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Submitted to the

NORTH PACIFIC ANADROMOUS FISH COMMISSION

By

JAPAN

April 2007

THIS PAPER MAY BE CITED IN THE FOLLOWING MANNER:

Sato, S., M. Yoon, S. Abe, and S. Urawa. 2007. Update of mitochondrial DNA baseline for stock identification of chum salmon. (NPAFC Doc. 1019) 26 p. National Salmon Resources Center, Fisheries Research Agency, 2-2 Nakanoshima, Toyohira-ku, Sapporo 062-0922, Japan.

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Abstract

A baseline of mitochondrial DNA frequencies covering the entire range of chum salmon was developed and evaluated for its resolution to estimate stock of origin of Asian, Russian, and North American chum salmon in complex mixtures. Tissue samples of chum salmon were collected from adult chum salmon in 107 populations around the Pacific Rim, and up to now the mtDNA haplotypes of 88 populations were detected by direct sequence analysis or DNA microarray methods. The simulation studies using the present baseline indicated that the accuracy of estimates was reliable for the Japanese (94.6%) and North American (92.8%) stocks, and 80.1% for Russian stock.

Keywords: chum salmon, mitochondrial DNA, genetic stock identification, baseline

Introduction

Chum salmon (*Oncorhynchus keta*) is the most widely distributed species of salmon in the Pacific Rim (Salo 1991). Chum salmon from Asia and North America migrate into the North Pacific Ocean and the Bering Sea. The eastward extension of Asian chum salmon shows a more distant migration than the chum salmon of North America, which are not commonly found west of 175°E (Salo 1991). This result indicates that mixed aggregations, often composed of both Asian and North American stocks, form during these ocean migrations. Identification of composite stocks in mixtures has become an important part of chum salmon management and conservation programs.

In the Pacific salmon, stock identification has been attempted by tagging, scale characteristics, parasite tagging, thermal otolith marking, and protein genetic (allozyme) characters (e.g. Ishida et al. 1989; Winans et al. 1994; Wilmot et al. 1998; Urawa et al. 1998). Recently, molecular genetic markers, e.g. mitochondrial (mt) DNA, microsatellite DNA, and single nucleotide polymorphisms (SNPs), are developed for several genetic stock identification (GSI) studies of Pacific salmon (e.g. Seeb et al. 1998, 2005; Seeb and Crane 1999; Sato et al. 2001, 2004; Smith et al. 2005; Moriya et al. 2006). Particularly, analysis of mtDNA has received considerable attention in GSI of Pacific salmon species because of the higher sequence variability than single copy nuclear genes (Brown et al. 1979) and clonal haploid inheritance.

Previous reports described the initial efforts to develop mtDNA baseline for chum salmon. Sato et al. (2004) reported that the mtDNA baseline samples of chum salmon included 2,154 individuals collected from 48 populations in the Pacific Rim. Additional

2,524 individuals from 45 populations in Russia and North America were analyzed by Yoon et al. (2004, 2005). Sato et al. (2005) reported that a total of 6,447 individuals from 93 populations were collected from around the Pacific Rim and 48 out of 116 sample groups were examined for mtDNA baseline populations. In this document, we update sample list, the mtDNA baseline, and resolution for GSI of chum salmon. We also review chum salmon baselines in different genetic stock identification methods: allozyme, mtDNA, and microsatellite DNA.

Methods of genetic stock identification of chum salmon

Sample collection

Fresh tissues (liver, muscle, blood or fin) were collected from adult chum salmon which returned to their natal river for spawning. Liver and blood tissues were stored at -80°C or -30°C. Muscle tissues were stored in 100% ethanol at room temperature. Fin tissues were stored at -30°C or in 100% ethanol at room temperature.

DNA extraction

DNA was extracted from the collected tissues using a conventional phenol-chloroform method (Sambrook et al. 1989) or Puregene™ DNA Purification kit (Gentra Systems, Minneapolis, MN) following the manufacturer's instructions. In brief, DNA was isolated from the stored specimens following the phenol-chloroform methods (Sambrook et al. 1989). Prior to extraction of DNA, the muscle samples were washed twice in 500 µl sodium tris EDTA buffer (STE; 0.1 M NaCl, 10mM Tris-HCl, and 1 mM EDTA, pH 8.0). The frozen liver samples were immediately homogenized in the same solution. About 50 µl of whole blood and homogenates of liver or muscle were added to 500 µl STE buffer containing 500 µg/ml proteinase K and 0.5% SDS, and incubated at 37°C overnight. DNA was extracted three times with a mixture of phenol (250 µl) and 24:1 chloroform:isoamylalcohol (250 µl), and then twice with 500 µl of the chloroform-isoamylalcohol alone. DNA in aqueous phase was recovered by ethanol precipitation, dried in air, and dissolved in tris EDTA buffer (TE; 10 mM Tris-HCl, 1 mM EDTA, pH 7.5).

PCR amplification and nucleotide sequence analysis

The PCR amplification and nucleotide sequence analysis of the mtDNA control region was followed as in the previous studies of Sato et al. (2001) and Sato et al. (2004). In brief, amplifications were performed in 100 µl volumes containing 25-100 ng of template DNA, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl₂, 250 nM each dNTPs, 250nM of forward and reverse primers, 0.001% gelatin, and 1.25 U of *Taq* polymerase (Takara, Tokyo). The PCR products were purified by the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) after confirmation of their sizes by gel-electrophoresis.

Nucleotide sequences were determined directly from PCR products. Approximately 500-bp in the variable position of 5' end of the mtDNA control region was sequenced with a Hitachi SQ-5500L DNA Sequencer (Hitachi, Tokyo). Sequence reaction was preformed using a Thermo Sequenase™ pre-mixed cycle sequencing kit (Vistra Systems, Sunnyvale, CA) according to the manufacturer's instruction.

MtDNA haplotype detection by DNA microarray system

The mtDNA haplotypes of several chum salmon samples were detected by DNA microarray system. DNA microarray analysis of approximately 500 bp in the variable position of the 5' end of the mtDNA control region followed the protocol developed by Moriya et al. (2004) with an OligoArray® (Chum salmon) Kit (Nisshinbo Industries, Tokyo),

according to the manufacturer's instruction. Amplification was carried out in a 25 μ l of reaction mixture containing 25-100 ng of template DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 0.25 mM each dNTP, 1 U Taq DNA polymerase (Sigma-Aldrich Corporation, St. Louis, MO), 1 μ M of forward and reverse primers. The condition of PCR amplification using a GeneAmp PCR System 2400 (Applied Biosystems, Foster City, CA) was as follows; preheating at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 45°C for 30 sec, and elongation at 72°C for 30 sec, and post-cycling extensions at 72°C at 3 min. The PCR products were examined of the fragment size with 1.5% agarose-gel electrophoresis and ethidium bromide staining.

Hybridization of PCR products and signal detection followed the protocol of Moriya et al. (2004), using reagents and buffers supplied in the above kit. 2 μ l each of reaction mixture was denatured at 95°C for 2 min, followed by quenching on ice until hybridization. The denatured PCR product was mixed with 16 μ l of hybridization buffer, mounted on a DNA microarray with cover film, and hybridized at 37°C for two hours in a moisture chamber. After hybridization, the DNA microarray was washed in a washing buffer at 37°C for 5 min. Then, 1.4 ml of conjugate solution, prepared according to the manufacturer's instruction, was mounted on the DNA microarray and incubated at room temperature for 30 min. The DNA microarray was washed twice a coloring buffer at room temperature for 5 min each, followed by incubation with 1.4 ml of coloring solution at room temperature for 30 min. Coloring reaction was stopped by rinsing of the DNA microarray in distilled water. Air-dried DNA microarray was scanned by a GT-8700F and GT-9300UFS scanner (Seiko Epson Corp., Tokyo) for visual analysis of the signal intensity on a computer. Haplotypes were determined according to the combination of signal positive-oligomer sites, which correspond to the previously identified SNPs in the target region (Sato et al. 2004).

Data analysis

A neighbor-joining tree inferred from mtDNA data was constructed with MEGA version 3.1 software (Kumar et al. 2004). Stock contributions of the mixed samples were estimated via a conditional maximum likelihood (Pella and Milner 1987; Masuda et al. 1991). A conjugate-gradient searching algorithm using a square root transformation was employed, because it provides good performance with large baselines and small stock differences (Pella et al. 1996). Standard deviations were estimated by 1,000 bootstrap resamplings of the baseline and 500 mixture samples. Estimates were made to individual stocks and then pooled to regional stock groups: Japan, Russia, and North America. These regional stock groups were categorized based on previous genetic analysis for data set of 88 samples of chum salmon in the Pacific Rim. Computations were performed with the Statistics Programs for Analyzing Mixtures (SPAM version 3.7b, Alaska Department of Fish and Game, Anchorage, AK).

Current baseline

Sampling locations, sampling date, kinds of tissue, number of collected and analyzed samples, archives, and references are given in Table 1 and Fig. 1. Several populations were sampled over multiple years and run timings. New 25 sample groups from 19 Japanese and one Russian populations were added in the collected sample list (Table 1). A total of 107 populations are included in the present sample list: 35 populations in Japan (19 in Hokkaido and 16 in Honshu), one population in Korea, 24 populations in Russia (one in Anadyr, 11 in Kamchatka, 5 in Sakhalin, 2 in Primorye, 4 in Magadan, one in Nikolaevsk-na-Amure), and 47 populations in North America (13 in Northwest Alaska, 6 in Southcentral Alaska, 5 in

Alaska Peninsula, 10 in Southeast Alaska, 8 in British Columbia, and 5 in Washington).

MtDNA haplotypes of 112 sample groups from 88 populations of chum salmon were detected by direct sequence analysis or DNA microarray methods. All analyzed sample groups of chum salmon were used for mtDNA baseline populations (Table 1, Appendix 1).

