NPAFC
Doc. No. 1274
Rev. No.

STUDIES IN GENETIC STRUCTURE OF PACIFIC SALMON POPULATIONS IN THE RUSSIAN FAR EAST WITH USE OF MICROSATELLITE MARKERS

Lev A. Zhivotovsky

Vavilov Institute of General Genetics,
Russian Academy of Sciences, Gubkina Str. 3, Moscow 119991, Russia

Submitted to the
NORTH PACIFIC ANADROMOUS FISH COMMISSION
by the
RUSSIAN NATIONAL SECTION

October 2010

THIS PAPER MAY BE CITED IN THE FOLLOWING MANNER:

Zhivotovsky, L.A. 2010. Studies in genetic structure of Pacific Salmon populations in the Russian Far East with use of microsattellite markers. NPAFC Doc. 1274. 14 pp. (Available at www.npafc.org).

STUDIES IN GENETIC STRUCTURE OF PACIFIC SALMON POPULATIONS IN THE RUSSIAN FAR EAST WITH USE OF MICROSATELLITE MARKERS

Lev A. Zhivotovsky

Vavilov Institute of General Genetics,
Russian Academy of Sciences, Gubkina Str. 3, Moscow 119991, Russia

ABSTRACT

The paper outlines the current state in creating population data bases on Pacific and other salmon in the Russian Far East based on DNA microsatellite markers. Microsatellite analysis is a very powerful genetic tool that is able to distinguish well population structures, which is necessary to know for fish identification, mixed stock analysis, ecological certification, etc. The most developed is a data base on chum salmon (*Oncorhynchus keta*), a commercially important species in Russia, with more than 200 population samples (30 to 50 fish each) from more than 80 rivers in the main geographic regions of the Russian Far East, characterized with ten microsatellite markers.

These markers reveal a relatively large differentiation between populations from geographically distant regions. As an example, F_{ST}-values, which estimate genetic differentiation, between chum salmon populations in South Kuril Islands and those in Sakhalin Island exceed 7%. The data base is being updated every year. Besides, we introduce additional DNA primers to fix deficiencies that are caused by null alleles due to mutations in the DNA primer regions, as well as develop an additional set of markers for a finer differentiation of geographically close populations of chum salmon.

Population data bases on other salmonid species in the Russian Far East are under construction. Among the latter is pink salmon (*O. gorbuscha*), commercially the most important salmon in the Russian Far East. However, previous rich data on allozyme variation and preliminary DNA data have revealed an exclusively low genetic differentiation of Asian pink salmon, with an indefinite, labile population structure, that was hypothesized to be caused by spatially and temporally varying strong between-stock gene flows (the 'pink salmon fluctuating stocks' hypothesis). Another direction of research is studying population structure of rare and endangered salmonid species, among which are sockeye salmon (*O. nerka*) in Iturup Island, where this species is rare and represents the most southern part of its habitat in the Russian Far East, Sakhalin taimen (*Parahucho perryi*) that inhabits the Sakhalin-Kuril and neighboring regions, and populations of other species for conservation genetics purposes.

INTRODUCTION
(WHY MICROSATELLITES?)

Stocks of Pacific salmon and other salmonids exhibit significant temporal and spatial differentiation that are caused by ecological divergence of spawning areas and reproductive isolation between populations. Addressing conservation and management questions need knowledge of population structure. This can be achieved by using natural physical markers, such as scale pattern (Bugaev et al. 2009), but this approach requires developing a new regional data base every year and can hardly distinguish local populations. A more advanced is using genetic markers (loci), at which variation is sufficiently great for distinguishing differences between salmon stocks.

Allozyme variation has revealed within-species subdivision of Pacific salmon from the Russian Far East (Altukhov et al. 2000, Varnavskaya 2006). However, low genetic variation and difficulties with collecting, storing and processing biological samples have made allozymes much less practical than DNA markers. For example, analyzing chum salmon populations with both allozymes and microsatellites indicated that the latter provided a better resolution of population subdivision (Rubtsova et al. 2008).

Mitochondrial DNA (mtDNA) is a very useful marker, with high variation and uniqueness of the majority of its mutations, which allows for inferring population structure and for a detailed analysis of relationship between individuals through constructing haplotypic networks (Sato et al., 2004, 2005; Polyakova et al. 2006, and many others). MtDNA may help in estimating population components in mixed-stock analyses; however, it is not powerful because it represents single-locus variation. Because of that, mtDNA is not appropriate for individual identification; in particular, it fails to determine the population origin of a single fish that is caught, say, in an open sea. Indeed, just a few major haplotypes constitute the majority of mtDNA variation within a species. These haplotypes are not unique and occur in many populations. Therefore, with a high chance, a given fish carries such a mtDNA haplotype, and thus the probability of that fish to be assayed to a definite population is equal to the frequency of this haplotype in this very population. This probability can not be increased without introducing additional marker systems.

