North Pacific Anadromous Fish Commission



TECHNICAL REPORT 5

Workshop on Application of Stock Identification in Defining Marine Distribution and Migration of Salmon

Edited by: Jim Irvine, Lisa Seeb, Shigehiko Urawa, Natalia Varnavskaya, and Richard Wilmot

Vancouver, Canada

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Workshop on Application of Stock Identification in Defining Marine Distribution and Migration of Salmon

Honolulu, Hawaii, USA, November 1-2, 2003

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Foreword

The International Workshop on Application of Stock Identification in Defining Marine Distribution and Migration of Salmon was held in Honolulu, Hawaii, U.S.A., on November 1-2, 2003. The Workshop was organized and sponsored by the North Pacific Anadromous Fish Commission (NPAFC). The Workshop Organizing Committee consisted of scientists from Canada, Japan, Russian Federation and U.S.A. All necessary arrangements were made by the NPAFC Secretariat in cooperation with the Organizing Committee.

Over 70 scientists, industry representatives, and fisheries officials attended the Workshop. There were 26 oral presentations including two keynote addresses, followed by a panel discussion session, and 25 poster presentations. Extended abstracts of the oral and poster presentations are included in this Technical Report, which also contains opening remarks by Ms. Fran Ulmer, the U.S. Representative to the NPAFC and short review of the Workshop by the co-chairs of the Workshop. The material presented in this Technical Report has not been peer reviewed, and does not necessarily reflect the views of the NPAFC or the Parties. Some work may be preliminary. The material has been edited for clarity and publication purposes only.

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Opening Remarks

It is my pleasure to welcome you today to the NPAFC Workshop on Application of Stock Identification in Defining Marine Distribution and Migration of Salmon.

On behalf of the other Representatives, I want to thank the Organizing Committee and the staff for all their hard work in preparing for this workshop. I also want to thank all of the scientists who have contributed papers and posters and presentations. By doing so you are providing us all the opportunity to advance our understanding of the complicated ecosystems and the precious resources of the North Pacific.

The focus of the NPAFC has been the conservation of anadromous fish and related species in the North Pacific. The treaty which created the Commission specifies two main missions: the enforcement of the ban of high seas fishing of salmon and the coordination of data, information and research.

The NPAFC has made extraordinary progress in both missions and I want to thank the Representatives, advisors, staff, participants and others who have invested time and money to improve our work and assure success.

Today is a result of the increased levels of cooperation among the parties. Scientists have worked together effectively to expand research and information availability to all who are concerned about the future of this region and its resources. There are many examples of this cooperation, but I believe the best one is the BASIS program which is now in its second year of work. You will hear more about it this afternoon, so I will not go into any detail now, but I do want to express my personal appreciation to those who have worked so hard to find creative ways to make this collaborative research effort a reality. I am hopeful that we will be able to obtain additional resources to support this international research project, because it is an excellent model for what must happen in other regions. No one country owns the North Pacific Ocean. Only when all the countries which have a stake in the health of the ocean and the ecosystems work together though joint research efforts will we obtain the kind of information and understanding that we need to achieve sustainable management policies and practices.

As this is my last meeting with you as a Representative, I want to make one request to each of you. Please take time to explain the work that you do and the science that you know so well to someone who is not familiar with the importance of integrating science and public policy. Adopt a legislator or mayor or reporter this week when you return home to your country, and help them understand what happened here at this workshop and why they should care about the work of the NPAFC. So many of the important decisions regarding the management of fisheries and other resources could be improved if key decisionmakers had a better understanding of the science and the implications of alternative actions. Very few of the key decisionmakers, either elected or appointed, have science backgrounds, at least in my country. At a time when the world is changing very rapidly and the cumulative impacts of decisions can have large and lasting consequences, it is more important than ever before that scientists talk to policy makers and vice versa. I hope you will help make that happen. You have much to contribute.

Thank you.

Fran Ulmer Representative of the U.S.A. to the NPAFC

Evaluation of Carrying Capacity of Pacific Salmon in the North Pacific Ocean for Ecosystem-Based Sustainable Conservation Management

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Keywords: Climate change, carrying capacity, hatchery/wild interactions

The change in biomass of Pacific salmon (*Oncorhynchus* spp.) indicates a 30- or 40-year periodicity in the North Pacific Ocean coinciding with long-term climate conditions. I estimated the carrying capacity (K) of sockeye (*O. nerka*), chum (*O. keta*), and pink salmon (*O. gorbuscha*) in the North Pacific Ocean using the replacement level of the Ricker's recruitment curve, and defined residual carrying capacity (RCC):

RCC = (Carrying capacity – Biomass) / (Carrying capacity) x 100

A significant positive correlation between the Aleutian low pressure index (ALPI) and the carrying capacity was observed at the species level. Factors affecting carrying capacity at the population level, such as reproductive regimes (e.g. survival rate and sea surface temperature (SST) in the early marine life period and hatchery technology), differed by population in Hokkaido chum salmon. The RCC was significantly positively correlated with body size and negatively related to age at maturity in Hokkaido chum salmon populations. The biomass of chum salmon wild populations in the 1990s decreased 50% below that of the 1930s, despite the significant increases in the biomass of hatchery populations. Biological interaction between wild and hatchery populations should be an important consideration in the sustainable management of Pacific salmon production based at the ecosystem level.

In the North Pacific Ocean, the annual catch of Pacific salmon increased from the late 1970s to the early 1990s due to favorable oceanic conditions associated with long-term climate change and successful hatchery programs (e.g. Beamish and Bouillion 1993; Kaeriyama 1998; Klyashtorin 1998). The period of the change in catch also coincided with the climatic regime-shift year. According to the prediction of Minobe (2000), the new climatic regime shift occurred in 1998–1999. This suggests that there may be a change in biomass of Pacific salmon in the near future resulting from the changes in oceanic conditions.

Pacific salmon occupy higher trophic levels in food webs of the Western and Eastern Subarctic Pacific Gyres. Chum, sockeye, and pink salmon obtained higher biomass in the Subarctic Pacific Gyres (Aydin et al. 2003). Relationship between carbon and nitrogen stable isotope of animals in the Gulf of Alaska shows that the Pacific salmon occupy the fourth trophic level, and that trophic levels of chinook salmon (O. tshawytscha) and steelhead trout (O. mykiss) are higher than those of sockeye, chum, pink, and coho (O. kisutch) salmon (Fig. 1). Thus, Pacific salmon are important key species not only as human food resources, but also to the ecosystem in the Subarctic Pacific Ocean.

The purpose of this paper is to evaluate carrying capacity of Pacific salmon in order to develop sustainable conservation management based on the ecosystem.

Pacific salmon catch data from the INPFC statistical yearbooks, the NPAFC statistical yearbooks, FAO yearbooks, and Fredin (1980), and the Aleutian low pressure index **Fig. 1.** Relationship between carbon and nitrogen stable isotope of animals in the Gulf of Alaska, 1999–2000. EU: Euphausiids, CO: Copepods, AM: Amphipods, DE: Decapods, PT: Pteropods, PO: Polychaetes, GE: Gelatinous zooplankton, SA: Sagiita, SQ: Squids, FI: Fishes.



(ALPI) from Beamish et al. (1997) and URL: http://www.pac.dfo-po.gc.ca/sci/samfpd/downloads/alpi.txt were used to evaluate biomass and carrying capacity of Pacific salmon and long-term climate change. The estimation of biomass from catch data was based on the method of expansion of terminal run to total biomass by D. Eggers (Alaska Department of Fish and Game, unpublished data). From the Ricker's recruitment curve (R  $= \alpha P e^{-\beta t}$ ), calculated by the Levenberg-Marquardt method, the replacement level  $(\ln(\alpha)/\beta$ , Ricker 1975) was defined as the index of carrying capacity (K). In the data set of Ricker's curve, pink salmon included 10 generations divided by odd- and even-year groups, while sockeye and chum salmon included 20 brood years. Residual carrying capacity (RCC) was defined as above.