Baseline evaluation

The 112 sample groups from 88 populations were clustered using a neighbor-joining method (Fig. 2). The 112 sample groups were grouped into three regions: Japan and Korea, Russia, and North America. Particularly, the Japanese and Korean sample groups were clearly separated from Russian and North American groups. The performance of the maximum likelihood model for chum salmon was investigated through simulation studies of clustered three regions, Japan, Russia, and North America. We used the mtDNA data baseline collected from 88 populations of chum salmon around the Pacific Rim described in the former session. In simulation studies where the true regional contributions were 100%, estimations for the Japanese and North American regions reached more than 90% (94.6% Japan and 92.8% North America), while estimate for Russian region was 80.1% accurate (Table 2).

Acknowledgment

The present study was supported in part by Grants-in-Aid from the North Pacific Research Board to the NPAFC Cooperative Research, from the Fisheries Agency of Japan, and from the Ministry of Education, Culture, Sports, and Technology, Japan.

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Table 1. River population names, sampling locations, sampling date, collect tissues, preservation, numbers of collected samples (Nc), numbers of analyzed samples (Na), and archives of chum salmon in the Pacific Rim. *NSRC, National Salmon Resources Center; **HU, Hokkaido University.

River population	Region	Sampling date	Tissue	Preservation	Nc	Na	Archives	Reference***
1. Tokushibetsu River 1997SEP	Okhotsk Sea Coast, Hokkaido, Japan	Sep. 23, 1997	Liver	Frozen	80	51	NSRC*, HU**	1
1. Tokushibetsu River 1997OCT	Okhotsk Sea Coast, Hokkaido, Japan	Oct. 27, 1997	Liver	Frozen	80	-	NSRC	Unpublished
1. Tokushibetsu River 2004SEP	Okhotsk Sea Coast, Hokkaido, Japan	Sep. 2004	Liver	Frozen	80	77	NSRC	Unpublished
1. Tokushibetsu River 2004OCT	Okhotsk Sea Coast, Hokkaido, Japan	Oct. 2004	Liver	Frozen	80	74	NSRC	Unpublished
1. Tokushibetsu River 2004NOV	Okhotsk Sea Coast, Hokkaido, Japan	Nov. 2004	Liver	Frozen	80	76	NSRC	Unpublished
2. Tokoro River 1998	Okhotsk Sea Coast, Hokkaido, Japan	Nov. 20, 1998	Liver	Frozen	44	44	HU	1
2. Tokoro River 1999	Okhotsk Sea Coast, Hokkaido, Japan	Oct. 13, 1999	Blood	Frozen	49	49	HU	1
2. Tokoro River 2005	Okhotsk Sea Coast, Hokkaido, Japan	Oct.3, 2005	Fin	Frozen	100	-	NSRC	Unpublished
3. Shari River	Okhotsk Sea Coast, Hokkaido, Japan	Oct. 11, 2001	Blood	Frozen	78	-	NSRC	Unpublished
4. Abashiri River	Okhotsk Sea Coast, Hokkaido, Japan	Oct. 19, 1998	Blood	Frozen	80	-	NSRC	Unpublished
5. Shibetsu River	Nemuro Stait, Hokkaido, Japan	Oct. 10, 2003	Liver	Frozen	80	76	NSRC	Unpublished
6. Touhoro River	Nemuro Stait, Hokkaido, Japan	Oct.5, 2006	Fin	Frozen	100	-	NSRC	Unpublished
7. Nishibetsu River 1997SEP	Nemuro Stait, Hokkaido, Japan	Sep. 25, 1997	Liver	Frozen	80	41	NSRC, HU	1
7. Nishibetsu River 1997OCT	Nemuro Stait, Hokkaido, Japan	Oct. 22, 1997	Liver	Frozen	80	-	NSRC	Unpublished
8. Teshio River	Japan Sea Coast, Hokkaido, Japan	Oct. 23, 2001	Liver	Frozen	80	74	NSRC	Unpublished
9. Syokanbetsu River	Japan Sea Coast, Hokkaido, Japan	Oct. 16, 2006	Fin	Frozen	100	-	NSRC	Unpublished
10. Chitose River 1996	Japan Sea Coast, Hokkaido, Japan	Oct. 14, 1996	Liver	Frozen	80	51	NSRC, HU	1
10. Chitose River 2003SEP	Japan Sea Coast, Hokkaido, Japan	Sep. 4, 2003	Liver	Frozen	80	76	NSRC	Unpublished
10. Chitose River 2003OCT	Japan Sea Coast, Hokkaido, Japan	Oct. 7, 2003	Liver	Frozen	80	78	NSRC	Unpublished
10. Chitose River 2003NOV	Japan Sea Coast, Hokkaido, Japan	Nov. 6, 2003	Liver	Frozen	80	80	NSRC	Unpublished
10. Chitose River 2003DEC	Japan Sea Coast, Hokkaido, Japan	Dec. 5, 2003	Liver	Frozen	80	77	NSRC	Unpublished
11. Shiribetsu River	Japan Sea Coast, Hokkaido, Japan	Oct. 17, 2006	Fin	Frozen	100	-	NSRC	Unpublished
12. Assabu River	Japan Sea Coast, Hokkaido, Japan	Oct. 6, 2006	Fin	Frozen	100	-	NSRC	Unpublished
13. Kushiro River	Pacific Coast East Area, Hokkaido, Japan	Oct. 22, 1998	Liver	Frozen	79	49	NSRC, HU	1
14. Tokachi River 1996	Pacific Coast East Area, Hokkaido, Japan	Oct. 17, 1996	Liver	Frozen	80	46	NSRC, HU	1
14. Tokachi River 2002	Pacific Coast East Area, Hokkaido, Japan	Oct. 22, 2002	Liver	Frozen	80	-	NSRC	Unpublished
15. Shizunai River	Pacific Coast West Area, Hokkaido, Japan	Oct. 17, 2002	Liver	Frozen	80	-	NSRC	Unpublished
16. Shiraoi River	Pacific Coast West Area, Hokkaido, Japan	Oct. 12, 2006	Fin	Frozen	100	-	NSRC	Unpublished
17. Shikyu River	Pacific Coast West Area, Hokkaido, Japan	Nov. 25, 1998	Liver	Frozen	80	-	NSRC	Unpublished
18. Yurappu River 1997SEP	Pacific Coast West Area, Hokkaido, Japan	Sep. 24, 1997	Liver	Frozen	80	-	NSRC	Unpublished
18. Yurappu River 1997NOV	Pacific Coast West Area, Hokkaido, Japan	Nov. 17, 1997	Liver	Frozen	80	-	NSRC	Unpublished
18. Yurappu River 1998	Pacific Coast West Area, Hokkaido, Japan	Nov. 17, 1998	Liver	Frozen	80	40	NSRC, HU	1
18. Yurappu River 2006SEP	Pacific Coast West Area, Hokkaido, Japan	Sep. 25, 2006	Liver	Frozen	80	75	NSRC	Unpublished
18. Yurappu River 2006OCT	Pacific Coast West Area, Hokkaido, Japan	Oct. 17, 2006	Liver	Frozen	80	77	NSRC	Unpublished
18. Yurappu River 2006NOV	Pacific Coast West Area, Hokkaido, Japan	Nov. 13, 2006	Liver	Frozen	80	75	NSRC	Unpublished
18. Yurappu River 2006DEC	Pacific Coast West Area, Hokkaido, Japan	Dec. 1, 2006	Liver	Frozen	80	75	NSRC	Unpublished

Table 1-continued.

