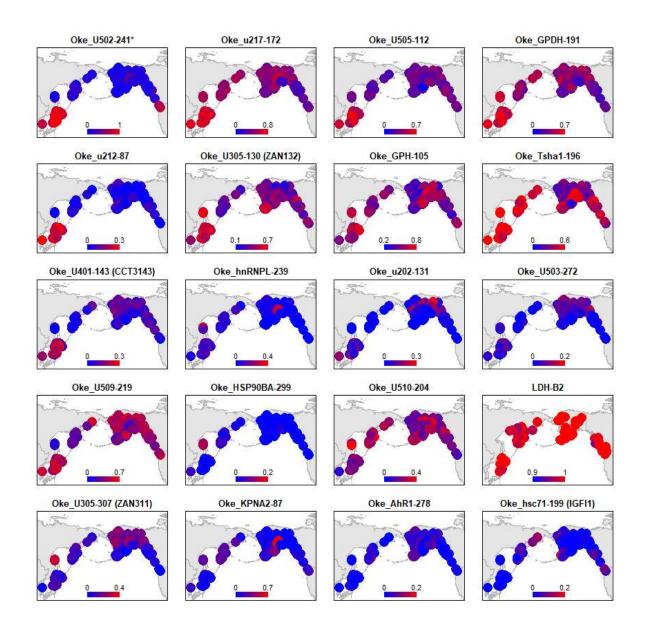


Figure S19-1 Distribution of frequencies of other alleles. Each point is the frequency of an allele visualized by a color gradient. The plots are ordered according to the absolute eigenvectors of PC1, as shown in Figure S16 and Table S10.

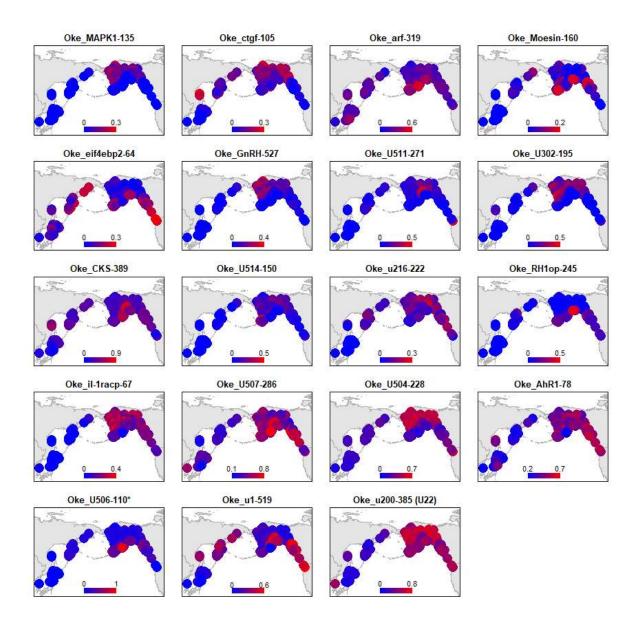
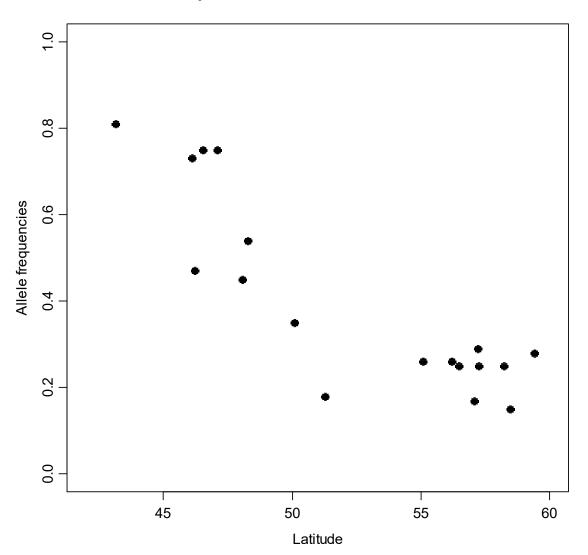


Figure S19-2 Distribution of frequencies of other alleles. Each point is the frequency of an allele visualized by a color gradient. The plots are ordered according to the absolute eigenvectors of PC1, as shown in Figure S16 and Table S10.



Clupeonella cultriventris LDH-A*100

Figure S20 LDH-A*100 frequencies of kilka (*Clupeonella cultriventris*) vs. latitude. Each point shows a sampling location. The data were obtained from Table 1 in Karabanov & Kodukhova (2018).