SNPs, or single nucleotide polymorphisms are very promising for population analysis (Smith et al. 2005, Smith and Seeb 2008) because they are numerous and, importantly, reliably reproduced in different laboratories. However, their analysis is still expensive, although may get cheaper (Garvin and Gharrett 2007), and their power in identification and distinguishing population components is not yet well compared relative to other genetic markers.

Microsatellites, or short tandem repeats (STR), or simple sequence repeats (SSR), are numerous markers, distributed across the genome, with multiple alleles at many loci, and thus can infer a fine population structure. However, they are less reliable than SNPs because of varying allele sizes at each locus, which need careful calibration. Also, there is a potential for null alleles caused by mutations in the primer regions that block amplification and introduce false homozygosity. Aside of these, microstellites are powerful in fish identification, analysis of mixed-stock fisheries and other genetic conservation and management purposes.

THE TEAM

In Russia, there are a few institutions in which genetic markers are being analyzed for purposes of population and evolutionary genetics on the Far East salmon. These are KamchatNIRO (Petropavlovsk-Kamchatskiy), VNIRO (Moscow), A.V. Zhirmunsky Institute of Marine Biology (Vladivostok), Institute of Biological Problems of the North (Magadan), and the Institute of General Genetics (Moscow).

A principal part of work on creation of within-species microsatellite data bases is being provided by the team of Laboratory of Genetic Identification at the Institute of General Genetics, Russian Academy of Sciences (Moscow). The team includes Lev A. Zhivotovsky (head), Konstantin I. Afanasiev, Galina A. Rubtsova, Marina V. Shitova, Tatiana V. Malinina, Tatiana A. Rakitskaya, and Valentina D. Prokhorovskaya. A brief sketch on the team can be viewed at http://www.vigg.ru/?cat_id=65 (in Russian).

We collaborate with colleagues from federal and regional fish-and-game research institutions in Russia, namely from VNIRO (Moscow), SakhNIRO at Yuzhno-Sakhalink, KamchatNIRO at Petropavlovsk-Kamchatskiy, MagadanNIRO at Magadan, ChukotNIRO at Anadyr, TINRO-Centre at Vladivostok, Khabarovsk branch of TINRO at Khabarovsk, from institutions of the Far East Branch of Russian Academy of Sciences: A.V. Zhirmunsky Institute of Marine Biology at Vladivostok, and the Institute of Biological Problems of the North at Magadan, from the Sakhalin Division of the Federal Fishery Agency (SakhRybVod), from fishery company “Gidrostroy”, hatcheries in Sakhalin and Iturup Islands, the independent organization “Sakhalin Salmon Initiative”, and other institutions. For many years we cooperate with our colleagues from School of Fisheries and Ocean Sciences at University of Alaska Fairbanks, and recently developed a productive association with Wild Salmon Center (Portland, OR) and some other organizations.

THE CURRENT DATA BASE

CHUM SALMON

Chum salmon and pink salmon are the most abundant and provide a major component of commercial catches of Pacific salmon in Russia. The most advanced is a data base on chum salmon (*Oncorhynchus keta*).

Ecology. Wild populations of Asian chum salmon become increasingly threatened as a result of habitat loss, fishing, and hatchery impact. In Hokkaido and Honshu Islands, chum salmon are almost exclusively reproduced artificially for more than a century. In the Russian Far East, chum salmon hatchery programs have been established as well, mainly in Sakhalin and South Kuril Islands, and started to rise fast last years. However, natural spawning coexists with artificial reproduction in South Kuril Islands and prevails in other regions of the Russian Far East, such as Chukotka, Kamchatka, the Amur River. Consequently, studies on genetic structure of chum salmon across these regions are important for conservation and management purposes.

In the Russian Far East, chum salmon stocks exhibit significant temporal and spatial differentiation. There is a latitude gradient in the time of chum salmon return to spawn, being earlier in Kamchatka Peninsula and later in South Sakhalin and South Kuril Islands. Additionally, there are distinguished two major temporal forms of chum salmon: early-run, or "summer", and late-run, or "autumn" forms. For example, chum salmon in Kamchatka Peninsula is mainly represented by the early form that spawns in July-August. In contrast, the late, autumn form of chum salmon is the most abundant in Sakhalin and South Kuril Islands and its spawning run lasts from September up to November and even December. In some areas, e.g. in the Amur River and Poronai River (eastern Sakhalin), both forms, the summer and the autumn, occur and exhibit significant ecological differentiation because the summer form lay eggs in spawning grounds with subsurface stream flow, whereas the later-run form prefer those with ground water upwelling (Volobuev et al. 1990). Such an ecological differentiation may provide genetic differentiation due to homing, the ability to return for spawning to the 'home' river, and because the time of return is inherited (McGregor et al. 1998).