River population	Region	Sampling date	Tissue	Preservation	Nc	Na	Archives	Reference
19. Torisaki River	Pacific Coast West Area, Hokkaido, Japan	Nov. 6, 2006	Fin	Frozen	100	-	NSRC	Unpublished
20. Niida River	Pacific Coast, Honshu, Japan	Oct. 18, 2006	Fin	Ethanol	124	-	NSRC	Unpublished
21. Iwate-kuji River	Pacific Coast, Honshu, Japan	Nov. 1, 2006	Fin	Ethanol	100	-	NSRC	Unpublished
22. Tarou River	Pacific Coast, Honshu, Japan	Nov. 6, 2006	Fin	Frozen	100		NSRC	Unpublished
23. Tsugaruishi River 1997	Pacific Coast, Honshu, Japan	Dec. 10, 1997	Liver	Frozen	80	44	NSRC, HU	1
23. Tsugaruishi River 1999OCT	Pacific Coast, Honshu, Japan	Oct. 1999	Blood	Frozen	47	47	HU	1
23. Tsugaruishi River 1999DEC	Pacific Coast, Honshu, Japan	Dec. 8, 1999	Liver	Frozen	80	-	NSRC	Unpublished
24. Oriksa River 1996	Pacific Coast, Honshu, Japan	Oct. 24, 1996	Liver	Frozen	80	-	NSRC	Unpublished
24. Oriksa River 2006	Pacific Coast, Honshu, Japan	Oct. 18, 2006	Fin	Frozen	100	-	NSRC	Unpublished
25. Otsuchi River	Pacific Coast, Honshu, Japan	Apr. 8, 1999	Muscle	Frozen	49	49	HU	1
26. Sakari River	Pacific Coast, Honshu, Japan	Nov. 19, 1997	Liver	Frozen	80	-	NSRC	Unpublished
27. Kesen-numa Okawa River	Pacific Coast, Honshu, Japan	Nov. 9, 2006	Fin	Frozen	100	-	NSRC	Unpublished
28. Koizumi River	Pacific Coast, Honshu, Japan	Nov. 21, 1996	Liver	Frozen	80	47	NSRC, HU	1
29. Uta River	Pacific Coast, Honshu, Japan	Oct. 27, 2006	Fin	Frozen	100	-	NSRC	Unpublished
30. Kawabukuro River	Japan Sea Coast, Honshu, Japan	Nov. 18, 1997	Liver	Frozen	54	30	NSRC, HU	1
31. Gakko River 1996	Japan Sea Coast, Honshu, Japan	Dec. 10, 1996	Liver	Frozen	45	45	NSRC, HU	1
31. Gakko River 2003	Japan Sea Coast, Honshu, Japan	Oct. 25, 2003	Liver	Frozen	80	-	NSRC	Unpublished
31. Gakko River 2006OCT	Japan Sea Coast, Honshu, Japan	Oct. 31, 2006	Liver	Frozen	80	74	NSRC	Unpublished
31. Gakko River 2006NOV	Japan Sea Coast, Honshu, Japan	Nov. 22, 2006	Liver	Frozen	80	78	NSRC	Unpublished
31. Gakko River 2006DEC	Japan Sea Coast, Honshu, Japan	Dec. 14, 2006	Liver	Frozen	80	78	NSRC	Unpublished
32. Miomote River	Japan Sea Coast, Honshu, Japan	Nov. 29, 2006	Fin	Frozen	100	-	NSRC	Unpublished
33. Uono River	Japan Sea Coast, Honshu, Japan	Oct. 23-24, 1996	Liver	Frozen	83	49	NSRC, HU	1
34. Jintsu River	Japan Sea Coast, Honshu, Japan	Nov. 7, 1995	Liver	Frozen	80	49	NSRC, HU	1
35. Shou River	Japan Sea Coast, Honshu, Japan	Nov. 24, 2006	Fin	Frozen	100	-	NSRC	Unpublished
36. Namde River 2000	Japan Sea Coast, Korea	Nov. 13, 2000	Liver	Frozen	-	46	HU	1
36. Namde River 2004	Japan Sea Coast, Korea	Oct. 8, 2004	Liver	Frozen	99	97	NSRC	Unpublished
37. Anadyr River 1990	Anadyr, Russia	1990	Liver	Frozen	-	45	HU	1
37. Anadyr River 1993	Anadyr, Russia	1993	-	DNA	-	33	HU	2
38. Apuka River	Kamchatka Peninsula, Russia	2002	Liver	Ethanol	-	50	HU	2
39. Olyutorskiy Bay	Kamchatka Peninsula, Russia	2002	Liver	Ethanol	-	50	HU	2
40. Tigil River	Kamchatka Peninsula, Russia	2002	Liver	Ethanol	-	44	HU	2
41. Hairusova River	Kamchatka Peninsula, Russia	1993	Liver	Frozen	-	41	HU	1
42. Kamchatka River 1990	Kamchatka Peninsula, Russia	1990	Liver	Ethanol	-	50	HU	2
42. Kamchatka River 1991	Kamchatka Peninsula, Russia	1991	Liver	Frozen	-	32	HU	1
43. Vorovskaya River	Kamchatka Peninsula, Russia	1990	Liver	Frozen	-	46	HU	1
44. Kol River	Kamchatka Peninsula, Russia	1991	Liver	Frozen	-	44	HU	1
45. Pymta River	Kamchatka Peninsula, Russia	2003	Fin	Ethanol	-	49	HU	2
46. Bolshaya Malki River	Kamchatka Peninsula, Russia	2001	Liver	Ethanol	-	50	HU	2

Table 1-continued.

River population	Region	Sampling date	Tissue	Preservation	Nc	Na	Archives	Reference
47. Utka River	Kamchatka Peninsula, Russia	2002	-	DNA	-	20	HU	2
48. Bolshaya River	Kamchatka Peninsula, Russia	1999	Liver	Ethanol	-	50	HU	2
49. Tymovo River	Sakhalin Island, Russia	2003	Liver	Ethanol	-	25	HU	2
50. Naiba River	Sakhalin Island, Russia	1995	-	DNA	-	16	HU	2
51. Belaya River	Sakhalin Island, Russia	2003	Liver	Ethanol	-	25	HU	2
52. Kalininka River 1994	Sakhalin Island, Russia	1994	Liver	Frozen	-	42	HU	1
52. Kalininka River 2003	Sakhalin Island, Russia	2003	Liver	Ethanol	-	25	HU	2
53. Taranay River	Sakhalin Island, Russia	2003	Liver	Ethanol	-	24	HU	2
54. Avakumovka River	Primorye, Russia	1994	Liver	Frozen	-	30	HU	1
55. Narva River	Primorye, Russia	1995			-	34	HU	2
56. Taui River	Magadan, Russia	1991	-	DNA	-	39	HU	2
57. Ola River 1990	Magadan, Russia	1990	Liver	Frozen	-	33	HU	1
57. Ola River 1991	Magadan, Russia	1991	Muscle	Ethanol	42	39	HU	Unpublished
58. Armanie River 1990	Magadan, Russia	1990	Liver	Frozen	-	37	HU	1
58. Armanie River 1991	Magadan, Russia	1991	-		-	42	HU	2
59. Okhotsk River	Magadan, Russia	2003	Liver	Ethanol	-	25	HU	2
60. Amur River	Nikolaevsk-na-Amure, Russia	Sep. 9, 2000	Muscle	Ethanol	-	50	HU	1
61. Salmon River	Northwest Alaska, United States	1991	Liver	Frozen	-	48	HU	1
62. Noatak River	Northwest Alaska, United States	1991	-	DNA	-	50	HU	2
63. Sheenjek River (fall-run)	Northwest Alaska, United States	1992	Liver	Frozen	-	45	HU	1
64. Tanana River	Northwest Alaska, United States	1993	Liver	Ethanol	-	50	HU	2
65. Toklat River	Northwest Alaska, United States	1992	Liver	Ethanol	100	50	HU	3
66. Unalakleet River	Northwest Alaska, United States	1992	-	DNA	-	50	HU	2
67. South Fork Kuskokwim River	Northwest Alaska, United States	1995	Liver	Ethanol	100	50	HU	3
68. Andraefsky River (summer-run)	Northwest Alaska, United States	1993	Liver	Frozen	-	45	HU	1
69. Kwethluk River	Northwest Alaska, United States	1994	Liver	Ethanol	-	50	HU	2
70. Upper Nushagak River	Northwest Alaska, United States	1993	Liver	Ethanol	-	49	HU	2
71. Togiak River	Northwest Alaska, United States	1993	Liver	Frozen	-	49	HU	1
72. Pelly River	Northwest Alaska, Canada	1993	Liver	Ethanol	84	50	HU	3
73. Donjek River	Northwest Alaska, Canada	1994	Liver	Ethanol	72	50	HU	3
74. Chulitna River	Southcentral Alaska, United States	1993	Liver	Ethanol	87	50	HU	3
75. Olsen Creek 1992	Southcentral Alaska, United States	1992	Liver	Frozen	-	45	HU	1
75. Olsen Creek 2004	Southcentral Alaska, United States	Jun. 8, 2004	Fin	Ethanol	70	50	HU	3
76. WHN Hatchery 1992	Southcentral Alaska, United States	1992	Liver	Ethanol	92	50	HU	3
76. WHN Hatchery 2002	Southcentral Alaska, United States	2002	Liver	Ethanol	100	50	HU	3
77. McNeil River	Southcentral Alaska, United States	1994	Liver	Ethanol	-	50	HU	2

Table 1-continued.

River population	Region	Sampling date	Tissue	Preservation	Nc	Na	Archives	Reference
78. Kitoi Hatchery	Southcentral Alaska, United States	1993	Liver	Ethanol	-	49	HU	2
79. Kizhuyak River	Southcentral Alaska, United States	1992	Liver	Frozen	-	44	HU	1
80. Chiginigak River	Alaska Peninsula, United States	1991	Liver	Ethanol	-	50	HU	2
81. Belkofski River	Alaska Peninsula, United States	1992	Liver	Frozen	-	46	HU	1
82. Joshua Green River	Alaska Peninsula, United States	1992	Liver	Ethanol	80	50	HU	3
83. Frosty Creek	Alaska Peninsula, United States	1992	Liver	Ethanol	100	50	HU	3
84. St. Catherine's Cove River	Alaska Peninsula, United States	1992	Liver	Ethanol	80	50	HU	3
85. Sawmill Creek	Southeast Alaska, United States	Jul. 28, 1993	Liver	Frozen	-	50	HU	1
86. Long Bay	Southeast Alaska, United States	Aug. 25-26, 1991	Liver	Frozen	-	49	HU	1
87. Whale Bay	Southeast Alaska, United States	Aug. 12, 1993	Liver	Frozen	-	48	HU	1
88. 9 Stream River	Southeast Alaska, United States	Jul. 8, 2004	Fin	DNA	80	44	HU	3
89. Little port Walter	Southeast Alaska, United States	Aug. 8, 2004	Fin	DNA	80	50	HU	3
90. Port Beauclerc	Southeast Alaska, United States	Aug. 20, 1995	Liver	Frozen	-	45	HU	1
91. Fish Creek	Southeast Alaska, United States	Sep. 25, 1988	Liver	Frozen	-	49	HU	1
92. Blossom River	Southeast Alaska, United States	1986	Liver	Ethanol	50	50	HU	3
93. Marten River	Southeast Alaska, United States	1986	Liver	Ethanol	50	50	HU	3
94. Disappearance Creek	Southeast Alaska, United States	Sep. 25, 1998	Liver	Frozen	-	50	HU	1
95. Ecstall River	British Columbia, Canada	Sep. 12, 1988	Liver	Frozen	-	45	HU	1
96. Bag Harbor	British Columbia, Canada	Mid-Oct. 1989	Liver	Frozen	-	50	HU	1
97. Nekite Channel	British Columbia, Canada	Sep. 15, 1989	Liver	Frozen	-	33	HU	1
98. Harrison River	British Columbia, Canada	2002	Operculum	DNA	50	50	HU	3
99. Vedder River	British Columbia, Canada	2002	Operculum	DNA	50	50	HU	3
100. Nanaimo River	British Columbia, Canada	2002	Operculum	DNA	50	32	HU	3
101. Nitinat River	British Columbia, Canada	1992	Blood	Ethanol	50	50	HU	3
102. Cowichan River	British Columbia, Canada	1997	Operculum	DNA	50	50	HU	3
103. Nooksack River	Washington, United States	1998	Liver	Frozen	-	47	HU	1
104. Quilcene Bay	Washington, United States	1998	Liver	Frozen	-	49	HU	1
105. Blackjack Creek	Washington, United States	1998	Liver	Frozen	-	50	HU	1
106. Satsop River	Washington, United States	1998	Liver	Frozen	-	49	HU	1
107. Hamilton Creek	Washington, United States	1998	Liver	Frozen	-	43	HU	1

***References: 1, Sato et al. (2004); 2, Yoon et al. (2004); 3, Yoon et al (2005).

Table 2. Mean estimated contribution for 1,000 bootstrap resampling where each region composes 100% of the mixtures (N=500 each). Bold italic cells denote correct regional allocation; Standard deviations (SD) are given in parentheses.