B. Supplemental Discussion

1. GnRH and straying of hatchery fish

Allele frequencies of *Oke_GnRH-37* were negatively correlated with latitude $(r = 0.81, t = -14.7, df = 112, p < 2.2 \times 10^{-16})$, with those of Japanese/Korean populations clearly distinct from other populations (Figure 8). GnRH is a key regulator of reproduction in vertebrates, including salmonids (Khakoo, Bhatia, Gedamu, & Habibi, 1994). In chum salmon, this gene is involved in gonadal maturation during the early and final phases of upstream migration (Kudo et al., 1996). Gene expression of GnRH increases in adult chum salmon brains during homing migration from the Bering Sea to the natal Chitose Hatchery, Japan, and GnRH improves stream odor discrimination in adult chum salmon (Ueda et al., 2016). The pulsatile secretion of GnRH is controlled by a clock gene, which may be a response of the circadian timing system to light (O'Malley, Camara, & Banks, 2007). Interestingly, the allele frequencies of *Oke_AhR1-78* were negatively correlated with those of *Oke_GnRH-37* (r = -0.67, t = -9.6, df = 112, $p < 2.6 \times 10^{-16}$) (Figure B1).

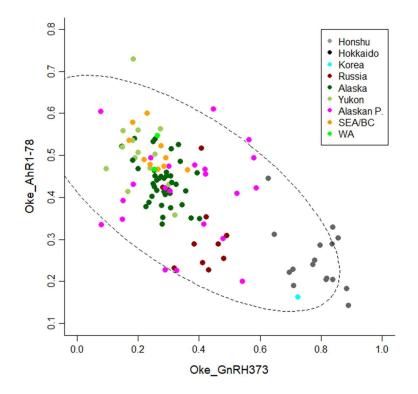


Figure B1 Plots of allele frequencies of *Oke_GnRH-37* vs. *Oke_AhR1-78*. Each point shows a sampling location (Figure S9). The dotted circle shows 95% confidence ellipse.

The aryl hydrocarbon receptor (AhR) is related to light-dependent regulation of circadian rhythm (Mukai & Tischkau, 2007). In Japanese hatcheries, fertilized eggs are kept undisturbed for \sim 2 months in incubation tanks. The hatched larvae are then reared for \sim 2 months, generally in indoor rearing ponds. The very dark environment experienced from egg incubation to emergence may affect the circadian rhythm of Japanese chum salmon. Efforts to

enhance early-run populations since the early 1980s changed the run timing of Hokkaido chum salmon, with late-run populations having almost disappeared by the late 1990s (Miyakoshi, Nagata, Kitada, & Kaeriyama, 2013). These factors may have also affected *Oke_AhR1-78* allele frequencies in Japanese chum salmon populations and the timing of homing in the North Pacific. Altered *Oke_GnRH-373* allele frequencies in Japanese chum salmon populations might reduce the ability to return to natal rivers and increased straying linked with *Oke_AhR1-78*, potentially leading to introgression from Japan into Russian and Alaskan rivers as discussed in Section 3.

2. Reproduction

Two genes related to reproduction were detected, namely, *Oke_GnRH-37* (gonadotropinreleasing hormone) and *Oke_TCP1-78* (T-Complex 1). Their allele frequencies were distributed along a north-to-south gradient (Figure 7). GnRH is a key regulator of vertebrate reproduction, including that of salmonids (Khakoo, Bhatia, Gedamu, & Habibi, 1994). In chum salmon, this gene is involved in gonadal maturation during the early and final phases of upstream migration (Kudo et al., 1996). TCP1 is related to sperm–zona-pellucida interaction and spermatozoan fertilization ability (Dun et al., 2011). Interestingly, the allele frequencies of the two genes were highly correlated ($r = 0.80, t = 13.9, df = 112, p < 2.2 \times 10^{-1}$) (Figure B2). In Japan, eggs and sperm are artificially taken from returning fish collected from enhanced rivers. Such artificial fertilization methods might select individuals better fit for artificial fertilization. Although the allele frequencies of these two genes were greatly different in Japanese/Korean populations, they exhibited north-to-south clinal variation. This result suggests that these reproduction-related genes have been adapted to hatchery environment.