**Fig. 2.** Changes in mean Aleutian low pressure index (ALPI) and total carrying capacity (K; million individuals) of sockeye, chum, and pink salmon in the North Pacific Ocean.



SST in the coasts around Hokkaido was provided by the Metrological Agency of Japan as a monthly mean of one degree latitude and longitude blocks. SST of each block was further averaged for spring (April–June).

Data on mean fork length of age-4 female adults returning to 11 rivers and mean age at maturity in Hokkaido chum salmon population (Kaeriyama 1998) were used to estimate the relationship between the RCC and the somatic growth reduction.

In three species of Pacific salmon (i.e. sockeye, chum, and pink salmon), the carrying capacity since the 1976 regime-shift year has increased to approximately double that of 1947–1975. The carrying capacity of chum salmon since 1976 was the same as it was in 1924–1946. However, carrying capacities of sockeye and pink salmon since 1976 were 1.2 and 1.4 times more than those in 1924–1946, respectively. Significant positive correlations between ALPI and total carrying capacity of sockeye, chum, and pink salmon were observed without a time lag. At minus values in the ALPI, carrying capacity tended to preserve the minimum level (Fig. 2). At the species level, therefore, carrying capacity was significantly synchronized with the long-term climate change.

In chum salmon, carrying capacities of Russia (r = 0.56, F = 32.1, P < 0.001) and southeastern Alaska populations (r = 0.58, F = 35.8, P < 0.001) were significantly linked with the ALPI. Carrying capacity of populations at the edge of distribution such as Hokkaido, Western Alaska, and British Columbia and Washington did not synchronize with the long-term climate change. The results of stepwise multiple regression analysis on carrying capacity of Hokkaido chum salmon showed that (1) the multiple regression identified body size of juvenile at the release, return rate, and the SST in spring, and that (2) relationship between ALPI or number of juvenile released and the carrying capacity did not indicate significant trends over time (Table 1). Kaeriyama (1999) reported that the relationship between return rate, which is the survival rate from the release to the return, and body size of juveniles at release showed significant-positive correlation in Hokkaido chum salmon population. Despite a positive correlation between SST in the coast and the return rate of Hokkaido chum salmon (r = 0.711, F = 30.75, P < 0.001), the relationship between the ALPI and the SST was not significantly positively correlated in all coasts around northern Japan, and showed a negative correlation in Okhotsk Sea and a part of Japan Sea near Hokkaido (A. Yatsu, National Research Institute of Fisheries Science, Japan. unpublished data)

| Dependent variable: Carrying capacity (K)      |                                |                                 |         |  |  |  |
|------------------------------------------------|--------------------------------|---------------------------------|---------|--|--|--|
| Predictor variable                             | Partial regression coefficient | Partial correlation coefficient | P-value |  |  |  |
| ALPI                                           | 0.409                          | 0.159                           | 0.586   |  |  |  |
| SST                                            | -9.763                         | -0.678                          | 0.008   |  |  |  |
| Nj                                             | 0.003                          | 0.072                           | 0.806   |  |  |  |
| RR                                             | 2.107                          | 0.696                           | 0.006   |  |  |  |
| BW                                             | 8.893                          | 0.798                           | 0.001   |  |  |  |
| $r^{2} = 0.052$ Alc = 22.027 Constant = 02.000 |                                |                                 |         |  |  |  |

Table 1. Summary of stepwise multiple regression on carrying capacity of Hokkaido chum salmon population.

r<sup>2</sup> = 0.952, AIC = 23.927, Constant = 82.869

\*ALPI: Aleutian low pressure index, SST: Sea surface temperature along the coast around Hokkaido, N<sub>j</sub>: number of released juvenile, BW: mean body weight (g) of juvenile released.

In Hokkaido chum salmon, wild population decreased since the late 1880s due to the negative impact of the poor hatchery technology, while hatchery populations increased exponentially since the late 1970s as a result of the development of progressive hatchery technology (Kaeriyama and Edpalina in press). As a result, the carrying capacity of Hokkaido chum salmon decreased abruptly at a negative critical point in the 1880s year-classes and dramatically increased at positive critical point since the 1960s year-classes (Fig. 3).





These results suggest that the carrying capacity of Hokkaido chum salmon does not relate to the ALPI and is affected by reproductive regimes such as hatchery technology, survival, and environmental factors (e.g. SST) in the early marine life period. Although the carrying capacity at the species level synchronizes with change in ecosystem linked with long-term climate change, the factors affecting carrying capacity at the population level will differ by population.

Relationship between the RCC and the fork length in Hokkaido chum salmon populations indicated a significant-positive correlation. On the other hand, relationship between the RCC and the age at maturity in Hokkaido chum salmon populations showed a significant-negative correlation (Fig. 4). The reduction in somatic growth with increase in population size is caused by the population density-dependent effect (Kaeriyama 1998). Therefore, these results suggest that carrying capacity of chum salmon may be closely related with both the long-term climate change and population dynamics.

In annual change in biomass of wild and hatchery chum salmon (Fig. 5), mean biomass of both wild and hatchery salmon in the 1990s (132 million individuals) is roughly the same as it in the 1930s (140 million individuals). However, wild salmon in the 1990s decreased 50% from that in the 1930s, despite the significant increase in the biomass of hatchery salmon. This phenomenon in chum salmon suggests that:

- (1) Despite the high carrying capacity, the biomass of wild salmon did not increase due to low survival rate (e.g. Russian, West-, and Central-Alaska populations).
- (2) Wild salmon were replaced with hatchery salmon such as pink salmon in Prince William Sounds (Hilborn and Eggers 2000). The primary reason is that, hatchery salmon have higher survival rate than wild salmon especially during their early marine life period.









Therefore, biological interaction between wild and hatchery population should be an important consideration in the sustainable management of Pacific salmon production based on the ecosystem level.

While it is true that salmon hatchery programs play an important role in meeting the demands of an expanding human population in the twenty-first century, we also would like to stress the negative impact of these hatchery programs to the wild salmon population such as the population density-dependent effect and the replacement between wild and hatchery populations. The following issues are extremely important:

- (1) harmonization with the ecosystem and
- (2) coexistence of wild and hatchery populations in the North Pacific.

To address these issues, the ecosystem-based sustainable conservation management should be adopted and implemented. A management plan should incorporate the following activities: (1) climatic and oceanic monitoring (e.g. long-term climate change, ecosystem structure), (2) biological monitoring (e.g. carrying capacity, individual somatic growth and age composition of a population, genetic and reproductive characters), and (3) sustainable management of Pacific salmon production (e.g. biological interaction between hatchery and wild populations, rehabilitation and conservation of wild salmon and riparian ecosystem) should be very important considerations.

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## Climate Change and Salmon: Some Thoughts from Outside the Box

#### **Robert C. Francis**

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Keywords: Modeling, oceanic life history, climate

This talk is meant to show how mathematical models, although they are gross abstractions of reality, can help us explore territory outside the box of conventional wisdom. I first gave four examples from my own personal experience of trying to relate climate to various aspects of marine biological production. The insights gained were:

- Time series analysis helped us understand that major changes in Alaska salmon production occurred suddenly, infrequently, and during the first several months in the ocean (Francis and Hare 1994).
- Multivariate analysis (EOF) enabled us to quantify spatial patterns in both climate (PDO) and NE Pacific salmon production and project these patterns on the time domain. We were able to demonstrate that, during the 20<sup>th</sup> century, Alaska and Pacific Northwest salmon production were out of phase (Mantua et al. 1997, Hare et al. 1999).
- Non-linear statistical modeling (GAM) helped reveal that off the Oregon coast, there is a string of relatively independent physical oceanographic processes, which, coupled together, affect coho salmon marine survival (Logerwell et al. 2003).
- Pacific Northwest coastal marine ecosystem modeling revealed that climate has both top down and bottom up effects on marine ecosystem dynamics (Field et al. 2001).