Regional allocation	Region		
	Japan	Russia	North America
Japan	<i>0.946 (0.035)</i>	0.045 (0.032)	0.009 (0.011)
Russia	0.016 (0.014)	<i>0.801 (0.086)</i>	0.182 (0.084)
North America	0.000 (0.000)	0.071 (0.034)	<i>0.928 (0.034)</i>

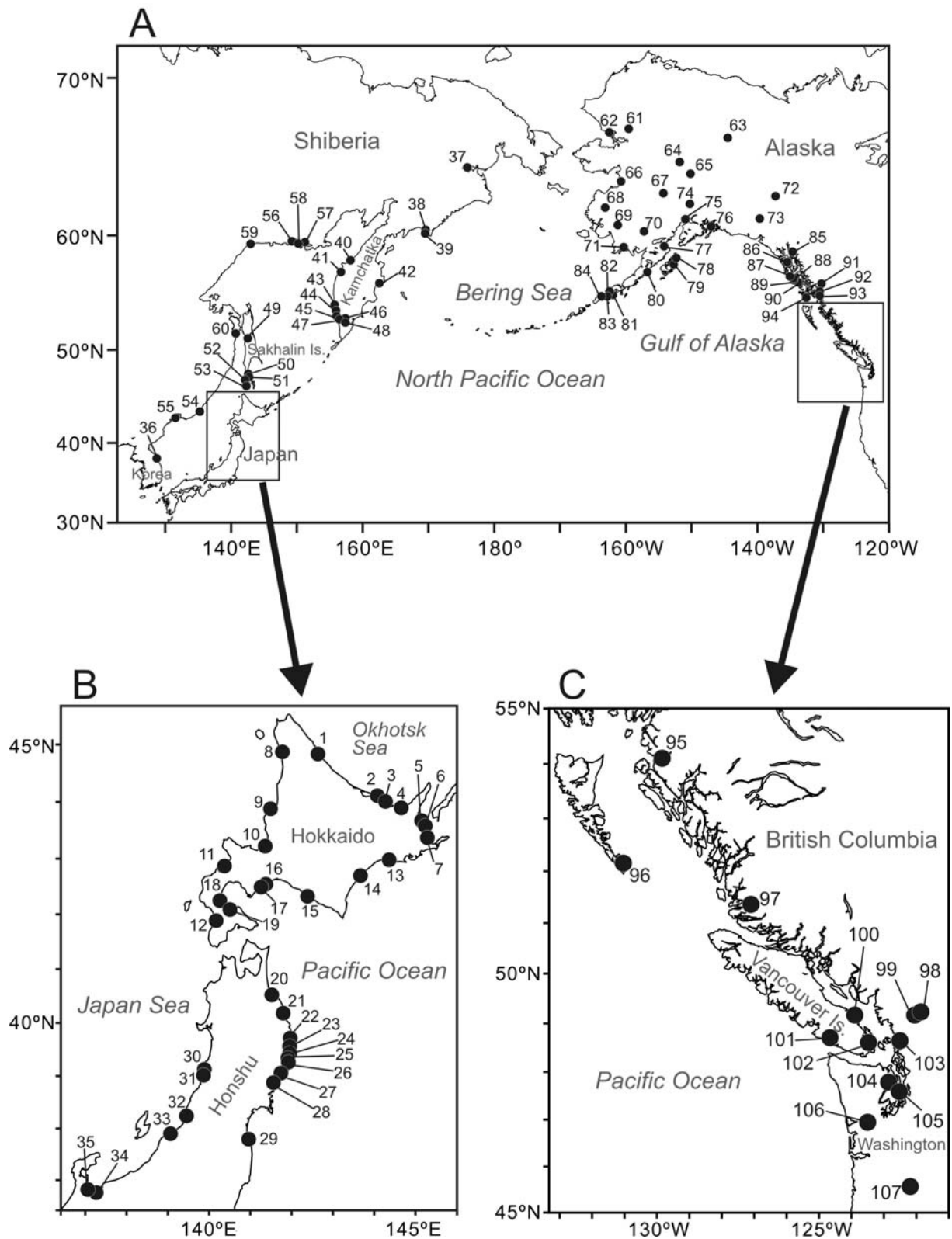


Fig.1. Sampling locations of chum salmon populations around the Pacific Rim. Numbers correspond to population names in Table 1. (A) Korea, Russia, and North America without British Columbia and Washington, (B) Japan, (C) British Columbia and Washington.

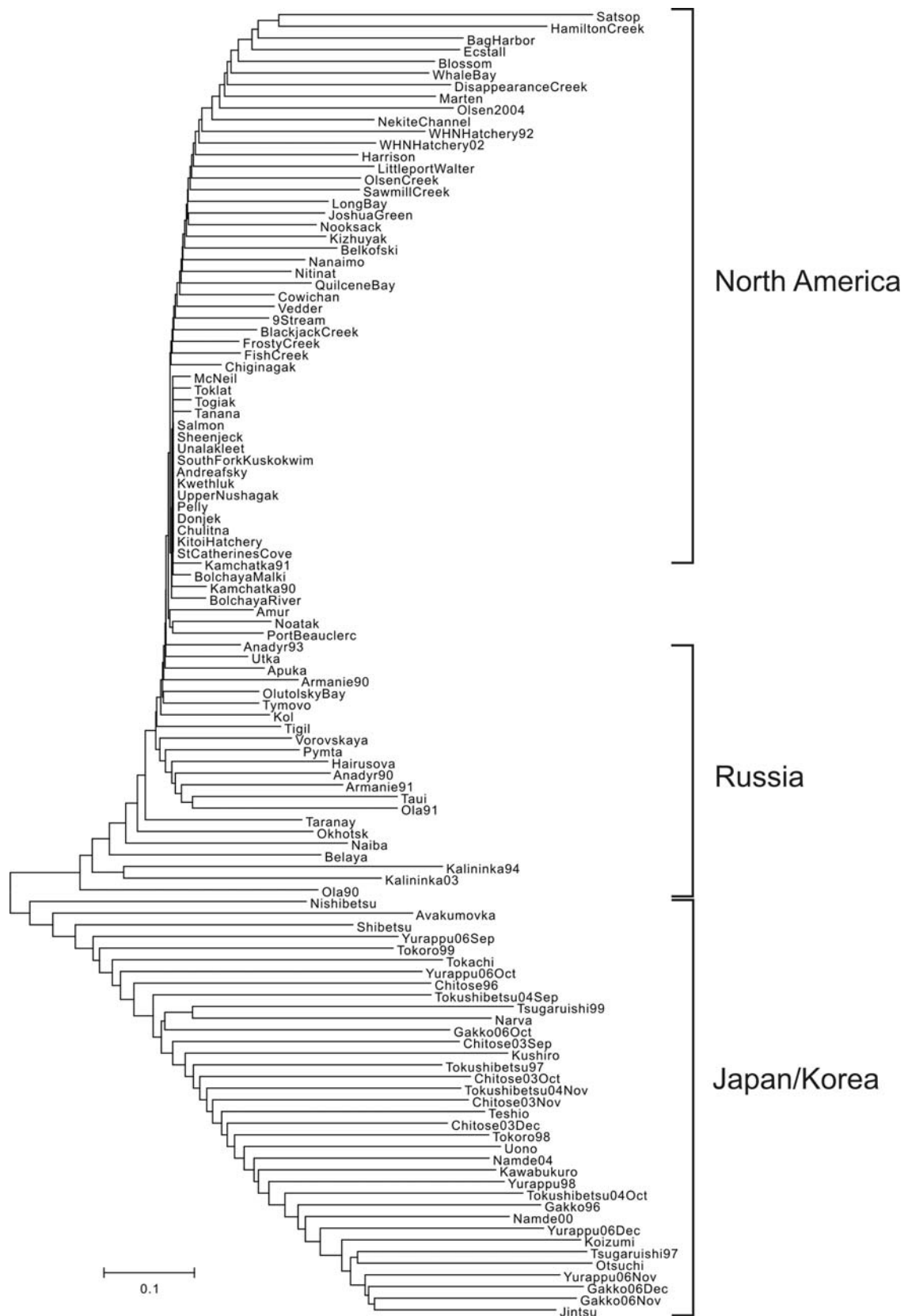


Fig.2. Neighbor-joining tree inferred from mtDNA data for the 112 sample groups from 88 populations of chum salmon. The 112 sample groups of chum salmon were grouped into three regions: Japan/Korea, Russia, and North America.