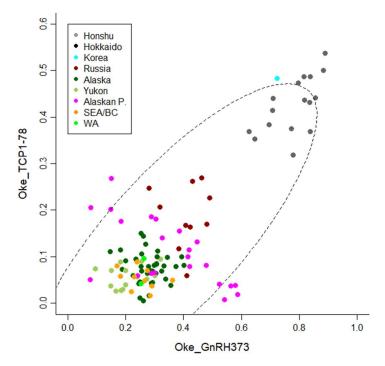


Figure B2 Plots of allele frequencies of *Oke_GnRH-37* vs. *Oke_TCP1-78*. Each point shows a sampling location (Figure S9). The dotted circles show 95% confidence ellipses.

3. Immune system and bacterial diseases

In contrast to the north-to-south clines, allele frequencies of *Oke_Tf-278** (transferrin), *Oke_IL8r-406* (interleukin) and *Oke_serpin-140* (serine protease inhibitors) increased in the opposite direction (Figure 7). Transferrin is related to iron storage and resistance to bacterial disease in Pacific salmon (Ford, 2001). Interleukins are a subgroup of cytokines that play a major role in the intercellular regulation of the fish immune system (Secombes, Wang, & Bird, 2011).

In Japanese salmon hatcheries, fish are often infected with bacterial kidney disease, infectious pancreatic necrosis and bacterial cold water disease (BCWD). In river surveys in Hokkaido, the causal bacteria of BCWD was found in the body cavity fluid of 85%–98% of females and the sperm of 78%–100% of males. Such bacterial infection rates may have altered the allele frequencies of bacterial disease resistance genes. Importantly, bacterial diseases are directly passed from parents to fertilized eggs (Urawa et al., 2013). Allele frequencies of *Oke_IL8r-406* were highly correlated with those of *Oke_GnRH-37* ($r = 0.80, t = 14.1, df = 112, p < 2.2 \times 10^{-16}$) and *Oke_TCP1-78* ($r = 0.84, t = 16.4, df = 112, p < 2.2 \times 10^{-16}$) (Figure B3), which suggests that genes associated with gamete production have developed resistance to bacterial disease.

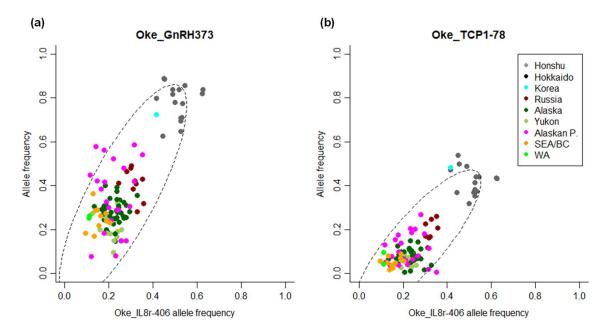


Figure B3 Plots of allele frequencies of *Oke_IL8r-406* vs. *Oke_GnRH-37* and *Oke_TCP1-78*. Each point shows a sampling location (Figure S9). The dotted circles show 95% confidence ellipses. (a) *Oke_GnRH-37* and (b) *Oke_TCP1-78*.

4. Parasites and DNA damage repair

Serpins are related to immune defense response against salmon louse in host fish (Maldonado-Aguayo, & Gallardo-Escárate, 2014). Japanese hatcheries have suffered from several parasites, such as an ectoparasitic flagellate (*Ichthyobodo necato*), trichodinids (*Trichodina truttae*) and *Chilodonella piscicola*. In particular, *Ichthyobodo necator*, whose parasitization of the body surface reduces the adaptability of juvenile chum salmon to

seawater, has an occurrence rate of 40% in Japanese hatcheries (Urawa et al., 2013). Although selection on *Oke_serpin-140* to defend against these parasites might change the allele frequencies of this gene, this does not explain why its allele frequencies in Japan/Korea are similar to those of Alaskan populations (Figure 7).

A serpin family gene (serpin B5) has a pathway related to DNA damage response and tumor suppressor (GeneCards). The mean egg-to-release survival rate of chum salmon has continued to increase since approximately 1990 and is currently ~90% (Supplemental Note). Such high egg-to-juvenile survival rates might relax natural selection, with less DNA repair required. In addition, mutation rates of genes might be lower in colder environments (Balloux, Handley, Jombart, Liu, & Manica, 2009; Koyano, & Kishino, 2010). In such cold environments, DNA damage at early life stages might be lowered, again reducing the necessity of DNA repair. These phenomena might explain the similarity of *Oke_serpin-140* allele frequencies between Japanese/Korean and Alaskan populations. If this is the case, deleterious genes could remain unselected.