I then presented several general lessons that were learned from these four modeling exercises:

- To be useful, models must be demonstrably consistent with history.
- The essence of modeling is to facilitate the acquisition of just enough detail to produce observed patterns.
- Feeding and being fed on are the same thing.
- Be wary of one-dimensional cuts through complex systems (e.g. PDO).

Next, I gave two cutting edge examples of where modeling has been used to begin to understand biophysical interactions occurring on complex space and time scales to create observable patterns in marine ecosystems. First, Lehodey et al. (1998) and Lehodey (2001) used coupled biogeochemical, general circulation, and simple food chain models to predict downstream development of skipjack tuna forage around equatorial Pacific convergence zones and fronts. They then went one step further, added in a spatially explicit skipjack tuna life history model, and were able to show how ENSO mediates a remarkable out-of-phase dynamic coupling between skipjack habitat (equatorial warm pool), equatorial Pacific productivity (cold tongue), and downstream forage production (warm pool). And second, moving north, Aydin (2000) was able to show that two processes could make a huge difference to salmon growth during their final year on the high seas: the availability of zooplankton in the winter when zooplankton abundance is at its lowest, and the temperature mediated spatial overlap in spring and summer between salmon and the pelagic squid, *Berryteuthis anonychus*.

I concluded with several thoughts:

- Modeling is a great tool for exploring outside the box.
- The new wave combines climate driven ocean circulation models with complex food web models.
- Looking back at history is one thing; looking forward into the future is another.
- It is false to assume that policymakers require reduced uncertainty in order to take action.
- Confronting uncertainty helps you to think outside the box.

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## A Sea Change: Genetics in Conservation of the Rich and Strange

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Keywords: Pacific salmon, genetics, stock identification, management, conservation, migration

Introduction of theoretical population genetics and practical molecular genetics to the biological sciences has wrought a sea-change to our understanding of species, their interactions with their environments and their potential for longterm stability, or at least persistence. Early 20<sup>th</sup> century genetic concepts envisioned environments as constant, or constantly variable, so that organisms evolved fixed, specialized adaptations to maximize fitness given their genetic potential. Much more recent is our understanding of environmental variability, both spatial and temporal, and its impact on resident organisms. This understanding led to the prediction that intraspecific genetic variation for all traits, including those directly affecting fitness, will persist both within and among populations of a species. Development of the quantitative and molecular genetic means to measure the genetic variation encompassed by species confirmed this view of species as connected but heterogeneous populations in which local adaptation has a major influence on the ability of organisms to not only survive, but to thrive over broad environmental spans. The ramifications of intraspecific 'genetic structure', as the distribution of genetic variation among organisms has come to be termed, have been enormous for ecological theory and for man's efforts to manage and conserve organisms.

Few species exemplify local adaptation in a more spectacular fashion than anadromous salmonids, known for their proclivity to return to natal spawning grounds bounded in kilometers, if not meters, after trans-oceanic migrations themselves independently forged by population-specific interactions with the environment. Many studies have demonstrated the genetic basis for differences in growth, life history, reproduction, and mate choice among salmonids and some have even demonstrated a variable genetic basis for traits that are phenotypically identical among populations. Conservation must now be viewed in an entirely new light – no longer a Noah's Ark type of proposition, in which minimal numbers ensure eternal, or even ephemeral, representation among earthly biota. Indeed, facing an era of environmental change that many believe will be the most rapid ever experienced on earth, Noah himself would be hard-pressed to identify populations with the genetic resources qualifying them as "most likely to succeed" over the next 100 years. In the absence of foresight, we choose to hedge our bets, attempting to first identify and then conserve in moderate abundance those segments of a species possessing unique genetic resources. Thus, we serve the future a banquet of evolutionary morsels - populations, ESUs or MUs - hoping that at least one will adapt throughout the upcoming environmental sea-change. In this manner, we are trying to preserve a status quo that evolution will certainly overthrow, but through which persistence may count as victory.

The finescale local adaptation characterizing Pacific salmonid species was recognized long before we had the means of quantifying it. Now that the molecular identification of population segments with at least the potential of unique evolutionary capacity has become a cottage industry for Pacific salmonid researchers, we enter an era in which genetic variation becomes simultaneously the resource to be conserved and the tool by which conservation is achieved. In Canada, modern molecular technologies have begun transformation of approaches to the management and conservation of Pacific salmon populations.

In the Molecular Genetics Lab at the Pacific Biological Station, salmonid research is most advanced for coho, chinook and sockeye salmon. For these species, molecular genetic data have been used to delineate management units for populations of conservation concern, manage mixed-stock fisheries to minimize harvest of vulnerable populations, provide forensic identification of illegally harvested and sold fish, provide otherwise unobtainable stock-specific ecological information including migration times and routes for both juvenile and adult fish and escapement estimates for remote populations. We have compared patterns of neutral and adaptive genetic variation in fish inhabiting complex, connected environments and identified the influence of selective forces that have both increased and decreased genetic variation within populations. Looking to the future, we are undertaking studies in which gene expression profiles, combined with genetic identification of fish sampled in the wild, will provide stock specific information on the physiological status of fish and insight into the mechanisms underlying environmental influences on growth and survival.

Genetic characterization of vulnerable population segments has been undertaken in coho salmon, for which sweeping fishery closures and restrictions were undertaken in 1998 and implemented based on the distinctiveness of the threatened Interior Fraser coho population. In sockeye salmon, management of the 2002 and 2003 Fraser River

commercial fishery, the most valuable fishery in BC, was based on conservation of the genetically defined Late Run stock grouping, which had been experiencing high pre-spawning mortality for the previous five years. Genetic identification of depressed west coast of Vancouver Island chinook salmon in the troll fishery conducted near the Queen Charlotte Islands enabled simultaneous conservation of target populations and exploitation of more numerically abundant populations.

Forensic identification of salmonid tissue to species and population of origin has been used in both the conviction and exoneration of individuals suspected of the illegal harvest and/or sale of salmon. The ability to identify fish sampled from high-class restaurants or from the tin after cannery processing has widened the scope of enforcement actions meant to stem the flow of illegal product. Species identification of juvenile and immature adult samples collected from freshwater and marine environments has revealed surprisingly frequent species misidentification, sometimes with serious consequences for management decisions.

Finally, investigations of functional genes such as those in the major histocompatibility complex (MHC) form the basis of molecular studies investigating the geographic scale of natural selection on salmon populations. Surveys of existing functional variation reveal the footprint of past selection and indicate that closely related populations within a watershed have experienced different selective histories, leaving them with equally different prospects for future adaptation.

Genetic data have transformed our views of species and how they are influenced by their environments. Consequently our understanding of, and attempts to achieve, conservation are experiencing a sea-change. Genetic information, collected and implemented wisely into management, enforcement and conservation measures will continue to enrich the endeavour to sustain Pacific salmon, already rich and strange.

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### Stock Identification Studies of High Seas Salmon in Japan: A Review and Future Plan

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Keywords: Genetic stock identification, chum salmon, Japan

To manage salmon internationally, stock identification is indispensable to determine ocean distributions and abundance. In Japan, tag release, scale pattern, parasites, genetic variation, and otolith mark are mainly used to estimate the geographical origin of salmon in the ocean. In collaboration with the North Pacific Rim countries, intensive salmon tagging experiments have been conducted on the high seas since 1956. Recovery information indicated ocean migration routes of major stocks of maturing sockeye, chum, pink, and coho salmon (Ogura 1994). However, the tagging information was insufficient to clarify the distribution and abundance of major salmon stocks throughout the entire ocean life including juvenile and overwintering periods.