Appendix 1. Comparison of chum salmon baselines in different genetic stock identification methods. NSRC, National Salmon Resources Center, FRA; NMFS, National Marine Fisheries Service, NOAA; ABL Auke Bay Laboratory, Alaska Fisheries Science Center, NOAA; ADFG, Alaska Department of Fish and Game; IMB RAN, Institute of Marine Biology, Russian Academy of Sciences; SFWS, U.S. Fish & Wildlife Service.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
JAPAN							
Tokushibetsu River Early Run	Okhotsk Sea Coast, Hokkaido	120	NSRC-unpublished			31	Yoon et al. 2006
Tokushibetsu River Mid Run	Okhotsk Sea Coast, Hokkaido			80	Sato et al. 2004 NSRC-unpublished		
Tokushibetsu River Late Run	Okhotsk Sea Coast, Hokkaido			80	NSRC-unpublished		
Tokoro River Early Run	Okhotsk Sea Coast, Hokkaido			49	Sato et al. 2004	41	Yoon et al. 2006
Tokoro River Late Run	Okhotsk Sea Coast, Hokkaido			44	Sato et al. 2004	43	Yoon et al. 2006
Shari River	Okhotsk Sea Coast, Hokkaido	78	NSRC-unpublished				
Abashiri River	Okhotsk Sea Coast, Hokkaido	80	NSRC-unpublished				
Yubetsu River	Okhotsk Sea Coast, Hokkaido	40	NSRC-unpublished				
Shokotsu River	Okhotsk Sea Coast, Hokkaido	80	NSRC-unpublished				
Shibetsu River	Nemuro Stait, Hokkaido			76	NSRC-unpublished		
Nishibetsu River Early Run	Nemuro Stait, Hokkaido	80	NSRC-unpublished	41	Sato et al. 2004	58	Yoon et al. 2006
Nishibetsu River Late Run	Nemuro Stait, Hokkaido	80	NSRC-unpublished				
Teshio River	Japan Sea Coast, Hokkaido			74	NSRC-unpublished		
Chitose River Early Run	Japan Sea Coast, Hokkaido	80	NSRC-unpublished	76	NSRC-unpublished		
Chitose River Mid Run	Japan Sea Coast, Hokkaido	40	NSRC-unpublished	129	Sato et al. 2004 NSRC-unpublished	54	Yoon et al. 2006
Chitose River Late Run	Japan Sea Coast, Hokkaido	80	NSRC-unpublished	157	NSRC-unpublished		
Assabu River	Japan Sea Coast, Hokkaido	79	NSRC-unpublished				
Toshibetsu River	Japan Sea Coast, Hokkaido	80	NSRC-unpublished				
Kushiro River	Pacific Coast, Hokkaido	80	NSRC-unpublished	49	Sato et al. 2004	45	Yoon et al. 2006
Tokachi River Early Run	Pacific Coast, Hokkaido	80	NSRC-unpublished	46	Sato et al. 2004	57	Yoon et al. 2006
Tokachi River Late Run	Pacific Coast, Hokkaido	80	NSRC-unpublished				
Shizunai River	Pacific Coast, Hokkaido	80	NSRC-unpublished				
Shikiu River	Pacific Coast, Hokkaido	80	NSRC-unpublished				
Yurrapu River Early Run	Pacific Coast, Hokkaido	80	NSRC-unpublished	72	NSRC-unpublished		
Yurrapu River Mid Run	Pacific Coast, Hokkaido	80	NSRC-unpublished	77	NSRC-unpublished		
Yurrapu River Late Run	Pacific Coast, Hokkaido	80	NSRC-unpublished	190	Sato et al. 2004 NSRC-unpublished	48	Yoon et al. 2006
Mabuchi River	Pacific Coast, Aomori Pref., Honshu	40	NSRC-unpublished				
Akka River	Pacific Coast, Iwate Pref., Honshu	80	NSRC-unpublished				
Hei River	Pacific Coast, Iwate Pref., Honshu	45	NSRC-unpublished				
Tsugaruishi River Early Run	Pacific Coast, Iwate Pref., Honshu	40	NMFS/ABL-unpublished	47	Sato et al. 2004	44	Yoon et al. 2006

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
Tsugaruishi River Late Run	Pacific Coast, Iwate Pref., Honshu	80	NSRC-unpublished	44	Sato et al. 2004	64	Yoon et al. 2006
Orikasa River	Pacific Coast, Iwate Pref., Honshu	80	NSRC-unpublished				
Otsuchi River	Pacific Coast, Iwate Pref., Honshu			49	Sato et al. 2004		
Katagishi River	Pacific Coast, Iwate Pref., Honshu	40	NMFS/ABL-unpublished				
Katagishi River	Pacific Coast, Iwate Pref., Honshu	79	NSRC-unpublished				
Koizumi River	Pacific Coast, Miyagi Pref., Honshu	80	NSRC-unpublished	47	Sato et al. 2004	24	Yoon et al. 2006
Hasama River	Pacific Coast, Miyagi Pref., Honshu	80	NSRC-unpublished				
Naruse River	Pacific Coast, Miyagi Pref., Honshu	80	NSRC-unpublished				
Kido River	Pacific Coast, Fukushima Pref., Honshu	80	NSRC-unpublished				
Kuji River	Pacific Coast, Ibaraki Pref., Honshu	69	NSRC-unpublished				
Kawabukuro River	Japan Sea Coast, Akita, Honshu	80	NSRC-unpublished	30	Sato et al. 2004		
Gakko River Early Run	Japan Sea Coast, Yamagata Honshu	120	NSRC-unpublished	74	NSRC-unpublished		
Gakko River Mid Run	Japan Sea Coast, Yamagata Honshu			78	NSRC-unpublished		
Gakko River Late Run	Japan Sea Coast, Yamagata Honshu	180	NSRC-unpublished	123	Sato et al. 2004 NSRC-unpublished	48	Yoon et al. 2006
Uono River	Japan Sea Coast, Niigata, Honshu	80	NSRC-unpublished	49	Sato et al. 2004	49	Yoon et al. 2006
Kurobe River	Japan Sea Coast, Toyama, Honshu	80	NSRC-unpublished				
Jintsu River	Japan Sea Coast, Toyama, Honshu			49	Sato et al. 2004	50	Yoon et al. 2006
Sho River	Japan Sea Coast, Toyama, Honshu	80	NSRC-unpublished				
Tedori River	Japan Sea Coast, Ishikawa, Honshu	40	NSRC-unpublished				
Reidova River	Etorofu Island	77	ADFG/KamchatNIRO -unpublished				
Reidova Bay	Etorofu Island	76	ADFG/KamchatNIRO -unpublished				
Kons Bay		35	ADFG/KamchatNIRO -unpublished				
KOREA							
Namde River	Japan Sea Coast			145	Sato et al. 2004 NSRC-unpublished	43	Yoon et al. 2006
RUSSIA							
Anadyr River	Anadyr	183	Winans et al. 1994 Wilmot et al. 1994	43	Sato et al. 2004		
Anadyr River Early Run	Anadyr			33	Yoon et al. 2004		

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
Apuka River	Eastern Kamchatka			50	Yoon et al. 2004	47	Yoon et al. 2006
Olyutorskiy Bay	Eastern Kamchatka			50	Yoon et al. 2004	43	Yoon et al. 2006
Kamchatka River	Eastern Kamchatka	120	Winans et al. 1994	32	Sato et al. 2004		
Kamchatka River Early Run	Eastern Kamchatka			50	Yoon et al. 2004	48	Yoon et al. 2006
Bolshaya River	Eastern Kamchatka	102	ADFG/KamchatNIRO -unpublished	50	Yoon et al. 2004	45	Yoon et al. 2006
Bolshaya Malki River	Eastern Kamchatka			50	Yoon et al. 2004		
Bolshaya Hachery	Eastern Kamchatka					45	Yoon et al. 2006
Ossora	Eastern Kamchatka	90	Winans et al. 1994 NMFS/KamchatNIRO -unpublished				
Nerpichi Lake	Eastern Kamchatka	40	Winans et al. 1994				
Paratunka River	Eastern Kamchatka	100	ADFG/KamchatNIRO -unpublished				
Tigil River	Western Kamchatka			44	Yoon et al. 2004	42	Yoon et al. 2006
Harusova River	Western Kamchatka	202	Winans et al. 1994 NMFS/ABL/KamchatNIRO -unpublished	41	Sato et al. 2004	46	Yoon et al. 2006
Vororskaya	Western Kamchatka	146	NMFS/ABL/KamchatNIRO -unpublished	46	Sato et al. 2004	40	Yoon et al. 2006
Kol River	Western Kamchatka	93	Winans et al. 1994	44	Sato et al. 2004		
Pymta River	Western Kamchatka	80	Winans et al. 1994 NMFS/ABL/KamchatNIRO -unpublished	49	Yoon et al. 2004	47	Yoon et al. 2006
Utka River	Western Kamchatka	79	Winans et al. 1994	20	Yoon et al. 2004		
Kikchik River	Western Kamchatka	40	Winans et al. 1994				
Bistraya River , Ozerki Hatchery	Western Kamchatka	150	ADFG/KamchatNIRO -unpublished			48	Yoon et al. 2006
Palana River	Western Kamchatka	98	ADFG/KamchatNIRO -unpublished				
Tymovo River	Sakhalin Island			25	Yoon et al. 2004		
Naiba River	Sakhalin Island	100	Wilmot et al. 1995, NMFS/ABL/KamchatNIRO/ TINRO-Center-unpublished	16	Yoon et al. 2004		
Belaya River	Sakhalin Island	40	NMFS/ABL/KamchatNIRO -unpublished	25	Yoon et al. 2004	22	Yoon et al. 2006