5. Growth

Oke_ras1-249 (ras) allele frequencies followed a north-south latitudinal gradient, while those of *Oke_GHII-3129* (growth hormone 2) were distributed in the opposite direction, thus becoming similar to those of Alaskan populations (Figure 7). The *ras* gene has a central role in cell growth (Rotchell, Lee, Chipman, & Ostrander, 2001), while GH is an important regulator of somatic growth in salmonids (Björnsson, 1997). Sizes of chum salmon at release have been increasing in Japan (Miyakoshi, Nagata, Kitada, & Kaeriyama, 2013), which could lead to the selection of individuals with higher growth rates and altered *Oke_GHII-3129* allele frequencies. GH also increases swimming activity and feeding behavior while diminishing the anti-predator behavior of juvenile salmonids (Björnsson, 1997). An ecological trade-off between high growth and longevity may be a cause of normal growth of natural populations at sub-maximal rates, and selection on GH may affect long-term survival of released juveniles (Björnsson, 1997).

6. Athletic performance and metabolic efficiency of Japanese chum salmon

In 1977, the Japanese Fishery Agency initiated a large research project to increase return rates of chum salmon, namely, by releasing larger juveniles in locations where released juveniles (~10 g) reared in sea cages had very high return rates (7.6%) (Sato, 1986). On the basis of the project findings, the agency recommended the release of larger juveniles throughout Japan, and the sizes of chum salmon at release have accordingly been increasing in Japan (Miyakoshi, Nagata, Kitada, & Kaeriyama, 2013). Kobayashi and Ohkuma (1983) have pointed out that large-sized seeds often have weak stamina and poor athletic performance for living in natural environments. Those authors measured the swimming endurance of hatchery-reared and wild-born chum salmon fry in the Chitose Hatchery, Hokkaido, using a stamina measurement experimental device. Wild chum salmon fry had a 1.4-fold higher swimming ability ($56.6 \pm 11.1 \text{ cm/s}$) than hatchery-reared fry (41.4 ± 12.3) (t = 2.45, df = 8.7, p = 0.038) (Figure B4a). Results consistent with those observations were obtained in a recent study, which found that downstream hatchery-reared chum salmon juveniles had a higher swimming ability than wild ones of the same body size in the Toyohira River, Sapporo, Hokkaido (Sasaki, 2018).

Otolith thermal-marking field experiments with all juveniles marked and released in the Chitose River revealed that hatchery-reared juveniles $(0.92 \pm 0.39 \text{ g}, \text{mean} \pm \text{SD})$ reduced their body weights ~1 month after release $(0.71 \pm 0.23 \text{ g})$ (z = 1.95, p = 0.0254), but these

weights were still greater than wild-born fish $(0.33 \pm 0.13 \text{ g})$ (z = 5.76, $p = 4.1 \times 10^{-9}$) (Shimizu et al., 2016) (Figure B4b). The authors also found that glycogen content (%) in the liver was very high in hatchery-reared juveniles before release $(2.76 \pm 0.96 \text{ g})$ but decreased substantially after release $(0.18 \pm 0.33 \text{ g})$ (z = 10.17, p = 0) to values much smaller than those of wild juveniles $(0.18 \pm 0.33 \text{ g})$ (z = 4.14, $p = 1.7 \times 10^{-5}$). Moreover, hatchery fish had a higher triglyceride content $(1.24 \pm 0.20 \text{ g})$, but this value decreased after release $(0.87 \pm 0.32 \text{ g})$ (z = 5.02, $p = 2.5 \times 10^{-7}$) to the same level as that of wild fish $(0.86 \pm 0.45 \text{ g})$ (z = 0.12, p = 0.4518). These results demonstrate that hatchery-reared juveniles experience a more pronounced reduction in body weight soon after release compared with wild fish due to the conversion of glycogen and triglyceride to energy. These results thus suggest that hatchery fish have a higher K_m^{PYR} (lower catalytic efficiency) and do not catch enough bait after release.

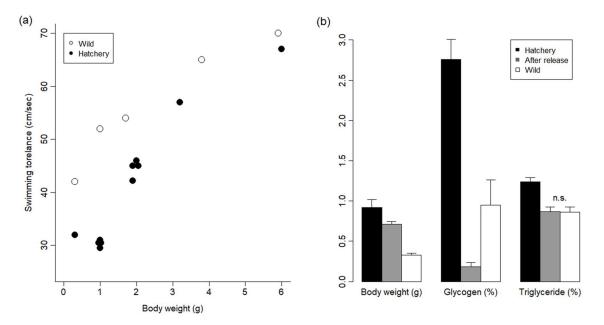


Figure B4 Athletic ability and nutritional condition of hatchery-reared and wild-born chum salmon juveniles in the Chitose River, Hokkaido. (a) Swimming performance by body weight measured in a stamina measurement experimental device (Kobayashi & Ohkuma, 1983). Redrawn from Figure 2 in the original study. (b) Body weight, glycogen weight in the liver (%) and triglyceride weight in a juvenile excluding the head, fins and digestive tract (%) (Shimizu et al., 2016). Data were taken from Table 3 in the original study. Error bars show standard errors; n.s. denotes no significant difference.