The migration route of Japanese chum salmon was estimated using recent information on fish abundance and genetic stock identification (GSI) of mixtures (n = 6,400) sampled on the high seas in 1993–99 (Urawa 2000). A genetic baseline of 20 allozyme loci from major stocks throughout the North Pacific Rim was employed. The GSI results showed that Japanese chum salmon globally shift their marine distribution depending on the life stage and season (Fig. 1). Juveniles are distributed in the Okhotsk Sea during summer and fall, but are confined to a narrow band (SST 4–6°C) of the western North Pacific during the first winter. Young salmon enter the Bering Sea by the next summer. In the late fall, immature chum salmon move southeast to the Gulf of Alaska for the second wintering. They migrate between summer feeding grounds in the Bering Sea and winter habitat in the Gulf of Alaska until they return to spawn through the Bering Sea and western North Pacific.

Nucleotide sequence analyses suggested that variation in the mitochondrial (mt) DNA control region of chum salmon will be useful for future stock identification (Sato et al. 2001; Abe et al. 2002). An oligonucleotide microarray hybridization method has been developed for rapid and accurate detection of 30 haplotypes in the mt DNA control region (Moriya et al. in press). In addition to GSI, an otolith mark program started in 1998. Approximately 45 million chum salmon fry with thermal marks were released from five hatcheries in northern Japan in the spring of 2002, and 14 marked juveniles were captured in the Okhotsk Sea in the following fall (Urawa et al. 2004). The number of otolith mark releases will increase year by year, with over 100 millions in 2004. Thus otolith marking is expected to be a practical tool for various salmon studies in Japan.

Future Japanese research plans include the long-term monitoring of salmon stocks in the major feeding habitats (the Okhotsk Sea and Bering Sea) to estimate their distribution and abundance during summer and fall. The stock identification of mixture samples will be conducted by allozyme, mt DNA, and otolith mark analyses. These identification methods should contribute to sustainable salmon fishery management.



Fig. 1. A seasonal migration model of Japanese chum salmon estimated by genetic stock identification.

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### The Perspectives of the Pacific Salmon Stock Investigations in Russia

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Keywords: Pacific Salmon, genetic stock identification, Russia, scales

The main goal of science associated with the fisheries industry is the exploitation and harvesting of fish populations without destroying them. It includes several important objectives: 1) to study the dynamics of populations and the mechanisms of their responses to environmental changes, 2) to forecast their responses and to estimate expected abundance in advance, and 3) to estimate the optimal size of future catches which stabilize population fluctuations and that will not destroy their ability to self-regulate. All of these objectives have one need in common – to determine the stock structure of exploited populations. The development of the "stock concept" of fish populations is especially important for the management of the salmon industry. This concept states that the whole species consists of reproductively isolated units – "stocks" or "Mendelian" populations that have their own genetically conserved specific traits and are organized into some kind of structure that allow them to maintain equilibrium for better adaptation and survival. Thus, the problem of revealing this structure and differentiation of fish populations is vitally important for salmon management.

In Russia such studies were started in the early 1970's by Yu. Altikhov and S. Konovalov. The main problem of these first studies of population structure was the lack of markers which could reveal differences between stocks and their responses to environmental influences. Now, 30 years later, the problem of good markers still stands before scientists studying population processes and micro-evolutionary changes. The first markers used were exterior morphological characteristics, but the genetic inheritance behind these characters was not understood. Some scientists used the bone structure of the skull, and this method was very useful for systematic studies. Other morphological method used more than 20 measurements of body composition such as the number of vertebra and gill rakers. There were significant morphological differences between populations, but they were not associated with interspecies structure on a permanent basis and varied from year to year. Parasite infestation was also used as a marker for some sockeye and pink stocks of Kamchatka and Sakhalin.

The most important technique involved the differences in scale structure. Scale analysis was first developed for determining the age of fish. However, it was discovered that there were differences between stocks in the structure of sclerites which formed in the first year at sea. Analysis of these differences resulted in successfully discriminating between some salmon stocks. In Russia this method was developed in the 1970's by M. Selifonov and V. Bugaev (sockeye), E. Nikolaeva (chum), J. Zorbidy (coho), N. Grachev (pink), Savvaitova (mykiss) and many others. Currently, using modern statistical procedures, stock identification by scale pattern analysis has been done by N. Antonov, A. Bugaev, O. Temnykh, N. Klovach, L. Zavarina, and others. However, scale analysis has its limitations. The main problem is that the scale structure is not genetically inherited and cannot be considered as a permanent population characteristic. Differences depend on the climate conditions of each particular year, and most importantly, reflect the specific environment of the estuaries and bays where juveniles spend their first summer and fall. Therefore, differences in scale structure reflect these particular environmental conditions and are not the result of genetic differences.

Protein polymorphism was the first set of markers which were able to reveal real genetic relationships between populations. In Russia we first studied the polymorphism of 2–5 markers which allowed us to determine many special traits of micro-evolutionary process, and to obtain important information on racial and spatial distributions of populations. Important studies in the 1980's were performed by the laboratories of population biology and genetics from Moscow Institute of General Genetics, Kamchatka Research Institute of Fisheries, TINRO-Center, Magadan Institute of Biological Problems of Arctic, Institute of Marine Biology, Vladivostok by Yu. Altukhov, E. Salmenkova, L. Jivotovsky, V. Omelchenko, V. Kirpichnikov, R. Victorovsky, A. Makoyedov, V. Kartavtcev, V. Efremov, N. Varnavskaya and others. The work of these scientists was very important, but the small number of markers used did not allow the use of reliable statistical procedures for discriminating individual populations.

Only when the ability to determine multi-loci genetic characteristics of populations was developed were we close to reaching the goal of identification by stock. The Laboratory of Population Biology and Genetics of KamchatNIRO, Petropavlovsk-Kamchatsky, began to collect data on multi-loci characteristics of salmon stocks throughout the Russian Far East. This data were analyzed by starch electrophoresis in co-operation with several North American agencies: Pacific Biological Station Nanaimo, Canada; NOAA Northwest Fisheries Center, Seattle;

Auke Bay Laboratory, Juneau; U.S. Fish & Wildlife Service, Anchorage; Alaska Department of Fish & Game, Anchorage, USA. One of the main objectives of this project was to standardize allele mobilities and electrophoresis techniques, and to create comprehensive genetic baselines including all salmon populations throughout the Pacific Rim. The main species of study were pink salmon – *Oncorhynchus gorbuscha*, chum salmon – *O. keta*, sockeye salmon – *O. nerka*, and chinook salmon – *O. tshawytscha*.

The work consists of two parts: first is the conservation of natural populations; and secondly the identification of natural and artificial populations. It includes the determination of genetic population structure within species, the possible levels of interspecies hierarchy, and the estimation of their divergence using the random selection of enzymes-coding genes. The next step should be monitoring of genetic changes occurring as a result of human activities such as fishing and artificial propagation. Stock identification requires the use of techniques that will identify specific populations in mixed-stock aggregations throughout their habitats and different life stages. Practical applications of Genetic Stock Identification consist of: 1) estimations of contributions of particular local stocks to the fisheries, and therefore, the survival, stock composition and relative abundance of salmon in the ocean, and 2) to determine migration timing and routes for the adults and juveniles of local stocks in the Pacific Ocean, adjacent seas, estuaries, and freshwater basins.