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
Kalininka	Sakhalin Island	49	Wilmot et al. 1995	42	Sato et al. 2004	46	Yoon et al. 2006
Kalininka River Early Run	Sakhalin Island			25	Yoon et al. 2004	19	Yoon et al. 2006
Taranay River	Sakhalin Island			25	Yoon et al. 2004	25	Yoon et al. 2006
Udarnitsa River	Sakhalin Island	98	Wilmot et al. 1995 IMB RAN/TINRO-Center -unpublished			23	Yoon et al. 2006
Tym River	Sakhalin Island	56	NMFS/ABL/KamchatNIRO -unpublished			24	Yoon et al. 2006
Avakumovka River	Primorye/Suifen	35	Wilmot et al. 1995 IMB RAN/TINRO-Center -unpublished	30	Sato et al. 2004	32	Yoon et al. 2006
Avakumovka River Early Run	Primorye/Suifen			26	Yoon et al. 2004		
Narva	Primorye/Suifen		Wilmot et al. 1995 IMB RAN/TINRO-Center -unpublished	34	Yoon et al. 2004		
Ryzanovka	Primorye/Suifen	51	Wilmot et al. 1995 IMB RAN/TINRO-Center -unpublished				
Suifen (Dongning Hatchery)	Primorye/Suifen	25	Wilmot et al. 1995 NMFS/ABL-unpublished				
Tau	Magadan			39	Yoon et al. 2004		
Ola River	Magadan	80	Winans et al. 1994	33	Sato et al. 2004		
Ola River Early Run	Magadan			42	HU-unpublished		
Armanie River	Magadan			37	Sato et al. 2004		
Armanie River Early Run	Magadan			42	Yoon et al. 2004		
Okhotsk River	Magadan			25	Yoon et al. 2004		
Tumani River 1991	Ohkotsk Sea	66	Winans et al. 1994				
Oklan	Ohkotsk Sea	77	NMFS/ABL/KamchatNIRO -unpublished				
Penzhina	Ohkotsk Sea	50	NMFS/ABL/KamchatNIRO -unpublished				
Amur River Summer	Nikolaevsk-na-Amure	100	NSRC-unpublished				
Amur River fall run, Heilong	Nikolaevsk-na-Amure	150	Wilmot et al. 1995 Urawa et al. 2002 ADFG/KamchatNIRO -unpublished	50	Sato et al. 2004		

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
North America							
Peel River	Arctic	38	NMFS/ABL-unpublished				
Noatak River	Kotzebue Sound	400	Seeb and Crane 1999	50	Yoon et al. 2004	38	Yoon et al. 2006
			NMFS/ABL unpublished				
Kelly Lake	Kotzebue Sound	100	Seeb and Crane 1999				
Kobuk River	Kotzebue Sound	206	Seeb and Crane 1999				
Salmon River	Kotzebue Sound			48	Sato et al. 2004	48	Yoon et al. 2006
Pilgrim River	North Sound	90	Seeb and Crane 1999				
N. Norton Sound	North Sound	404	Seeb et al. 1997				
			Seeb and Crane 1999				
SW Norton Sound	North Sound	300	Seeb and Crane 1999				
Unalakleet River	North Sound			50	Yoon et al. 2004	48	Yoon et al. 2006
Andreafsky River	Yukon River-Summer	200	Seeb and Crane 1999	45	Sato et al. 2004	48	Yoon et al. 2006
Innoko River	Yukon River-Summer	88	Seeb and Crane 1999				
Anvik River	Yukon River-Summer	650	Seeb and Crane 1999				
Kaltag River	Yukon River-Summer	100	Crane et al. 2001				
Nulato River	Yukon River-Summer	100	Seeb et al. 1997				
Upper Koyukuk River	Yukon River-Summer	362	Seeb et al. 1997				
			¹ USFWS-unpublished				
			NMFS/ABL-unpublished				
Lower Koyukuk River	Yukon River-Summer	400	Seeb et al. 1997				
			Seeb and Crane 1999				
			Crane et al. 2001				
Melozitna River	Yukon River-Summer	100	Seeb et al. 2001				
Tozitna River	Yukon River-Summer	71	USFWS-unpublished				
Chena River	Yukon River-Summer	186	Seeb et al. 1997				
			Seeb and Crane 1999				
Salcha River	Yukon River-Summer	200	Seeb et al. 1997				
			Seeb and Crane 1999				
Black River	Yukon River-Summer	96	USFWS-unpublished				
			NMFS/ABL-unpublished				
Toklat River	Yukon River-Fall Run	815	Sarfin 1995	50	Yoon et al. 2005	47	Yoon et al. 2006
			Seeb et al. 1997				
			Seeb and Crane 1999				
Upper Tanana River	Yukon River-Fall Run	597	Seeb et al. 1997	50	Yoon et al. 2004	49	Yoon et al. 2006
			Seeb and Crane 1999				

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
Sheenjek River	Yukon River-Fall Run	164	Seeb and Crane 1999	45	Sato et al. 2004	42	Yoon et al. 2006
Fishing Branch	Yukon River-Fall Run	200	Crane et al. 1992 Seeb et al. 1997				
Big Creek	Yukon River-Fall Run	200	Crane et al. 1992 Seeb et al. 1997				
Tatchun	Yukon River-Fall Run	98	Crane et al. 1992				
Pelly River	Yukon River-Fall Run	84	Seeb et al 1997	50	Yoon et al. 2005	45	Yoon et al. 2006
White River	Yukon River-Fall Run	174	Crane et al. 1992 Seeb et al. 1997				
Teslin River	Yukon River-Fall Run	100	Crane et al. 1992				
Donjek River	Yukon River-Fall Run			50	Yoon et al. 2005	42	Yoon et al. 2006
Kwethluk River	Kuskokwim Bay and Lower River	100	Seeb and Crane 1999	50	Yoon et al. 2004	46	Yoon et al. 2006
Kasigluk River	Kuskokwim Bay and Lower River	70	Seeb et al 1997				
Kisaralik River	Kuskokwim Bay and Lower River	100	Seeb et al 1997				
Tuluksak River	Kuskokwim Bay and Lower River	100	Seeb and Crane 1999				
Aniak River	Kuskokwim Bay and Lower River	100	Seeb and Crane 1999				
Holokuk River	Kuskokwim Bay and Lower River	48	Seeb et al 1997				
Oskawalik River	Kuskokwim Bay and Lower River	58	Seeb et al 1997				
George River	Kuskokwim Bay and Lower River	100	Seeb et al 1997				
Kogruklu River	Kuskokwim Bay and Lower River	125	Seeb and Crane 1999				
Stoney River early and late	Kuskokwim Bay and Lower River	156	Seeb et al 1997				
Tatlawiksuk River	Kuskokwim Bay and Lower River	100	Seeb and Crane 1999				
Nunsatuk River	Kuskokwim Bay and Lower River	100	Seeb et al 1997				
4 th of July Creek	Kuskokwim Bay and Lower River	100	Seeb and Crane 1999				
Kuskokwim River at McGrath	Kuskokwim Bay and Lower River	100	Seeb et al 1997				
Kanektok River	Kuskokwim Bay and Lower River	214	Wilmot et al. 1994 Seeb et al. 1997 Seeb and Crane 1999				
Goodnews River	Kuskokwim Bay and Lower River	100	Seeb and Crane 1999				
South Fork Kuskokwim-late	Kuskokwim River-Upper	100	Seeb et al 1997	50	Yoon et al. 2005	47	Yoon et al. 2006
Big River	Kuskokwim River-Upper	100	Seeb et al 1997				
Togiak River	Bristol Bay	200	Seeb and Crane 1999	49	Sato et al. 2004	46	Yoon et al. 2006
Upper Nushagak River	Bristol Bay	103	Seeb and Crane 1999	49	Yoon et al. 2004	49	Yoon et al. 2006
Mulchatna River	Bristol Bay	100	Seeb and Crane 1999				
Stuyahok River	Bristol Bay	88	Seeb and Crane 1999				
Alagnak River	Bristol Bay	84	Seeb and Crane 1999				

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
Naknek/Big Creek	Bristol Bay	80	Seeb and Crane 1999				
Egegik Bay/Whale Mountain Creek	Bristol Bay	98	Seeb and Crane 1999				
Ugashik Bay/Pumice Creek	Bristol Bay	100	Seeb and Crane 1999				
Meshik/Plenty Bear Creek	Bristol Bay	93	Seeb and Crane 1999				
Meshik/Braided Creek	Bristol Bay	78	Seeb and Crane 1999				
Wiggly Creek	North Alaska Peninsula	100	Seeb et al. 1997				
First stream N. of Cape Seniavin	North Alaska Peninsula	55	ADFG-unpublished				
Franks Lagoon	North Alaska Peninsula	18	ADFG-unpublished				
Right Hand Moller Bay	North Alaska Peninsula	100	ADFG-unpublished				
Lawrence Valley Creek	North Alaska Peninsula	100	Seeb and Crane 1999				
Moffitt Creek	North Alaska Peninsula	100	Seeb et al. 1997				
Joshua Green River	North Alaska Peninsula	180	Seeb and Crane 1999 Seeb et al. 1997	50	Yoon et al. 2005	42	Yoon et al.2006
Frosty Creek	North Alaska Peninsula	100	Seeb and Crane 1999	50	Yoon et al. 2005	44	Yoon et al. 2006
Alligator Hole	North Alaska Peninsula	100	Seeb et al. 1997				
Trader's Cove Creek	North Alaska Peninsula	100	Seeb and Crane 1999				
St. Catherine's Cove	North Alaska Peninsula	80	Seeb and Crane 1999	50	Yoon et al. 2005	46	Yoon et al. 2006
Peterson Lagoon	North Alaska Peninsula	86	Seeb and Crane 1999				
Little John Lagoon	South Alaska Peninsula	87	Seeb and Crane 1999				
Sandy Cove	South Alaska Peninsula	100	Seeb et al. 1997				
Russell Creek	South Alaska Peninsula	200	Seeb et al. 1997 Seeb and Crane 1999				
Delta Creek	South Alaska Peninsula	100	Seeb et al. 1997				
Belkofski River	South Alaska Peninsula	87	Seeb and Crane 1999	46	Sato et al. 2004	46	Yoon et al. 2006
Volcano Bay	South Alaska Peninsula	106	Seeb et al. 1997				
Ruby's Lagoon	South Alaska Peninsula	100	Seeb et al. 1997				
Canoe Bay	South Alaska Peninsula	100	Seeb and Crane 1999				
Zachary Bay	South Alaska Peninsula	80	Seeb et al. 1997				
Coleman Creek	South Alaska Peninsula	100	Seeb et al. 1997				
Foster Creek (Balboa Bay)	South Alaska Peninsula	100	Seeb et al. 1997				
Chichigof Bay	South Alaska Peninsula	100	Seeb et al. 1997				
Stepovak Bay	South Alaska Peninsula	50	Seeb et al. 1997				
Stepovak River	South Alaska Peninsula	100	Seeb and Crane 1999				
Ivanoff River	South Alaska Peninsula	94	Seeb and Crane 1999				
Portage Creek	South Alaska Peninsula	100	Seeb et al. 1997				
North Fork River	South Alaska Peninsula	72	Seeb et al. 1997				