We found several YouTube videos—seven from Japan and one from the state of Washington, USA—that recorded the athletic performance of returning chum salmon. These eight videos were all we could find without any intentional (biased) selection. In the Okushibetsu River, Shiretoko, Hokkaido, a representative enhanced river to which thousands of chum salmon return every year (Hokkaido Shimbun Press, 2017), fish movement looked slow. Jumping trials of returning Japanese chum salmon at a small fall in Hokkaido at Esashi Town facing the Sea of Okhotsk (Sapporo TV House Stock Footage, 2015) resulted in no

success after 64 jumps, excluding two jumps that were difficult to judge. When we counted the two jumps as successful ones, the rate of success was 3.0% (= 2/66). At another small fall in northern Hokkaido (Kawamura, 2008), the success rate was 1 out of 47 jumps (2.1%). At a small weir in the Hamamasu River, Hokkaido (Onishi, 2009), only one chum salmon succeeded out of 219 jumps (success rate = 0.5%). Even at a much lower small fall in the Haboro River, Hokkaido (haruma1108, 2017), only three jumps were successful out of eight (success rate = 37.5%). Judging from information on locations of hatcheries (Supporting Information Figure C2 in Supplemental Notes), none of these four small rivers in Hokkaido had a hatchery. The chum salmon in these rivers in Hokkaido were therefore hatchery strays or natural-born fish. In the hatchery-enhanced Kido River, Fukushima, Honshu, none of the eight observed jumps were successful (The Asahi Shimbun, 2013). In this river, which is located in the southern distribution area in Honshu, body damage was severe (KyodoNews, 2014), which suggests that a higher K_m^{PYR} (lower catalytic efficiency) operated to convert body mass to energy, with no intake of food when spawning upstream, compared with colder areas such as Hokkaido. Chum salmon originally were powerful swimmers, even swimming across roads during floods on the coast and along Puget Sound, WA (Allison, 2016). The results of scientific experiments, field surveys and our observations of social-network videos consistently show the weaker athletic performance of Japanese chum salmon.

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C. Supplemental Note: the Japanese chum salmon hatchery propagation program

Several reviews have been published on Japanese chum salmon, mainly focusing on ecological issues (e.g., Kaeriyama, 1999; Kaeriyama, Seo, Kudo, & Nagata, 2012; Morita et al., 2006) and hatchery releases (Kitada, 2014; Miyakoshi, Nagata, Kitada, & Kaeriyama, 2013; Nagata et al., 2012). We have reviewed available literature, data and statistics on Japanese chum salmon and briefly summarize the results of investigations related to this study.

1. Release of juveniles

Artificial propagation of chum salmon was first conducted in 1877 and 1878 in Honshu and Hokkaido, Japan, respectively. The Hokkaido government constructed the Chitose Hatchery in 1888 (Kobayashi, 1980). In Hokkaido, the number of released chum salmon was only 330,000 in 1888 and first exceeded 100 million in 1916 (Miyakoshi, Nagata, Kitada, & Kaeriyama, 2013). This number increased to 549 million in 1966 and exceeded 1 billion in 1981. The rise was positively correlated with that of Honshu, which released numbers ~80% that of Hokkaido (Figure C1a). As demonstrated by cumulative release numbers, stocking intensity was much stronger beginning in the 1980s (Figure C1b).

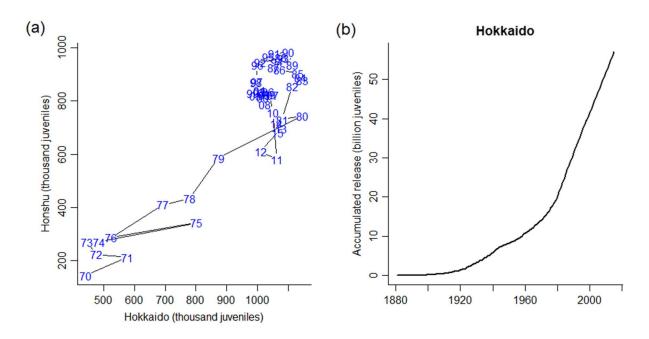


Figure C1 Number of chum salmon released in Japan. (a) Hokkaido vs. Honshu ($r = 0.90, t = 44, df = 44, p = 2.2 \times 10^{-16}$). Data from the Japan Fisheries Research and Education Agency, http://salmon.fra.affrc.go.jp/, accessed January 2020. (b) Cumulative number of released chum salmon in Hokkaido. Data from Kitada (2020).