During the last 15 years more than 20,000 individual fish were analyzed from natural populations from all over Russian Pacific coast, and more than 3,500 from the Pacific Ocean. Forty-three enzyme systems coding up to 100 loci were scored using starch gel electrophoresis. Variation at more than 30 polymorphic loci in stocks including those from the North America were analyzed, and significant differences between and within regional population complexes were detected. Cluster and multifactor analysis were performed for all main stocks throughout the Pacific Rim for four species. High levels of genetic divergence and characteristics of interspecies population hierarchy were obtained, and gene flow and migration were studied. Genetic data were used for detecting the mechanisms of micro-evolutionary changes such as natural selection, genetic drift, intensity of genetic migration, and reproductive isolation. The interactions of population dynamics and genetic structure were revealed. The relative abundance and biological characteristics of main regional population complexes throughout the Pacific Rim were summarized in comparison with interspecies structuring. Several sets of experiments were conducted revealing the mechanisms of natural selection influence on genetic polymorphism and its adaptive values. The international datasets on allozyme polymorphism were created, and a numerous studies on stock composition in mixed-stock fisheries in the Pacific Ocean were performed.

Enzyme polymorphism has been very useful for population genetic studies, but it has limitations. Many discriminating enzyme loci were found in chinook and chum salmon populations, but only a limited number in sockeye that have not allowed accurate stock discrimination. In pink salmon, many polymorphic loci were detected, but the spatial differention by population units was shown to be low, and regional groups of populations could not be satisfactorily identified. As a result, scientists are now investigating DNA polymorphism techniques for salmonid species.

In Russia, mitochondrial DNA polymorphism in salmon and other species is studied at the genetic laboratory of the Institute of Marine Biology since the early nineties under the supervision of Dr. V. Brykov. This work is conducted in association with University of Alaska, Juneau. They discovered polymorphism in haplotype variation in pink, chum, and coho salmon of Eastern Kamchatka, Sakhalin, Primorie and Kuril Islands. They found significant differences between pink salmon regional groups and significant differences between regional groups of Sakhalin and Primorie chum salmon. Within regional groups, they found no significant differences in both pink and chum salmon.

Variation in microsatellite nuclear DNA was shown to have some attributes that are useful in population analysis and stock identification. Alleles show co-dominant Mendelian inheritance, high levels of spatial variation, and no significant annual differences resulting in high levels of accuracy and precision for stock identification. Studies of microsatellite DNA in sockeye salmon of Asia were started recently in co-operation with the Pacific Biological Station, Nanaimo. Data for 25 local stocks were collected on variation of 13 microsatellite DNA loci: *Ots3*, *Ots100*, *Ots103*, *Ots107*, *Ots108*, *Omy77*, *Oki1a*, *Oki1b*, *Oki6*, *Oki10*, *Oki16*, *Oki29*, and *One8*. Stock identification studies in the Bering Sea were performed using microsatellite nuclear DNA variation in sockeye and the proportions of American and Russian stocks in that area during summer and fall were obtained.

## International Data Bases Help Resolve Migration and Survival of Pacific Salmon in the North Pacific Ocean and Bering Sea

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Keywords: Allozyme, allele standardization, microsatellite, SNP, stock identification

In the summer of 2003, the United States completed its fifth decade of research into the distribution and migration of salmonids in the North Pacific Ocean and Bering Sea. These basins provide major feeding habitats for various salmon stocks originating from both Asia and North America (e.g., Kaeriyama 1998; Seeb et al. in press). Information from tags, parasites, or scale patterns provides important insights (Wood et al. 1989; Patton et al. 1998; Urawa et al. 2000); here we review the emergence of genotyping approaches used by United States laboratories that have led to finer resolution in stock identification studies.

Unanticipated fluctuations in the productivity of some regional aggregations of stocks (see Anon. 1998) increased interest into the effects of environmental variables. We anticipate that stock identification using gene markers will provide a better understanding of migration patterns that, when coupled with existing ecological studies, may clarify mechanisms that cause variable production in the patchy marine environment (see Sukhanova et al. 1999). Pertinent hypotheses include: stocks are segregated both geographically and temporally, stocks utilize similar geographic areas, but are temporally segregated, stocks overlap both geographically and temporally but respond differently to existing environmental conditions, competition of wild fish with larger hatchery fish, or interceptions in near-shore fisheries (Brannon 1984, cf. Welch et al. 2003).

The usefulness of gene markers, initially in the form of allozyme data developed by the NOAA Fisheries laboratory in Seattle, emerged in the 1970s (Utter et al. 1976). During the 1980s several laboratories, ranging from University of California Davis to the NOAA Fisheries Auke Bay Laboratory in Alaska, started their own stock identification programs using allozymes. It was soon realized that the synergy provided by laboratory to laboratory standardization could generate huge and enormously valuable databases for gene markers from populations throughout the species range (see Shaklee and Phelps 1990). The laboratories named above, along with laboratories from the States of Washington and Alaska, formed a consortium of cooperating agencies who met frequently to run allele comparisons and merge data sets from their respective jurisdictions. Cooperation and support by laboratories from Japan and Russia yielded some of the largest data sets of this kind for any organism (chinook salmon data maintained by NOAA Fisheries, Seattle [Teel et al. 1999]; sockeye salmon data maintained by NOAA Fisheries, Auke Bay [Habicht et al. 2001]; and chum salmon data maintained by Alaska Department of Fish and Game [Kondzela et al. 2002]).

During the 1980s and 1990s laboratories began comparing various DNA techniques to improve the accuracy offered by allozyme data and to streamline the sample collection procedures (allozyme analysis requires frozen tissue and organs while DNA can be extracted from fins stored at room temperature; interesting comparisons may be seen in Park et al. 1994; Scribner et al. 1998; Allendorf and Seeb 2000). Numerous techniques were developed and evaluated including detection of (1) DNA sequences (Park et al. 1993), (2) restriction fragment length polymorphisms (RFLPs) of mitochondrial and nuclear DNA (Cronin et al. 1993), (3) randomly amplified polymorphic DNA (RAPDs; e.g., Allendorf and Seeb 2000), and (4) length polymorphisms of minisatellite and microsatellite DNA (Beacham 1996; Beacham and Wood 1999; Allendorf and Seeb 2000). Each offered some advantages over allozyme techniques, but data from highly polymorphic microsatellite loci emerged as the favored technique of many laboratories. However, various issues such as slow throughput or the non-transportability of data from laboratory to laboratory retarded the general application of these DNA approaches for most high-seas studies.

The NPAFC Science Plan 2001-2005 (http://www.npafc.org) renewed the mandate for expanded stock identification research and increased at-sea research on the effects of anomalous ocean conditions. Consortium agencies are now collaborating to replace the allozyme data bases by (1) standardizing an inter-agency database for microsatellite DNA for chinook salmon and (2) shifting to analysis of single nucleotide polymorphisms (SNP DNA) whose attributes lend themselves to high throughput and easy standardization among laboratories and among nations (see Brumfield et al. 2003; Melton 2003).

Standardizing data from microsatellite DNA across laboratories and analysis platforms has proven to be extremely difficult. An attempt to standardize microsatellite data for chinook salmon was begun in 1999, but that effort was dropped, and to date no interagency databases exist for any Pacific salmon. Recently, a large and costly

program to standardize microsatellite loci for chinook salmon was funded by the Pacific Salmon Commission. This program shows substantial promise, but the standard data set will be limited to those populations inhabiting the eastern Pacific Ocean.

In contrast, the various SNP assays now available all produce genotype data that is automatically standardized across laboratories. Additionally, the SNP techniques capture some of the same genetic data that was collected by slower-throughput sequencing and RFLP techniques used in the past. For chum salmon, for example, historical sequence data (Park et al. 1993), RFLP data (Cronin et al. 1993), contemporary sequence data collected by different techniques (Abe et al. 2002) or microarray analysis (Moriya et al. 2002) are all captured by the high-throughput SNP techniques of Smith et al. (2003). In some organisms, SNPs have demonstrated diagnostic differences between populations that could not be differentiated by microsatellites (Bensch et al. 2002). Several United States laboratories are developing SNP assays for chum, chinook, and sockeye salmon, anticipating that issues such as data quality and transportability will make these the markers of choice for the study of these migratory species (cf. Morin et al. in press).