The history. Addressing conservation and management questions needs knowledge on population-genetic structure of chum salmon. Allozyme variation revealed subdivision of chum salmon from the Russian Far East (Salmenkova et al. 1992, 2008, Bachevskaya and Pustovoit 1996, Seeb et al. 2004). However, low genetic variation and difficulties with collecting, storing and processing biological samples have made allozymes less practical than DNA markers. Moreover, allozyme variation failed to determine a fine structure of chum salmon. Indeed, typing a set of samples from chum salmon populations with both allozymes and microsatellites

has shown that the latter provide much better resolution of population subdivision (Rubtsova et al. 2008). Although other kinds of DNA markers are useful as well, among which are mitochondrial DNA (Sato et al. 2004, 2005, Polyakova et al. 2006) and single nucleotide polymorphisms, or SNPs (Smith et al. 2005, Garvin and Gharrett 2007, Smith and Seeb 2008), microsatellite markers seem to be more powerful in inferring population structure and for identification purposes because of their high polymorphism and better differentiation of closely related populations (Scribner et al. 1998, Chen et al. 2005, Small et al. 2006, Afanasiev et al. 2006, Narum et al. 2008, Zhivotovsky et al. 2008, Shitova et al. 2009).

The first study of chum salmon populations from the Russian Far East have been made on Sakhalin stocks using eight microsatellite markers (Afanasiev et al. 2006). Later the Sakhalin hatchery stocks and some Kuril Islands populations of chum salmon have been analyzed with a bit larger set of microsatellites, ten markers (Afanasiev et al. 2008, Shitova et al. 2009). Detailed analysis of the Iturup Island chum salmon, for which samples were collected across its spawning run, have been provided by Zhivotovsky et al. (2008).

Using a set of fourteen microsatellite loci, Beacham et al. (2008) estimated that divergence of chum salmon populations in the Russian Far East is low, F_{ST}=1.7%, with no statistically significant differentiation between stocks within large geographic regions, such as Sakhalin Island, Kamchatka, Amur River, the Magadan coast of the Sea of Okhotsk, etc. In contrast, using a different set of microsatellite markers, that slightly overlaped with the Beacham's et al. (2008) set, Afanasiev et al. (2008) and Zhivotovsky et al. (2008) found significant variation between chum salmon populations in Sakhalin and Iturup Islands and a different picture of population subdivision than those in Beacham et al. (2008). Such a discordance between the latter and our studies could be due to different abilities of different markers to differentiate populations or/and different strategies of sampling from chum salmon populations across the Russian Far East.

Molecular markers. For DNA analysis, tissue samples (mainly, a piece of the pectoral fin) were fixed in 96% ethanol. Total DNA was extracted with a standard isolation procedure with the Diatom DNA Prep reagent kit (IzoGen, Russia). PCR amplification was performed using the Gene Pak PCR Core reagent kit (IzoGen, Russia), with addition of 5 µl of primer mixture (final concentration 0.5 µM) and 5 µl of DNA template (100 ng). Microsatellite loci were amplified in a MJ Research PTC-100 thermal cycler. Amplification products were fractionated by electrophoresis in the 6% nondenaturing polyacrylamide gel in 1xTBE buffer at 300V for 2 to 3 h. The gels were stained with ethidium bromide and photographed in the UV light. The 25-bp and 100-bp molecular weight standards (Promega, the United States) and pBr322 plasmid DNA digested with the *Hae* III restriction endonuclease were used as molecular weight markers. Allele sizes for each locus were determined using the 1D Image

Analysis Software, version 3.5 (Kodak). All individuals were typed with microsatellites *Ssa197* (O'Reilly et al., 1996), *Ssa20.19* (Sanchez et al., 1996), *Ogo2* (Olsen et al., 1998), *Oke3* (Buchholz et al., 2001), *Oke11* (Buchholz et al., 2001), *One101* (Olsen et al., 2000), *One103* (Olsen et al., 2000), *One109* (Olsen et al., 2000), *Ots3* (Small et al., 1998), and *Oki1* (Smith et al., 1998).

The fragment profiles at *One101* occurred to be identical to those at *One103*, with a constant difference of 18 bp, and thus we used a shorter variant, *One103*. Using the primers for *Oki1*, we obtained two distinct zones on a gel that can be interpreted as a result of duplication followed by evolutionary molecular divergence into two microsatellite loci with identical flanking regions; both loci were used in this study with notation *Oki1-1* (180-270 b.p.) и *Oki1-2* (90-110 b.p.).