Releases on the scale of a billion have continued for 30 years. At present, 262 salmon hatcheries operate in Japan (129 in Hokkaido and 133 in Honshu) (Kitada, 2020) (Figure C2). Hatchery propagation of chum salmon with very high stocking rates has thus lasted over 130 years, and its genetic impacts on wild populations probably became substantial beginning in

the 1980s, when the cumulative number of releases first exceeded 20 billion and then accelerated. According to NPAFC release statistics (NPAFC, 2019), Japan released 88 billion juveniles from 1952 to 2018.

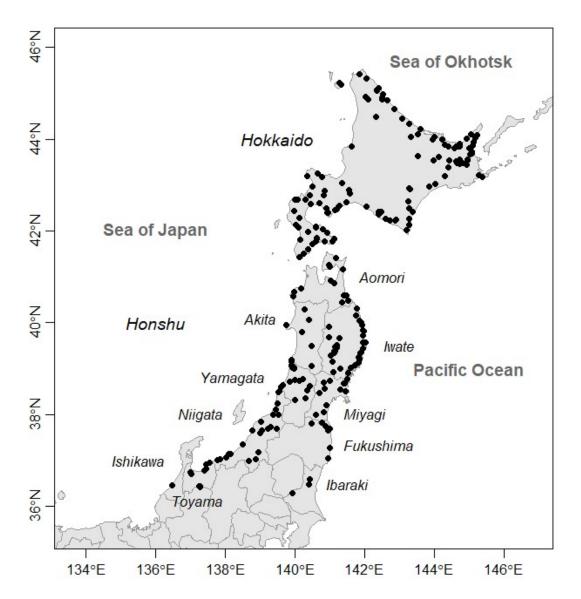


Figure C2 Locations of salmon hatcheries in Japan. A total of 262 hatcheries operate in 11 prefectures in northern Japan. Redrawn from Kitada (2020).

2. Returning chum salmon; stray and gene flow

Japanese chum salmon returning to spawn are caught by commercial set-net fisheries in the coastal waters of Hokkaido and northern Honshu. In Hokkaido, the major production area responsible for more than 80% of chum salmon catch, ~1,000 salmon set nets are operated from late August to late December; landings peak in late September and early October, with variation among regions (Nagata et al., 2012). On the Pacific coast of Honshu, the return of chum salmon takes place between September and early February according to the catch statistics of Iwate Prefecture, with landings peaking in late November to December. On the

Sea of Japan coast of Honshu, chum salmon return between October and January, and a peak run occurs in November and December (Saito, Okamoto, & Sasaki, 2015). Returning fish are caught in weirs, which are set in the enhanced rivers and used for seed production every year. Returning chum salmon comprise five age classes, ranging from 2 to 6 years old, and 4-yearold fish constitute the major age class in Hokkaido (Miyakoshi, Nagata, Kitada, & Kaeriyama, 2013). The age composition of returning chum salmon varies among years, but the variation is insubstantial.

According to field surveys, natural spawning occurs in non-enhanced and enhanced rivers in Hokkaido (Miyakoshi et al., 2012) and Honshu (Iida, Yoshino, & Katayama, 2018). In Hokkaido, roughly 140 out of ~1,500 rivers receive hatchery releases (Miyakoshi et al., 2012). Hatcheries are located on major rivers, whereas non-enhanced rivers are generally small (Figure C3a, b). Miyakoshi et al. (2012) reported that natural spawning occurred in 31%–37% of 238 non-enhanced rivers surveyed in Hokkaido. Iida, Yoshino, & Katayama (2018) also observed natural spawning in 94% of 47 enhanced rivers and 75% of 47 non-enhanced rivers surveyed on the northern Sea of Japan coast of Honshu. A thermal-marking study also estimated that the proportion of naturally spawned chum salmon to total production was 16%–28% in eight rivers in Hokkaido (Morita, Takahashi, Ohkuma, & Nagasawa, 2013). Because escapement for natural spawning in enhanced rivers is not large (Kitada, 2014) and non-enhanced rivers are generally small, the contribution of natural spawning may not be substantial.