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
North Fork Creek	South Alaska Peninsula	100	Seeb et al. 1997				
Main Creek	South Alaska Peninsula	92	Seeb et al. 1997				
Chiginigak River	South Alaska Peninsula	75	Seeb and Crane 1999	50	Yoon et al. 2004	45	Yoon et al. 2006
Wide Bay	South Alaska Peninsula	100	Seeb and Crane 1999				
E. Bear Bay Creek	South Alaska Peninsula	100	Seeb et al. 1997				
Alagoshak River	South Alaska Peninsula	95	Seeb and Crane 1999				
Gull Cape Creek	South Alaska Peninsula	100	Seeb et al. 1997				
Big River	South Alaska Peninsula	100	Seeb and Crane 1999				
McNeil River	South Alaska Peninsula	109	Seeb et al. 1997	50	Yoon et al. 2004	49	Yoon et al. 2006
American River	Kodiak Island	145	Seeb et al. 1997				
Dog Bay	Kodiak Island	100	Seeb et al. 1997				
Big Sukhoi Creek	Kodiak Island	100	Seeb and Crane 1999				
Sturgeon River	Kodiak Island	171	Seeb et al. 1997 Seeb and Crane 1999				
Uganik River	Kodiak Island	100	Seeb et al. 1997				
Kizhuyak River	Kodiak Island	138	Seeb and Crane 1999 NMFS/ABL-unpublished	44	Sato et al. 2004	40	Yoon et al. 2006
Kitoy Hatchery	Kodiak Island			50	Yoon et al. 2004	45	Yoon et al. 2006
Little Susitna	Susitna River	79	NMFS/ABL-unpublished				
Yentna/Lake Creek 1996	Susitna River	100	Seeb et al. 1997				
Chulitna, Susitna River	Susitna River	237	Seeb et al. 1997 Seeb and Crane 1999	50	Yoon et al. 2005	45	Yoon et al. 2006
WHN Hatchery	Prince William Sound	92	Seeb and Crane 1999	192	Yoon et al. 2005	86	Yoon et al. 2006
Wells River	Prince William Sound	100	Seeb et al. 1997				
Olsen Creek	Prince William Sound	350	Seeb et al. 1997 NMFS/ABL-unpublished	95	Sato et al. 2004 Yoon et al. 2005	88	Yoon et al. 2006
Constantine Creek	Prince William Sound	100	Seeb et al. 1997				
Keta Creek	Prince William Sound	100	Seeb et al. 1997				
Alsek River, lower river slough	Northern Southeast Alaska	100	NMFS/ABL-unpublished				
Lynn Brothers	Northern Southeast Alaska	50	Kondzela et al. 1994				
Excursion River	Northern Southeast Alaska		NMFS/ABL-unpublished				
Tyndall	Northern Southeast Alaska	102	NMFS/ABL-unpublished				
Vivid	Northern Southeast Alaska	100	NMFS/ABL-unpublished				
Well's Bridge	Northern Southeast Alaska	90	NMFS/ABL-unpublished				
Herman Creek	Northern Southeast Alaska	159	Kondzela et al. 1994				
Sawmill Creek	Northern Southeast Alaska	100	NMFS/ABL-unpublished	50	Sato et al. 2004	45	Yoon et al. 2006

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
Eagle River	Northern Southeast Alaska	54	NMFS/ABL-unpublished				
Taku River, lower river slough	Northern Southeast Alaska	45	NMFS/ABL-unpublished				
Lace River	Northern Southeast Alaska	25	NMFS/ABL-unpublished				
Donkey Creek	Northern Southeast Alaska	162	Kondzela et al. 1994				
Pybus Bay East	Northern Southeast Alaska	65	Kondzela et al. 1994				
Eliza Harbor	Northern Southeast Alaska	61	Kondzela et al. 1994				
Chaik Bay	Northern Southeast Alaska	100	NMFS/ABL-unpublished				
Hood Bay, N arm, E head	Northern Southeast Alaska	110	NMFS/ABL-unpublished				
Greens Creek	Northern Southeast Alaska	100	NMFS/ABL-unpublished				
Humpback Creek	Northern Southeast Alaska	102	NMFS/ABL-unpublished				
Spasski Creek	Northern Southeast Alaska	145	NMFS/ABL-unpublished				
Saltery River	Northern Southeast Alaska	101	NMFS/ABL-unpublished				
Black Bay, N head	Northern Southeast Alaska	200	NMFS/ABL-unpublished				
Ford Arm	Northern Southeast Alaska	71	NMFS/ABL-unpublished				
Ushk Bay	Northern Southeast Alaska	145	NMFS/ABL-unpublished				
Kennel Creek	Northern Southeast Alaska	100	NMFS/ABL-unpublished				
Long Bay	Northern Southeast Alaska	169	NMFS/ABL-unpublished	49	Sato et al. 2004	48	Yoon et al. 2006
Lover's Cove Creek	Northern Southeast Alaska	131	Kondzela et al. 1994				
Saook Bay	Northern Southeast Alaska	103	NMFS/ABL-unpublished				
W. Crawfish Inlet	Northern Southeast Alaska	105	NMFS/ABL-unpublished				
Whale Bay, head of Great Arm	Northern Southeast Alaska	100	NMFS/ABL-unpublished	48	Sato et al. 2004	46	Yoon et al. 2006
Irish Creek	Northern Southeast Alaska	98	Kondzela et al. 1994				
Saginaw Creek	Northern Southeast Alaska	97	NMFS/ABL-unpublished				
Port Camden	Northern Southeast Alaska	100	NMFS/ABL-unpublished				
No Name Bay	Northern Southeast Alaska	84	NMFS/ABL-unpublished				
Port Beauclerc	Northern Southeast Alaska	100	NMFS/ABL-unpublished	45	Sato et al. 2004	45	Yoon et al. 2006
Gastineau Hatchery	Northern Southeast Alaska	300	NMFS/ABL-unpublished				
Sheep Creek Hatchery	Northern Southeast Alaska	100	NMFS/ABL-unpublished				
Hidden Falls Hatchery	Northern Southeast Alaska	150	NMFS/ABL-unpublished				
Medvejie Hatchery	Northern Southeast Alaska	150	NMFS/ABL-unpublished				
Little Port Walter	Northern Southeast Alaska			50	Yoon et al. 2005	40	Yoon et al. 2006
9 Stream River	Northern Southeast Alaska			44	Yoon et al. 2005		
Herman River	Southeast Mainland Alaska	100	Kondzela et al. 1994				
North Arm Creek	Southeast Mainland Alaska	81	Kondzela et al. 1994				
Harding River	Southeast Mainland Alaska	95	Kondzela et al. 1994				
Fish Creek	Southeast Mainland Alaska	202	Kondzela et al. 1994	49	Sato et al. 2004	49	Yoon et al. 2006

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
Tombstone River	Southeast Mainland Alaska	98	Kondzela et al. 1994				
Marten River	Southeast Mainland Alaska	105	Kondzela et al. 1994	50	Yoon et al. 2005	50	Yoon et al. 2006
Keta River	Southeast Mainland Alaska	101	Kondzela et al. 1994				
Blossom River	Southeast Mainland Alaska	101	Kondzela et al. 1994	50	Yoon et al. 2005	50	Yoon et al. 2006
Wilson River	Southeast Mainland Alaska	103	Kondzela et al. 1994				
Traitor River	Southeast Mainland Alaska	100	Kondzela et al. 1994				
Carroll River	Southeast Mainland Alaska	100	Kondzela et al. 1994				
Portage Creek	Southeast Mainland Alaska	173	Kondzela et al. 1994				
King Creek	Southeast Mainland Alaska	152	Kondzela et al. 1994				
Klahini River	Southeast Mainland Alaska	102	Kondzela et al. 1994				
Eulachon Creek	Southeast Mainland Alaska	90	Kondzela et al. 1994				
Grant Creek	Southeast Mainland Alaska	127	Kondzela et al. 1994				
Neets Bay Hatchery (summer)	Southeast Mainland Alaska	150	NMFS/ABL-unpublished				
Kugel Creek	Prince of Wales Island	104	Kondzela et al. 1994				
Aiken Creek	Prince of Wales Island	100	Kondzela et al. 1994				
Disappearance Creek	Prince of Wales Island	300	Kondzela et al. 1994 NMFS/ABL-unpublished	50	Sato et al. 2004	50	Yoon et al. 2006
Lagoon Creek	Prince of Wales Island	102	Kondzela et al. 1994				
Old Tom Creek	Prince of Wales Island	195	Kondzela et al. 1994 NMFS/ABL-unpublished				
Cabin Creek	Prince of Wales Island	103	Kondzela et al. 1994				
Karta River	Prince of Wales Island	100	Kondzela et al. 1994				
Coco Harbor	Prince of Wales Island	200	Kondzela et al. 1994 NMFS/ABL-unpublished				
Breezy Bay	Prince of Wales Island	254	Kondzela et al. 1994				
Port Real Marina	Prince of Wales Island	58	NMFS/ABL-unpublished				
Cruz Cove	Prince of Wales Island	258	Kondzela et al. 1994 NMFS/ABL-unpublished				
Neets Bay Hatchery (fall)	Prince of Wales Island	150	NMFS/ABL-unpublished				
Awun River	Queen Charlotte Islands	75	Kondzela et al. 1994				
Mace Creek	Queen Charlotte Islands	100	Kondzela et al. 1994				
Peel Creek	Queen Charlotte Islands	100	Kondzela et al. 1994				
Tasu Creek	Queen Charlotte Islands	100	Kondzela et al. 1994				
Lagins Creek	Queen Charlotte Islands	91	Kondzela et al. 1994				
Deena River	Queen Charlotte Islands	82	Kondzela et al. 1994				
Lagoon Creek	Queen Charlotte Islands	83	Kondzela et al. 1994				