A comprehensive microsatellite data base. During seven years, from 2003 to 2009, we collected more than 200 population samples (30 to 50 fish each) from chum salmon stocks from more than 80 sampling sites across the Russian Far East (fig.1) and have genotyped those with ten microsatellite markers. The objective of this work was to develop a microsatellite baseline information, based on samples distributed throughout the main regions of the Russian Far East in a finer geographic scale than those that have been previously provided, that allowed for an increased resolution of population structure of chum salmon spawning stocks and was aimed to fish identification, analysis of mixed-stock fisheries and other genetic conservation and management purposes. Analysis of a part of these data has been done in a recent publication by Afanasiev et al. (2010). The current data base still has lacunas in the sample range, and in 2010-2011 we are going to collect more chum salmon samples from Khabarovsk region, Primorje, and Kunashir Island.

Application of the data base. This data base includes very detailed samples from chum salmon populations of Iturup Island. A part of these samples has been used for a Marine Stewardship Certification (MSC) process on fishery company "Gidrostroy", Iturup Island (Zhivotovsky et al. 2008, 2010a). Following the MSC criteria, we have developed a detailed specific sub-database on the island chum salmon populations, both wild and hatchery, with multiple samples from each population both across years (totally, from 2004 to 2009) and during the spawning runs (fig.2). This data base can also be used for identification of chum salmon in marine catches and for mixed-stock analysis.

Further development of the data base on chum salmon populations. We have found that individual identification achieves large values with respect to chum salmon stocks that are reproduced in the main regions of the Russian Far East. For example, a fish that is caught in Sakhalin Island rivers can be successfully identified as belonging to the Sakhalin genetic cluster *versus* the South Kuril Islands genetic cluster, and *vice versa*, with an error of about 2%.

Such a small error is due to a relatively high level of genetic differentiation between these two regions, F_{ST} ~ 7%. However, within-region genetic differentiation of chum salmon populations is lower, and thus errors of individual identification are larger. In order to increase the power of individual identification for within-region comparisons, we are increasing the data base power by developing additional microsatellite markers.

Another direction of our research on the chum salmon data base is development of additional primer sets for loci with substantial frequency of null alleles. One of the ten microsatellite loci that constitute the data base, *Oke*3, significantly deviates from the Hardy-Weinberg expectations in some chum salmon populations from Kuril and Sakhalin Islands; a detailed analysis with several novel sets of primers discovered null alleles in the populations (Kordicheva et al. 2010). These null alleles are caused by mutations in both, forward and reverse primer zones that flank the microsatellite body; the various null alleles at this locus are distributed differently in chum salmon populations across geographic regions (Zhivotovsky et al. 2010b). Developing such primer sets removes the deviation caused by null alleles and, additionally, introduces null mutations as a new marker linked to the microsatellite.

PINK SALMON

In the Russian Far East, pink salmon (*O. gorbuscha*) is the most abundant species of Pacific salmon and constitutes the majority of commercial fisheries. It is mainly reproduced as wild stocks, except for Sakhalin and Iturup Islands where up to 20% of local pink salmon juveniles release from hatcheries.

The Sakhalin Island pink salmon has two distinct season forms, summer and autumn. The autumn form is numerous, with high oscillation in abundance, and spends its marine stage in the Pacific Ocean, while the summer form does in the Sea of Japan. The abundance of the latter form decreased significantly during the last decades, and now it can be considered as an endangered form that needs urgent measures to be treated carefully.

Asian pink salmon is genetically homogeneous across long distance. Although large regional stocks differ from each other in allele frequencies at a statistically significant level within the odd- or even-year broodline, say Sakhalin *vs* Kamchatka stocks, the rate of their differentiation in terms of the F_{ST}-statistic is very small (Zhivotovsky et al. 1989), almost negligible compared to differentiation of chum salmon. According to the concept of "fluctuating stocks of pink salmon" developed by Glubokovsky and Zhivotovsky (1986), such a low genetic subdivision of pink salmon populations is caused by a high rate of straying. In particular, this is applicable to the Sakhalin pink salmon which autumn populations are almost

homogeneous genetically across the island and thus can be considered as a single management unit.

However, the concept of fluctuating stocks has been formulated based on data on both allozyme variation and rates of straying that were estimated using physical markers by removing one or two fins. Now pink salmon in the Sakhalin and Kuril Islands is under the process of otolith marking, which is supposed to bring additional estimates of the straying rate. Together with that, we are going to investigate the genetic structure of pink salmon with use of microsatellites, toward which we have developed an appropriate set of microsatellite markers, jointly with our colleagues from KamchatNIRO, and begun to collect population samples.

OTHER SPECIES

We are studying population structure of rare and endangered salmonid species in the Russian Far East. One of those is Sakhalin taimen (*Parahucho perryi*) that inhabits the Sakhalin-Kuril and neighboring regions. As well, we survey populations of other species for conservation genetics purposes. For example, sockeye salmon (*O. nerka*) is abundant in Kamchatka Peninsula; however, this species is rare in the Sakhalin-Kuril region and can be met in Iturup Island only, where it represents the most southern part of the sockeye salmon habitat in the Russian Far East; we are studying these populations.