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## A History of U.S. High Seas Salmon and Steelhead Stock Identification Research

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Keywords: High seas, salmon, steelhead, U.S. history, stock identification

This abstract provides a brief history of U.S. high seas salmon and steelhead stock identification research. Jackson and Royce (1986) and Harris (1989) reviewed the history of the Japanese high seas salmon driftnet fisheries and the international treaties that regulated them. After World War II, interceptions of Alaska salmon by Japan's rapidly expanding high seas salmon driftnet fisheries were a major concern to the U.S. salmon fishing industry. A Japanese mothership fleet operated in the Bering Sea and in the North Pacific Ocean (north of 46°N), and a landbased fleet operated south of 46°N in the North Pacific Ocean. In 1953 the International Convention for the High Seas Fisheries of the North Pacific Ocean established the International North Pacific Fisheries Commission (INPFC, 1953–1992), and set a provisional abstention line that restricted the Japanese fisheries to areas west of 175°W. The major goal of INPFC research was to determine the areas of intermingling of Asian and North American salmon in the North Pacific Ocean and adjacent seas.

Burgner (1992) reviewed the history of INPFC research by the United States, which involved a coordinated effort among federal, university, and state scientists, as well as cooperation with Canadian, Japanese, and Soviet scientists. A U.S. tagging study conducted in the first three years of the program (1956–1958) showed that Asian and North American salmon intermingle in the vicinity of 175°W, and established the necessity for a major international high seas salmon research program (Thompson 1954, 1962; Hartt 1962). The INPFC research plan included four major elements: (1) sampling to determine salmon distribution, (2) tagging to study salmon movements, (3) oceanographic studies to relate environmental factors to salmon migration, distribution, and growth, and (4) racial studies to identify Asian and North American salmon. The U.S. racial studies involved the development and application of new methods to identify Asian and North American salmon, including: (1) meristics and morphometrics, (2) scales, (3) serology, and (4) parasites. These methods met with varying degrees of success. The most frequent problem encountered was the lack or insufficiency of baseline data for Russian salmon. The major results of this pioneering research are summarized in INPFC joint comprehensive reports for coho (Godfrey et al. 1975), sockeye (French et al. 1976), chum (Neave et al. 1976), chinook (Major et al. 1978), and pink (Takagi et al. 1981) salmon.

The INPFC Convention was changed in 1978 and again in 1986 to reduce Japanese interceptions of North American salmon, and INPFC research focused on determination of the continent of origin of salmon and steelhead migrating in the Japanese landbased driftnet fishery area. Myers et al. (1993) summarized the methods and results of this research. The primary stock identification tools used were: (1) scale patterns, (2) tags, and (3) parasites. The U.S. scale pattern research provided the first quantitative estimates of the relative proportions of Asian and North American sockeye, coho, and chinook salmon in the region south of 46°N, as well as estimates of interceptions of Russian and North American salmon by the Japanese landbased fishery. The final results indicated intermingling of Asian and western Alaska stocks in this region, however, the majority of sockeye and chinook salmon intercepted by the landbased fishery were of Russian (Kamchatka Peninsula) origin. Bias was suspected in the results of coho salmon scale pattern analyses because of insufficient baseline data. High seas tagging results significantly increased the known limits of ocean distribution of many Asian and North American salmon and steelhead stocks, and provided indisputable evidence of the presence of western Alaska sockeye, coho, chum, pink, U.S. West Coast stocks of steelhead, and Russian sockeye, chum, and coho salmon in the area closed to high seas salmon driftnet fishing in 1978. Coded-wire tag recoveries showed that U.S. Pacific Northwest coho and steelhead stocks migrated far offshore into the landbased fishery area, as well as into the area of a rapidly expanding Asian driftnet fishery for flying squid (Ommastrephes bartramii). Parasite studies showed that U.S. steelhead ranged across the area south of 46°N to as far west as about 162°E.

In 1993 the Convention for the Conservation of Anadromous Stocks in the North Pacific Ocean established the North Pacific Anadromous Fish Commission (NPAFC, 1993-present). The Convention prohibits salmon fishing in international waters of the North Pacific Ocean and Bering Sea, and emphasizes the importance of scientific research for the conservation of anadromous salmon stocks. The development of the NPAFC science plan was coordinated with the North Pacific Marine Sciences Organization (PICES). The overarching goal of this plan is to

investigate the effects of change in the productivity of the North Pacific Ocean on Pacific salmon, including: (1) current trends in ocean productivity and effects on carrying capacity, and (2) changes in biological characteristics of salmon (growth, size and age at maturity, oceanic distribution, survival, and abundance). Myers et al. (2000) reviewed the results of U.S. research in the 1990s under this plan, including the development and application of new stock identification tools, comprehensive genetic baselines, and statistical techniques in cooperation with Canadian, Japanese, and Russian scientists. The primary stock identification tools used by U.S. scientists included genetics (allozyme and DNA), thermal otolith marks, tags, and parasites. The results provided new information on the distribution, migration patterns, and relative proportions of regional stocks of Asian and North American salmon in coastal and offshore waters, as well as information on the origins of salmon seized from vessels fishing illegally in NPAFC Convention waters.

In 2000 the NPAFC adopted a new five-year science plan (2001–2005) that emphasizes cooperative science activities in three areas: (1) Bering Sea salmon research, (2) juvenile salmon research in eastern and western North Pacific waters, and (3) winter salmon research. An important aspect of U.S. research in all three areas is to investigate stock-specific growth and other biological characteristics of Asian and North American salmon. Some of the new stock identification methods being developed and the preliminary results of this research were presented in other papers at this workshop.

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## Status of the Bering-Aleutian Salmon International Survey (BASIS)

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Keywords: Salmon, Bering Sea, climate change, trawls, forage fish

North American and Asian salmon that utilize the Bering Sea have declined in recent years. Changes in ocean conditions have occurred and these changes may be responsible for the declines in salmon. In order to understand how these changes influence abundance of salmon, a new research program was initiated in 2002 and coordinated through the North Pacific Anadromous Fish Commission (NPAFC). The Bering-Aleutian Salmon International Survey (BASIS) was developed to learn how salmon respond to variations in ocean conditions caused by climate change. For the first time, scientists from the NPAFC - Canada, Japan, Russia, and the United States - developed a scientific plan and followed through with coordinated surveys of salmon distribution across the entire Bering Sea in 2002 and 2003. Oceanographic observations were made simultaneously with fishing operations. Large trawls, towed near the surface, were used to sample salmon and associated marine forage and prey species. The RV Kaiyo maru from Japan, the RV TINRO from Russia, and the FV Northwest Explorer and FV Sea Storm from the United States participated in the surveys. Ships from the three countries rendezvoused north of Attu Island in 2002 near the Russian boundary and made side-by-side tows to compare catches. Genetic methods are used to identify country and location of origin of salmon captured at sea so that stock-specific migrations can be determined. Samples from catches at sea are saved for studies on age, growth, food habits, lipid content, and bioenergetics as well for genetic samples. Data on salmon, forage and prey species, and oceanographic data are shared between the countries in common databases.

#### The Problem of Pacific Salmon Stock Identification during the Marine Period of Life (Results and Prospects)

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Keywords: Pacific salmon, stock identification, tagging

The problem of stock identification of Pacific salmon caught on the high seas was important from the very outset of fishing at sea. However, the priorities for the problem have varied considerably through time. Initially, during the period of large – scale high seas fishing, the stocks of salmon needed to be identified in order to establish the scientific basis for further diplomatic, legal, or enforcement efforts. In recent years, with the drastic reduction in high seas fisheries, the need to track the origin of the salmon taken from some specific regions of the ocean was dictated primarily by ecological considerations.