Almost all chum salmon returning to Japan are hatchery-reared fish (Kaeriyama 1999). Moderate spawning-river fidelity ($50 \pm 22\%$ in Hokkaido) can facilitate gene flow between rivers, which is very high, and almost all chum salmon returning to Japan are hatchery-released fish or possibly wild-born hatchery descendants (Kitada, 2020).

3. Seed production

The number of parent fish captured in Hokkaido increased to ~5 million in 2004, when the catch was at a historical maximum, and decreased to ~2 million in 2018 (http://sakemasu.or.jp/html/07-01.html). Although not all collected fish are used for artificial fertilization, ~1.2 billion eggs are obtained annually (Figure C4). In hatcheries (Figure C3c, d), eggs and sperm are artificially taken from parent fish collected from enhanced rivers. Fertilized eggs are kept undisturbed in hatcheries to avoid vibration until hatching (http://salmon.fra.affrc.go.jp/). The rearing water is maintained at a stable temperature, typically an average of ~8°C (Shimizu, 2013); this temperature is similar to that of water near redds in springtime but much higher than that of natural streams during this period—for example, 1.1–2.8°C in the tributary of the Ishikari River, Hokkaido (Ando et al., 2014). Hatching begins when the accumulated temperature reaches 480 degrees in hatcheries maintained at 8°C (~2 months after fertilization) (https://www.hro.or.jp/). Hatched larvae (body length (BL) ~19 mm; body weight (BW) ~0.05 g) are reared for ~2 months, generally in indoor rearing ponds; they emerge 40 days after absorption of yolksack (BL ~38 mm; BW ~0.28 g) (Kaeriyama, 1986) and are fed to their release size (BL ~50 mm; BW ~1 g) in rearing ponds during January and May until the sea water temperature exceeds 5°C (https://www.hro.or.jp/). Although the duration of rearing primarily depends on temperature, juveniles are thus hatchery-reared for more than 6 months. The mean egg-to-release survival rate \pm SD in hatcheries between 1967 and 2017 was very high, $81 \pm 7\%$; it has continued to increase since approximately 1990 and is currently ~90% (Figure C5).

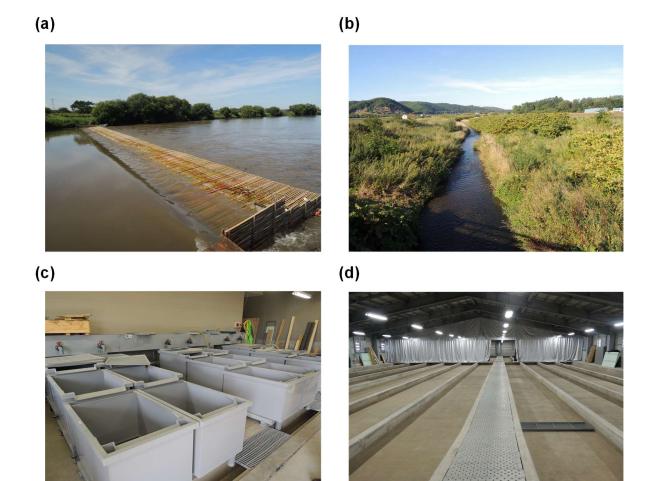


Figure C3 Chum salmon stocking in Hokkaido. (a) A representative enhanced river with a weir for collecting brood fish. (b) A non-enhanced river where natural spawning has been observed. (c) Tanks for incubation of fertilized eggs. (d) Indoor rearing tanks, where hatched larvae are generally reared for ~2 months until emergence. Photographs were taken by S.K.