ACKNOWLEDGMENTS

The study was funded by the grant of the RAS Prezidium Program ‘Molecular and Cell Biology’ to LAZh.

REFERENCES

Afanasiev KI, Rubtsova GA, Malinina TV, Salmenkova EA, Omelchenko VT, and Zhivotovsky LA (2006) Microsatellite variability and differentiation of hatchery stocks of chum salmon *Oncorhynchus keta* Walbaum in Sakhalin. Genetika (Russian J Genetics), **42**, 1431-1438 [in Russian].

Afanasiev KI, Rubtsova GA, Shitova MV, Malinina TV, and Zhivotovsky LA (2008) Interregional differentiation of chum salmon from Sakhalin and South Kurils inferred with microsatellite markers Genetika (Russian J Genetics), **44**, 833–840 [in Russian].

Afanasiev K.I., Rubtsova G.A., Shitova M.V., Malinina T.V., Rakitskaya T.A., Prokhorovskaya V.D, Shevlyakov E.A., Zavarina L.O., Bachevskaya L.T., Chereshnev I.A., Brykov V.A., Kovalev M.Yu., Shevlyakov B.A., Sidorova S.V., Borzov S.I., Pogodin V.P., Fedorova L.K., and Zhivotovsky L.A. 2010. Population structure of chum salmon (*Oncorhynchus*

keta) in the Russian Far East revealed with microsatellite markers. Biologia\ya Morya (*Marine Biology*) (in Russian, with English summary). [in press]

Altukhov Y.P., Salmenkova E.A., Omelchenko V.T. 2000. Salmonid fishes: population biology, genetics and management. Blackwell Science. Fish and Aq. Res. Series 2, 368 p.

Bachevskaya LT, Pustovoyt SP (1996) Genetic variation of populations of chum salmon *Oncorhynchus keta* from rivers bordering the northern coast of the Sea of Okhotsk and its change under natural and artificial reproduction Voprosy Ichthyologii (Russian J of Ichthyology), **36**, 660-666 [in Russian].

Beacham TD, Varnavskaya NV, Le KD, and Wetklo M (2008) Determination of population structure and stock composition of chum salmon (*Oncorhynchus keta*) in Russia determined with microsatellites Fishery Bulletin, **106**, 245-256.

Bucholz WG, Miller SJ, Spearman WJ (2001) Isolation and characterization of chum salmon microsatellite loci and use across species Animal Genetics, **32**,162-165.

Bugaev AV, Zavolokina EA, Zavolokin AV, Zavarina L.O., Kireev I.N., Shubin A.O., Ignatiev Yu.I., Zolotukhin S.F., Kaplanova N.F., Bolobuev M.V. 2009. Origin and distribution of local stocks of the chum salmon *Oncorhynchus keta* in the western Bering Sea on the data of trawl surveys by RV TINRO in 2004 and 2006 Izv TINRO, **157**, 3-33 [in Russian, with English summary].

Chen J-P, Sun D-J, Dong Ch-Zh, Liang B, Wu W-H, Zhang S-Yi (2005) Genetic analysis of four wild chum salmon *Oncorhynchus keta* populations in China based on microsatellite markers Environmental Biology of Fishes, **73**, 181-188.

Garvin MR, and Gharrett AJ (2007) DEco-TILLING, An inexpensive method for SNP discovery that reduces ascertainment bias. Molecular Ecology Notes, **7**, 735-746.

Glubokovsky MK and Zhivotovsky LA. 1986. Population structure of pink salmon: A system of fluctuating stocks. Biologiya Morya #2 [in Russian, with English summary]

Kordicheva S.Yu., Rubtsova G.A., Shitova M.V., Shaikhaev GO, Afanasiev K.I., and Zhivotovsky L.A. 2010. A search for null alleles at the microsatellite locus of chum salmon (*Oncorhynchus keta* Walbaum). Genetika (Russian J Genetics) 46 (in press).

McGregor AJ, Lane S, Thomason MA, Zhivotovsky LA, Smoker WW, and Gharrett AJ (1998) Migration timing, a life history trait important in the genetic structure of pink salmon NPAFC Bull, **1**, 262-273.

Narum SR, M Banks, TD Beacham, et al (2008) Differentiating salmon populations at broad and fine geographical scales with microsatellites and single nucleotide polymorphisms Molecular Ecology, **17**, 3464-3477.

Olsen JB, P Bentzen, JE Seeb (1998) Characterization of seven microsatellite loci derived from pink salmon Molecular Ecology, **7**, 1087-1089.