At present the task of studying the population structure is being accomplished by applying several sets of techniques: morphological, genetic, parasitological, and marking or tagging. A collation of the potentials of these methods, even in most general terms, shows that the identification of local fish populations is not simple enough to entrust to any of the above sets of methods alone if we are to obtain an unambiguous solution. This makes it extremely important to compare and give an unbiased interpretation to the results of studies made using various techniques.

The technique of tagging stands out from all methods used because it ensures unambiguous results which do not need interpretation. That is why the results of tagging can be reasonably used as a kind of reference point or null hypothesis in interpreting the results obtained by other methods. It is appropriate to remember that the problems of population structure have been successfully resolved in ornithology where ring tagging was applied for a long period time.

Let us consider the results of tagging made between 1956 and 2000 (Gritsenko 2002). Both the abundance of salmon and their sea habitat conditions have varied widely throughout that period. That is why the pattern of distribution of individual stocks obtained has a generalized format. For the most part it shows the range of adult Pacific salmon during their spring and summer feeding and the pre-spawning migrations. Given below are the essentials of the spatial interaction among the Asian and American stocks.

In spring and summer a great number of the Asian pink salmon is found west of 180°. However, a considerable portion of is still east of 180°. The major concentrations of the Asian pink salmon in May–July occur on the high seas; an important part of them is found in the U.S. zone, north and south of the Aleutians, and in the Central Bering Sea. The bulk of American pink salmon stay east of 160°W. For the most part, it is the pink salmon of the north-western coast of Alaska.

Consequently, the feeding ranges of the Asian and American stocks of pink salmon overlap in a small area limited by 170°W and 180° because of the wider distribution range of the Asian pink salmon.

The Asian coast chum salmon is more frequent in the North Pacific than the other salmon species. It feeds in the Pacific waters off the Aleutians within the U.S. zone, the high seas area of the Bering Sea, the U.S. EEZ northeast of the eastern boundary of the Central Bering Sea, and the high seas areas in the Pacific Ocean north of 40– 41°N. Besides, large amounts of the Japanese chum salmon migrate between June and October north to south within the Russian EEZ. Most of the American chum salmon stay within the zones of the USA and Canada or the high seas area of the Gulf of Alaska. The feeding ranges of the Asian and American chum salmon overlap mostly inside the U.S. zone off the Aleutians; east of the donut hole in the Bering Sea; and insignificantly in the Gulf of Alaska.

The Asian and American sockeye salmon stay together in a vast territory between 168°E and 175°W. However, less than 1% of the total number of fish of the Asian and American stocks feed in this far-reaching region. It should be pointed out that their ranges coincide mostly on the high seas and in the U.S. zone.

One important feature of distribution of coho salmon at sea is its relative proximity to the continents which is probably related to the shortness of its marine period of life. The overlapping zone of the Asian and North American stocks of coho salmon is located between 167°E and 172°W where there is not more than 32% of the

Asian and 1.54% of the North American stocks. The North American coho salmon remain far away from the Russian EEZ whereas small quantities of the Asian coho salmon occur in the U.S. zone south of the Aleutians, while most of it stick to the high seas areas of the Pacific.

Unlike the other species of salmon, the American stocks of chinook are distributed across a relatively small area of the ocean. The inshore stocks of chinook in the Gulf of Alaska, off British Columbia and the state of Washington cling to shores near Washington and Oregon, as well as the Alaskan Peninsula and the Aleutians. One exception is the chinook off the Bering Sea coast of Alaska which is widely distributed throughout the Bering Sea. We have heard of individual cases of it entering the EEZ of Russia (off Koryak hills) in July.

Of all the salmon near the American coast the steelhead has the longest westwardly range. Unlike the other species, the concentration density in the steelhead salmon does not decrease along with the increasing distance from the American continent. Its range is attached to the North Pacific drift and is located over its main branch.

According to S.A. Kovalenko et al. (2003), some individual steelhead marked by removal of fins occur in the Russian EEZ around the Kuril Islands.

Consequently, the tagging data indicate that the American chum and pink salmon are actually not observed within the EEZ of Russia. It is the sockeye and chinook of the American stocks that feed in small numbers in the northeast of the western Bering Sea within the Russian EEZ during summer and autumn. On the whole, it is mostly the eastern part of the common range that the regions of distribution of the Asian and American stocks overlap. Hence, the fish belonging to most of the Asian salmon stocks inhabit in winter and spring the high seas area of the Pacific Ocean and the U.S. zone.

The data on the migratory routes and their length obtained from tagging appear to be most useful for making hypotheses regarding the potential presence of some or other representatives of local populations in catches. Such judgments make it possible to use a far smaller number of parameters employed in the statistical procedures of the maximum likelihood method and to avoid errors in conclusions on the composition of mixed concentrations obtained by analyzing the morphological or genetic data (Wood et al. 1987).

The choice of methods is dictated by the task set. In some cases simple express-methods are enough. For example, differentiation between the Russian and Japanese chum in the Kamchatkan waters of the Pacific and the Bering Sea could well be made using the technique of counting the sclerites on the fish scale in the summer growth zone of the first year of life at sea (Klovach and Zavarina 2001). The differences detected in the biological characteristics of individual stocks of pink salmon during the same spawning season are usually applied to subdividing the migrating groups both into large sets of stocks, e.g. northern and southern stocks in the Sea of Okhotsk (Temnykh 1996) and smaller stocks, e.g. West Kamchatka and East Sakhalin stocks of pink (Shubin and Kovalenko 2000). The length, weight, number, as well as the shape of spots on the tail, GSI, sex ratio, etc. were used as indicators. Analysis of the age structure of the migrating concentration is sufficient to differentiate between sockeye stocks on a specifically local level (West Kamchatka and North Kuril Islands sockeye) since among the West Kamchatka approach there are virtually no fish which had lived in fresh water for less than two years, while such individuals make up over 80% in the North Kuril Islands sockeye stock (Gritsenko 2000). Seeking and application of simple and cheap methods remains currently central since the situations as mentioned above can emerge in various areas of the ocean, and in various species.

Conversely, not only express methods, or complicated scale structure analysis techniques, but also the complex and expensive genetic method of isoterment analysis turned out to be of little use in dealing with the global challenge of distinguishing between the Asian and American sockeye.

In that respect we lay great hopes on the method of polymerase chain reaction using random primers or random amplified polymorphic DNA (RAPD), i.e. amplification of polymorphic DNA. It is exactly this technique that has often been used in recent years for DNA fingerprinting (Welsh and McClelland 1990; Williams et al. 1990).

The major objective in developing a molecular-genetic identification scheme is to establish a reference collection of DNA samples. In 2003 we began to set up such a collection for sockeye and Chinook since these species are the most debatable.

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## Origin of Juvenile Chum Salmon from Gulf of Alaska Coastal Waters, 2000 and 2001 Determined from Genetic Variation and Hatchery Thermal Marks

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Keywords: Juvenile chum salmon, migration, stock identification, genetic variation, thermal marks, Gulf of Alaska

Summer surveys (July–August) of juvenile salmon ecology along the continental shelf of the Gulf of Alaska are conducted annually by scientists from the Ocean Carrying Capacity program of the National Marine Fisheries Service's Auke Bay Laboratory. These surveys are an effort to link changes in salmon production to biological and physical factors in the ocean environment. An improved understanding of salmon distribution is one objective of this research. We identified the origin of juvenile chum salmon collected in transects from around the Gulf of Alaska in 2000 and 2001, using the presence of thermal marks in hatchery fish and the divergence of genetic characteristics among regional groups of populations.