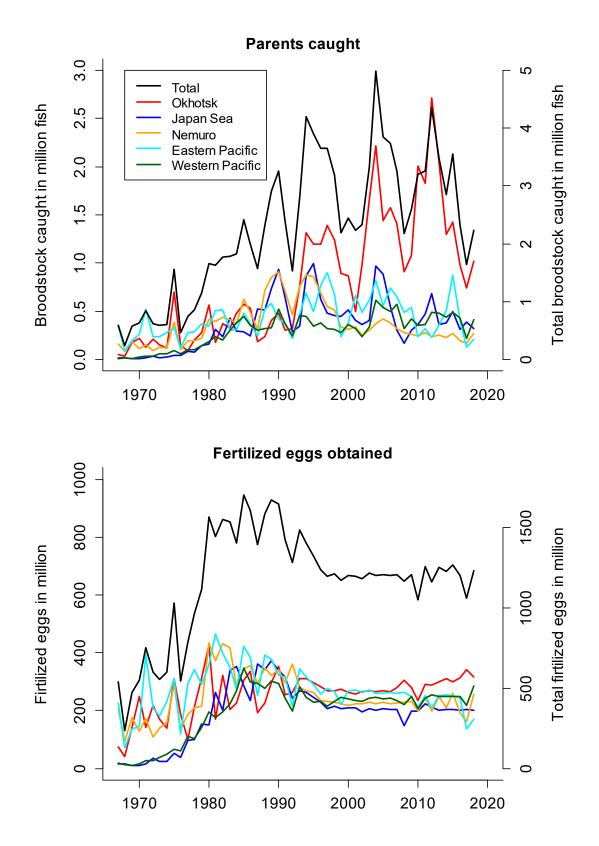


Figure C4 Number of chum salmon parents caught (upper) and fertilized eggs obtained for seed production in Hokkaido (1967–2018). Data were retrieved from the Hokkaido Salmon Enhancement Association, <u>http://sake-masu.or.jp/html/07-01.html</u>

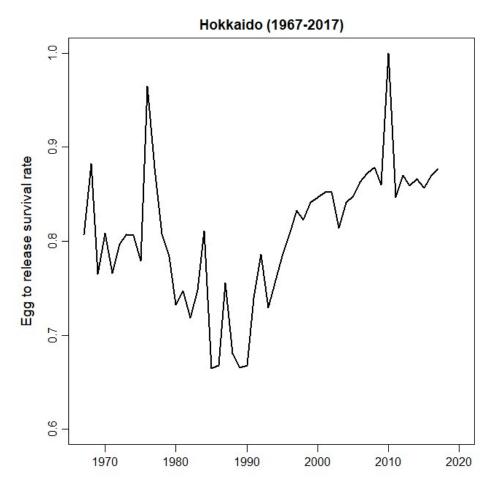


Figure C5 Changes in egg-to-release survival rates in Hokkaido (1967–2017). Eggto-release survival rates were estimated by dividing the number of released juveniles by the number of fertilized eggs. Data were retrieved from the Hokkaido Salmon Enhancement Association (<u>http://sake-masu.or.jp/html/07-01.html</u>).

4. Migration of released juveniles

Hatchery-reared fry are released into rivers during March and May and migrate down to the sea in late winter to early summer (Ueno, & Ishida, 1996). Juveniles leave the coasts for the Sea of Okhotsk, an important nursery area for juvenile salmon originating in Russia and Japan (Mayama, & Ishida, 2003; Urawa et al., 2004; Urawa, 2015). In a 5-year offshore survey (1977–1981) of Ishikari Bay on the Japan Sea coast of Hokkaido, juveniles arose in April, increased rapidly from late May to early June—when the water temperature at a 5-m depth was 11–12°C—and disappeared by mid-June (Mayama, & Ishida, 2003). On the Abashiri coast facing the Sea of Okhotsk, juvenile chum abundance was found to be rich in coastal areas from May to June, when SSTs ranged from 8 to 13°C; the juveniles disappeared from coastal waters after late June, when SSTs exceeded 13°C (Nagata et al., 2007). On the Sanriku coast on the Pacific side of Honshu, early-migrating juveniles have been reported to remain in coastal waters in early spring (February–March) and then migrate offshore as the cool Oyashio Current approaches the coast (April–May, when SSTs are 8–11°C). In contrast, late-migrating juveniles remained in coastal waters until late spring (April–May) and migrated offshore when SSTs ranged from 11 to 13°C (Kaeriyama, 1986). Environmental

DNA surveys in Otsuchi Bay, Iwate Prefecture, confirmed the presence of juvenile chum salmon in mid-April, but no fish were found in mid-June (Minegishi et al., 2019). The SST in the bay was 15.7°C in late May, which suggests that juveniles left the bay before mid-June when the SST exceeded $\sim 15^{\circ}$ C.

Taking the results of all the above-mentioned studies into consideration, it may be concluded that juvenile chum salmon can stay in Japanese coastal waters until the SST exceeds 11–15°C. Juveniles stay in coastal waters until early August on the Pacific coast and the Okhotsk coast of Hokkaido (Ueno, & Ishida, 1996). They then migrate to the Northern Pacific Ocean in the middle of November as the sea temperature decreases (Ueno, 1998). After feeding migration for several years in the high seas, mature fish leave the Bering Sea starting around July and return to the Japanese coast in September and December for spawning (Urawa, 2015).

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