Olsen JB, SL Wilson, EJ Kretschemer, KC Jones, JE Seeb (2000) Characterization of 14 tetranucleotide microsatellite loci derived from sockeye salmon Molecular Ecology, **9**, 2185-2187.

O'Reilly PT, LC Hamilton, SK McConnell, JM Wright (1996) Rapid analysis of genetic variation in Atlantic salmon (Salmo salar) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites Can J Fish Aquat Sci, **53**, 2292-2298.

Polyakova NE, AV Semina, VlA Brykov (2006) Variability of mitochondrial DNA chum salmon *Oncorhynchus keta* (Walbaum) and its relationship to paleogeological events in the northwestern Pacific Genetika (Russian J Genetics), **42**, 1388–1396 [in Russian].

Rubtsova GA, KI Afanasiev, TV Malinina, MV Shitova, TA Rakitskaya, VD Prokhorovskaya, and LA Zhivotovsky (2008) Differentiation of chum salmon *Oncorhynchus keta* Wallbaum populations as revealed with microsatellite and allozyme markers, A comparative study. Genetika (Russian J of Genetics), **44**, 841-848 [in Russian].

Salmenkova EA, VT Omelchenko, KI Afanasiev, GA Rubtsova (2008) Genetic variability of Asian chum salmon *Oncorhynchus keta* Walbaum (Salminidae, Salmoniformes) and population-genetic dynamics under artificial reproduction Voprosy Ichthyologii (Russian J of Ichthyology), **48**, 361-373 [in Russian].

Salmenkova EA, VT Omelchenko, YuP Altukhov (1992) Genetic-geography study of populations of chum salmon *Oncorhynchus keta* (Walbaum) in the Asian part of the habitat Genetika (Russian J Genetics), **28**, 76-91 [in Russian].

Sanchez JA, Clabby C, Ramos D, Blanco G, Flavin F, Vazquez E, Powell R (1996) Protein and microsatellite single locus variability in *Salmo salar* L (Atlantic salmon) Heredity, **77**, 423-432.

Sato S, Kojima H, Ando J, et al (2004) Genetic population structure of chum salmon in the Pacific Rim inferred from mitochondrial DNA sequence variation Environ Biol Fish, **69**, 37−50.

Sato S, M-GYoon, S Urawa, A Urano, and S Abe (2005) Mitochondrial DNA Phylogeography of Chum Salmon in the Pacific Rim NPAFC Technical Report, **6**, 84-85.

Scribner KT, PA Crane, WJ Spearman, LW, and Seeb (1998) DNA and allozyme markers provide concordant estimates of population differentiation, analyses of Yukon River fall-run chum salmon (*Oncorhynchus keta*) Can J Fish Aquat Sci, **55**, 1748-1758.

Seeb LW, Crane PA, Kondzela ChM, Wilmot RL, Urawa Sh, Varnavskaya NV, and Seeb JE (2004) Migration of Pacific Rim chum salmon of the high seas, insights from genetic data Environmental Biology of Fishes, **69**, 21-36.

Shitova M.V., Afanasiev K.I., Rubtsova G.A., Malinina T.V., Sidorova S.V., and Zhivotovsky L.A. 2009. Microsatellite variation of hatchery populations of chum salmon (*Oncorhynchus keta* Walbaum) in Sakhalin Island. Problems of Fisheries 10: 102-115 (in Russian, with English summary).

Small MP, Frye AE, Von Bargen JF, and Young SF (2006) Genetic structure of chum salmon (*Oncorhynchus keta*) populations in the lower Clumbia River, Are chum salmon in Cascade tributaries remnant populations? Conservation Genetics, **7**, 65-78.

Small MP, Beacham TD, Withler RE, Nelson RJ (1998) Discriminating coho salmon (*Oncorhynchus kisutch*) populations within Fraser River, British Columbia, using microsatellite DNA markers Molecular Ecology, **7**, 141-155.

Smith CT, Koop BF, Nelson RJ (1998) Isolation and characterization of coho salmon (*Oncorhynchus kisutch*) microsatellites and their use in other salmonids. Molecular Ecology, **7**, 1614-1616.

Smith CT, Baker J, Park L, Seeb LW, Elfstrom C, Abe S, Seeb JE (2005) Characterization of 13 single nucleotide polymorphism markers for chum salmon. Molecular Ecology Notes, **5**, 259-262.

Smith CT, and Seeb LW (2008) Number of alleles as a predictor of the relative assignment accuracy of STR and SNP baselines for chum salmon Transactions of the American Fisheries Society, **137**, 751-762.

Varnavskaya N.V. 2006. Genetic Differentiation of Pacific Salmon Populations The Kamchatka Institute of Fishery and Oceanography (KamchatNIRO), 487 p [in Russian]

Volobuev VV, Rogatnykh AYu, Kuzishchin KV (1990) Intraspecific forms of chum salmon, *Oncorhynchus keta*, along the continental coast of the Sea of Okhotsk Voprosy Ikhtiologii (Russian J Ichthyology), **30**, 104-114 [in Russian].