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
Salmon River	Queen Charlotte Islands	57	Kondzela et al. 1994				
Sedgewick Creek	Queen Charlotte Islands	74	Kondzela et al. 1994				
Bag Harbor Creek	Queen Charlotte Islands	89	Kondzela et al. 1994	50	Sato et al. 2004	47	Yoon et al. 2006
Surprise Creek	Queen Charlotte Islands	85	Kondzela et al. 1994				
Kshwan River	Northern British Columbia	88	Kondzela et al. 1994				
Kitsault River	Northern British Columbia	95	Kondzela et al. 1994				
Stagoo Creek	Northern British Columbia	128	Kondzela et al. 1994				
Khutzeymateen	Northern British Columbia	94	Kondzela et al. 1994				
Toon River	Northern British Columbia	98	Kondzela et al. 1994				
Kitimat River/Mussel	Northern British Columbia	123	Kondzela et al. 1994				
Neekas Creek	Northern British Columbia	54	Kondzela et al. 1994				
Klownik Creek	Northern British Columbia	100	Kondzela et al. 1994				
Nekite River and Channel	Northern British Columbia	197	Kondzela et al. 1994	33	Sato et al. 2004	48	Yoon et al. 2006
Ecstall River	Northern British Columbia			45	Sato et al. 2004	48	Yoon et al. 2006
Nanaimo River	Georgia Strait	100	Phelps et al. 1994	32	Yoon et al. 2005		
Cowichan River	Georgia Strait	100	Phelps et al. 1994				
Cowichan Bay	Georgia Strait			50	Yoon et al. 2005	37	Yoon et al. 2006
Chemainus River	Georgia Strait	100	Phelps et al. 1994				
Goldstream River	Georgia Strait	100	Phelps et al. 1994				
Big Qualicum FH	Georgia Strait	200	Phelps et al. 1994				
Little Qualicum	Georgia Strait	100	Phelps et al. 1994				
Puntledge FH	Georgia Strait	100	Phelps et al. 1994				
Cheakamus River	Georgia Strait	100	Phelps et al. 1994				
Indian Arm	Georgia Strait	100	Phelps et al. 1994				
Mamquam River	Georgia Strait	100	Phelps et al. 1994				
Tzoonie River	Georgia Strait	100	Phelps et al. 1994				
Sliammon FH	Georgia Strait	100	Phelps et al. 1994				
Alouette River	Fraser River	100	Phelps et al. 1994				
Stave River	Fraser River	100	Phelps et al. 1994				
Chilliwack-Vedder FH	Fraser River	100	Phelps et al. 1994	50	Yoon et al. 2005	43	Yoon et al. 2006
Chehalis at Harrison FH	Fraser River	100	Phelps et al. 1994				
Inch Creek FH	Fraser River	100	Phelps et al. 1994				
Weaver River	Fraser River	100	Phelps et al. 1994				
Chehalis FH	Fraser River	100	Phelps et al. 1994				
Harrison River	Fraser River	100	Phelps et al. 1994	50	Yoon et al. 2005	25	Yoon et al. 2006
Squakum Creek	Fraser River	100	Phelps et al. 1994				

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
Wahleach Creek	Fraser River	100	Phelps et al. 1994				
Nitinat River and FH	West Coast Vancouver Island	380	Phelps et al. 1994	50	Yoon et al. 2005	41	Yoon et al. 2006
Nahmint River	West Coast Vancouver Island	100	Phelps et al. 1994				
Sarita River	West Coast Vancouver Island	127	Phelps et al. 1994				
Sooke River	Strait of Juan de Fuca	100	Phelps et al. 1994				
Deep Creek	Strait of Juan de Fuca	100	Phelps et al. 1994				
Lyre River	Strait of Juan de Fuca	100	Phelps et al. 1994				
Pysht River	Strait of Juan de Fuca	255	Phelps et al. 1994				
Squire Creek	Northern Puget Sound	140	Phelps et al. 1994				
Jim Creek	Northern Puget Sound	151	Phelps et al. 1994				
Fortson Creek	Northern Puget Sound	211	Phelps et al. 1994				
N.F. Stillaguamish	Northern Puget Sound	140	Phelps et al. 1994				
Skagit River	Northern Puget Sound	100	Phelps et al. 1994				
Illabot Creek	Northern Puget Sound	98	Phelps et al. 1994				
Dan Creek	Northern Puget Sound	153	Phelps et al. 1994				
Finney Creek	Northern Puget Sound	41	Phelps et al. 1994				
Samish FH	Northern Puget Sound	100	Phelps et al. 1994				
Thomas Creek	Northern Puget Sound	101	Phelps et al. 1994				
Bob Smith Creek	Northern Puget Sound	100	Phelps et al. 1994				
Nooksack River	Northern Puget Sound	36	Phelps et al. 1994	47	Sato et al. 2004	49	Yoon et al. 2006
Kendall FH	Northern Puget Sound	200	Phelps et al. 1994				
Maple Creek	Northern Puget Sound	100	Phelps et al. 1994				
Chuckanut Creek	Northern Puget Sound	35	Phelps et al. 1994				
Schoolhouse Slough	Northern Puget Sound	100	Phelps et al. 1994				
Wallace River	Northern Puget Sound	144	Phelps et al. 1994				
Skykomish River	Northern Puget Sound	200	Phelps et al. 1994				
Swift Creek	Southern Puget Sound	250	Phelps et al. 1994				
Perry Creek	Southern Puget Sound	350	Phelps et al. 1994				
Elson Creek	Southern Puget Sound	350	Phelps et al. 1994				
Carbon River	Southern Puget Sound	150	Phelps et al. 1994				
Fennel Creek	Southern Puget Sound	100	Phelps et al. 1994				
Skookum Creek	Southern Puget Sound	100	Phelps et al. 1994				
Little Creek	Southern Puget Sound	51	Phelps et al. 1994				
Reitdorf Creek	Southern Puget Sound	50	Phelps et al. 1994				
Upper Skookum Creek	Southern Puget Sound	100	Phelps et al. 1994				
Sherwood, Rocky, Coulter Cks,	Southern Puget Sound	106	Phelps et al. 1994				

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
Mill Creek	Southern Puget Sound	176	Phelps et al. 1994				
Kennedy Creek	Southern Puget Sound	200	Phelps et al. 1994				
Goldsborough Creek	Southern Puget Sound	200	Phelps et al. 1994				
Chico Creek	Southern Puget Sound	196	Phelps et al. 1994				
Gorst Creek	Southern Puget Sound	100	Phelps et al. 1994				
Johns Creek FH	Southern Puget Sound	100	Phelps et al. 1994				
Sherwood Creek	Southern Puget Sound	100	Phelps et al. 1994				
Coulter Creek FH	Southern Puget Sound	100	Phelps et al. 1994				
Blackjack Creek	Southern Puget Sound	100	Phelps et al. 1994	50	Sato et al. 2004		
Chambers Creek FH	Southern Puget Sound	100	Phelps et al. 1994				
Nisqually River	Southern Puget Sound	300	Phelps et al. 1994				
Big Mission Creek	Hood Canal	67	Phelps et al. 1994				
Little Mission Creek	Hood Canal	53	Phelps et al. 1994				
Big Beef Creek	Hood Canal	100	Phelps et al. 1994				
Dewatto River	Hood Canal	263	Phelps et al. 1994				
Tahuya River	Hood Canal	83	Phelps et al. 1994				
Enetai FH	Hood Canal	100	Phelps et al. 1994				
Hood Canal FH	Hood Canal	450	Phelps et al. 1994				
Lilliwaup Creek	Hood Canal	100	Phelps et al. 1994				
Walcott FH	Hood Canal	100	Phelps et al. 1994				
McKernan FH	Hood Canal	100	Phelps et al. 1994				
Big Quilcene FH	Hood Canal	100	Phelps et al. 1994				
Vance	Hood Canal	101	Phelps et al. 1995				
NF. Skokomish	Hood Canal	111	Phelps et al. 1996				
Hamma Hamma River	Hood Canal	186	Phelps et al. 1994				
Dosewallips River	Hood Canal	105	Phelps et al. 1994				
Tulalip FH GM	Hood Canal	197	Phelps et al. 1994				
Salmon Creek	Hood Canal Summer	50	Phelps et al. 1994				
Snow Creek	Hood Canal Summer	50	Phelps et al. 1994				
Jimmycomelately Creek	Hood Canal Summer	100	Phelps et al. 1994				
Union River Summer	Hood Canal Summer	103	Phelps et al. 1997				
Duckabush River Summer	Hood Canal Summer	125	Phelps et al. 1998				
Dosewallips River Summer	Hood Canal Summer	102	Phelps et al. 1994				
Hamma Hamma River Summer	Hood Canal Summer	37	Phelps et al. 1994				
Quilcene Bay & FH	Hood Canal Summer	240	Phelps et al. 1994	49	Sato et al. 2004		
Quilcene River	Hood Canal Summer					46	Yoon et al. 2006

Appendix 1.-continued.

Population	Region	Allozyme (20 loci)		mtDNA (Control region)		msDNA (4 loci)	
		N	Reference	N	Reference	N	Reference
Lilliwaup Creek Summer	Hood Canal Summer	120	Phelps et al. 1994				
Satsop River	Coastal, Columbia River	100	Phelps et al. 1994	49	Sato et al. 2004		
Wynoochee River	Coastal, Columbia River	22	Phelps et al. 1994				
Stevens Creek	Coastal, Columbia River	100	Phelps et al. 1994				
Ellsworth Creek	Coastal, Columbia River	100	Phelps et al. 1994				
Bitter Creek	Coastal, Columbia River	100	Phelps et al. 1994				
Hamilton Creek	Coastal, Columbia River	100	Phelps et al. 1994	43	Sato et al. 2004	48	Yoon et al. 2006
Grays River	Coastal, Columbia River	100	Phelps et al. 1994				
Coal Creek	Coastal, Columbia River	100	Phelps et al. 1994				