Zhivotovsky LA, Afanasiev KI, Rubtsova GA, Shitova MB, Malinina TV, Rakitskaya NF, Prokhorovskaya VD, Salmenkova EA, Fedorova LK, Borzov SI, Pogodin VP. (2008) On development of a DNA database for reproduction, identification and certification of populations of Pacific salmon: An example from chum salmon of Iturup Island. Problemy Rybolovstva (Problems of Fisheries), **9**, 96-109 [in Russian, with English summary].

Zhivotovsky LA, Rubtsova GA, Shitova MB, Malinina TV, Rakitskaya NF, Prokhorovskaya VD, Afanasiev KI. (2010a). Genetic principles of ecological certification on Pcific salmon. In: Shuntov VP (ed.) "The Program for Studies in Pacific Salmon". TINRO-CENTER, Vladivostok. Bull.#4: 117-125. [in Russian].

Zhivotovsky LA, Kordicheva S.Yu, Rubtsova GA, Fuller SA, Gharrett AJ, Shaikhaev GO, Afanasiev KI. (2010b) Local geographic expansion of microsatellite null alleles: An example from chum salmon populations. [Paper in progress]

Appendix figures

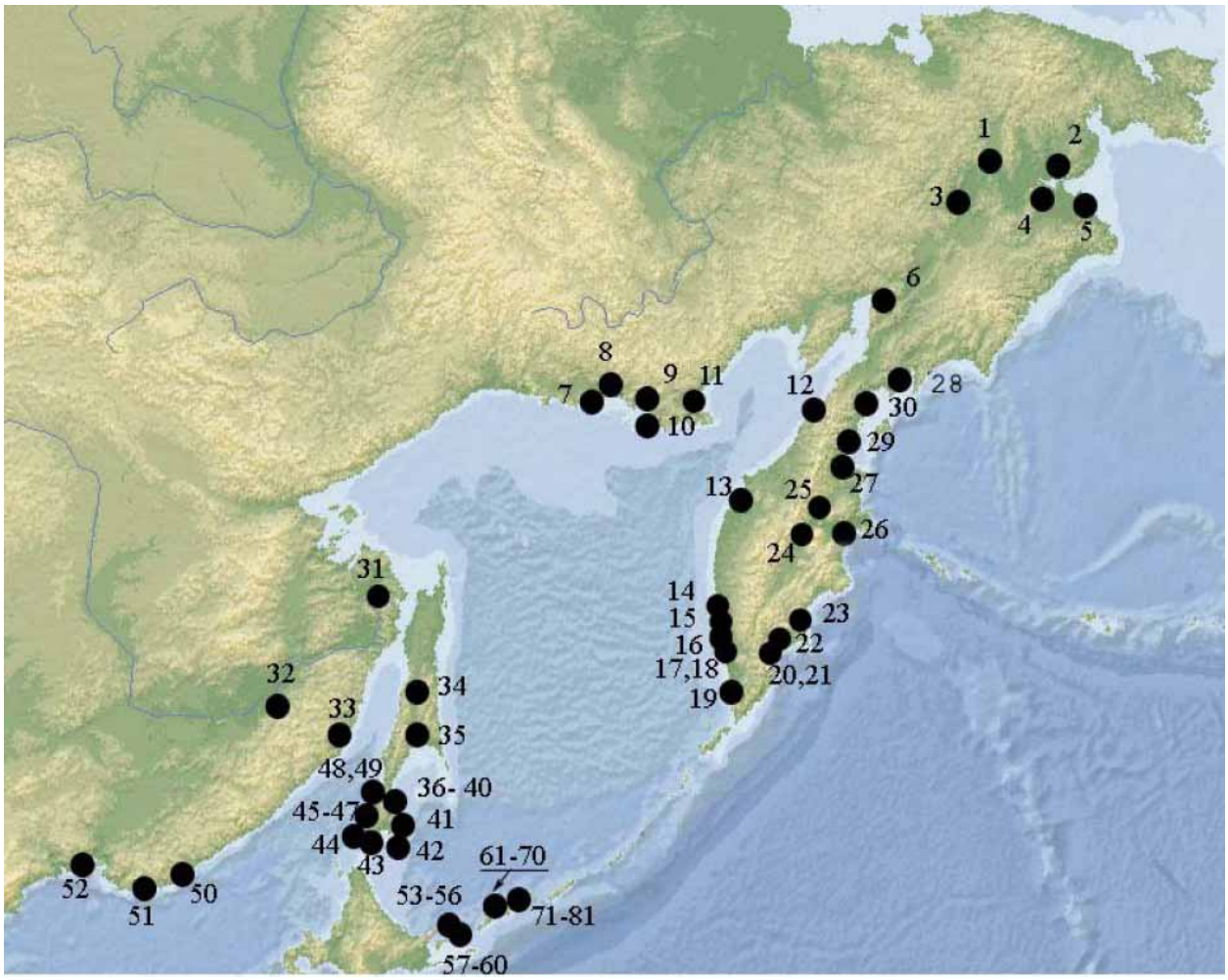

Fig.1. Sites of sampling from chum salmon spawning in rivers of the Russian Far East.

Notation: Chukotka Peninsula (1-5; 13 samples), Magadan region (7-11; 13 samples), Kamchatka Peninsula (6, 12-30; 26 samples), Khabarovsk region (31-33; 4 samples), Sakhalin Island (34-49; 25 samples), Primorje (50-52; 9 samples), Kunashir Island (53-60; 12 samples), Iturup Island (61-81; 100 samples).

Note. Each site represents an area with one or more geographically close rivers. The majority of samples were collected from 2003 to 2009. Some rivers are represented by a few samples taken during spawning runs or/and across years, such multiple samples have been collected from the chum salmon of Iturup Island (fig. 2)

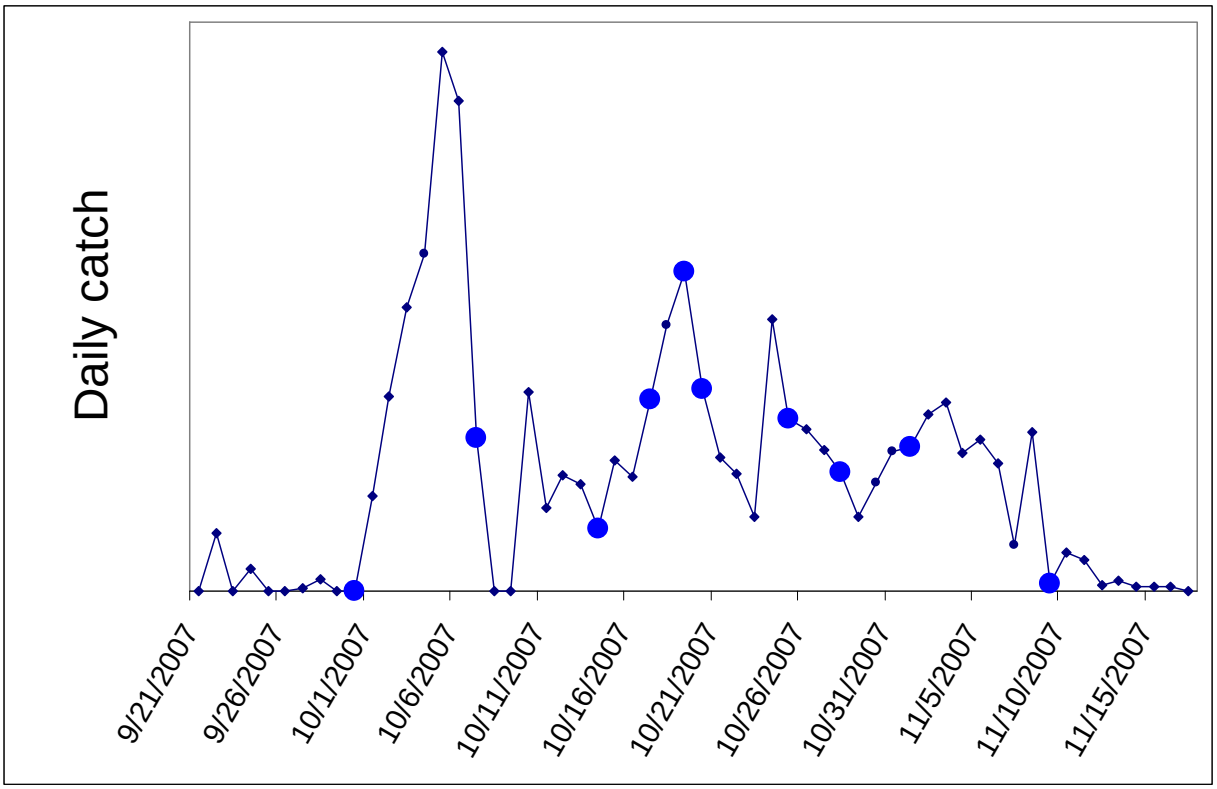

Fig.2. Example of multiple samples during a spawning run.

The figure represents daily catches of chum salmon in the Prostor Bay (Iturup Island) in 2006. The stock is mainly reproduced in the Reidovaya River (a hatchery and spawning grounds), where ten samples were taken that year (dates are indicated by circles). Altogether, this site is represented by 35 samples collected from 2004 to 2009