Juvenile chum salmon were caught on every surveyed transect along the continental shelf between nearshore and slope stations; very few fish were recovered from oceanic stations (Fig. 1). In both years, 36% of the juvenile chum salmon caught during the survey had been thermal-marked from three Alaska hatcheries, the Macaulay and Hidden Falls facilities in Southeast Alaska, and the Wally Noerenberg facility in Prince William Sound. By transect, the proportion of thermal-marked fish ranged from 0 to 85%. The majority of thermal-marked fish were captured just beyond the nearest coastal exit corridor; few thermal-marked fish were recovered from Shelikof Strait or south of Kodiak Island. Thermal-marked fish from Macaulay Hatchery were recovered between Icy Point and Gore Point, with one recovery in Shelikof Strait at Cape Kekurnoi. Hidden Falls fish were restricted to transects east of Prince William Sound, except for one fish recovered on the Gore Point transect. Thermal-marked fish from the Wally Noerenberg facility were recovered on every transect west of Prince William Sound, with the majority caught on the Seward and Gore Point transects.

Identification analyses based on genetic characteristics revealed similar regional estimates across the Gulf of Alaska between the two years. Overall, the contribution of southern North American populations was low: significant, but limited contribution was only found in transects off the Kenai Peninsula. Two distinct size categories of fish along the Gore Point transect occurred in 2000. At least 90% of the larger fish were estimated to be from the southern British Columbia-Washington region, whereas 85% of the smaller fish were from Prince William Sound. As with the thermal-marked fish results, the genetic estimate of contribution of Southeast Alaska-northern British Columbia populations was greatest in collections east of Prince William Sound, and the Prince William Sound contribution was concentrated just west of Prince William Sound, Fish

**Fig. 1.** Juvenile chum salmon transects surveyed in the Gulf of Alaska by the Ocean Carrying Capacity program during the summers of 2000 and 2001.



from northern Cook Inlet (Susitna and Yentna rivers), and the Alaska Peninsula–Kodiak Island regions made up a large proportion of fish caught in the Shelikof Strait transects. These results provide the most detailed view to date on the distribution of juvenile chum salmon migrating through this coastal corridor. Upon entering coastal waters, at least some juvenile chum salmon from southern North America stocks turn northward along the outer coast and migrate in a narrow band along the continental shelf, where they are met by more northern stocks. This summer migration continues in a counterclockwise direction around the continental shelf of the northern Gulf of Alaska at least as far as Kodiak Island. Fall surveys in the northern Gulf of Alaska and southern Alaska Peninsula will be necessary to determine where and when stocks move further offshore to oceanic waters.

## Spatial Distributions of Juvenile Chum Salmon in the Coastal Waters of Eastern Hokkaido Determined with Otolith-Marking in Relation to Zooplankton Community

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Keywords: ALC marking, hatchery chum salmon, spatial distribution and abundance, SST, zooplankton

The number of hatchery-origin chum salmon in Hokkaido increased from 10 million in the middle 1970s (brood year) to 40 million in the 1980s. During the 1990s, the chum population fluctuated between 27 and 65 million with return rates varying between 2.6% and 5.9%. There have also been great differences in return rates among chum returning to the Okhotsk Sea, the Japan Sea, and the Pacific Ocean. Recent return rates to the streams and coast of the Okhotsk Sea have been much higher than those in other areas. More interestingly, great differences in return rates between early and late run groups were found; return rates for early runs were higher than for late runs. We conclude that these fluctuations and differences are caused by coastal water conditions in relation to food production and predation. Hokkaido chum salmon populations have been maintained by hatcheries with similar numbers of juveniles released (approximately ten billion in Hokkaido) every year during the past twenty years. In contrast, wild salmon tend to fluctuate in recruitment. Therefore, we started a project in 2002 to clarify mechanisms responsible for population fluctuations in hatchery-produced chum salmon in relation to ocean conditions, especially the coastal environments. This project has been undertaken in collaboration with Hokkaido Fisheries Experimental Stations, Hokkaido Tokai University, and Hokkaido University. The project is comprised of three parts: zooplankton community structure, population dynamics of chum salmon, and prey-predator interactions.

We chose the Abashiri coast in the southwestern part of Okhotsk Sea to investigate the spatial distribution and abundance of juvenile chum salmon because there were large chum populations in the area that had highly variable survivals, but little recent information on chum salmon biology. On December 19–20, 2001, otoliths from chum salmon at the eyed egg stage in the Abashiri River were mass-marked with 200 ppm ALC (alizarin complexone) solution for 24 hrs in the Aioi Private Salmon Hatchery. ALC marking was used since it is very easy to detect the

marks under a fluorescent microscope without polishing the otolith. In mid May 2002 we stocked two million otolith-marked (47 mm mean FL) juveniles in the Abashiri River where a total of 35 million hatchery juveniles were stocked in May. Twelve study sites were established in the Abashiri coastal waters (Fig. 1). Four sites were set up 1 km off coast (~ 10 m deep), near the shoreline. Four study sites were established at 4 km (20–30 m deep) and 7 km (30–40 m deep) off the coast.

Although the mean SST in late April was < 5 °C, temperatures rapidly increased and exceeded 8 °C in early May, and then increased gradually in June, eventually reaching to 14–15 °C in mid July when we finished our investigation (Fig. 2). A total of 60,000 chum juveniles were collected with a surface trawl net towed 2 km at 4–6 km·hr<sup>-1</sup> from late April to mid July, 1–7 km off the coast. Approximately 2% of the catch were comprised of ALC marked juveniles. Most juvenile chum salmon were in the coastal waters where SST ranged between 9 and 14 °C. Although high CPUE (catch-per-unit effort; number of juveniles per 2 km towing) of unmarked chum







Fig. 2. Changes in mean values of SST and CPUE (catch per unit effort, the number of juveniles per 2 km towing) of no-marked and ALC marked juvenile chum salmon captured at the 1 km, 4 km and 7 km off the Abashiri coast in the Okhotsk Sea.

salmon was recorded at the four sites 1 km off the coast from mid May to early June, fish densities decreased rapidly afterwards (Fig. 2). In contrast, fish densities 4 and 7 km off the coast were much lower than at the sites 1 km off the coast, peak CPUEs occurred 10–20 days later, and densities decreased gradually after the peak. ALC marked juveniles were captured ten days after stocking. Marked juveniles showed similar patterns in fish abundance and distribution as unmarked fish. Fork lengths of juvenile chum salmon varied between 3 and 15 cm, with most from 4–8 cm. Although fork lengths at every site tended to increase with elapsed time, fish 1 km off the coast were significantly smaller than for those caught 4 and 7 km off the coast (Fig. 3). Although the fork length distribution of ALC marked juveniles was unimodal at the study sites, the length distribution of unmarked juveniles was bimodal or polymodal, indicating that groups of hatchery-produced fry emigrate at different times and/or grow at different rates.

Zooplankton were collected using a Norpac net (45cm mouth diameter, 0.33 mm mesh size) towed vertically from the bottom to the surface at each site. Zooplankton was most abundant in the inshore waters, 1 km off coast. Mean zooplankton abundance at all sites decreased gradually from May to July (Fig. 4). Copepods (mainly *Peudocalaunus newmani*) that predominated in May decreased in June when cladocerans (mainly *Podon leuckarti, Evadne nordmanni*) and

appendicularians (mainly *Oikopleura longicauda*, *Fritillaria borealis* f. *typica*) numbers increased. Stomach content indices (stomach content x 100 / body weight) for juvenile chum were high in late May and early June, but they decreased rapidly in late June (Fig. 4). Diet composition by number showed juvenile chum salmon consumed mainly copepods early in the season, switching to cladocerans and appedicularians as these became more abundant in the sea.

We know from results from the same area twenty years previously that most chum salmon fry tended to occur in nearshore areas where SST was from 5 to 13 °C and subsequently Fig. 3. Changes in mean fork length of no-marked and ALC marked juvenile chum salmon captured at the 1 km, 4 km and 7 km off the Abashiri coast in the Okhotsk Sea.


**Fig. 4.** Changes in mean values of zooplankton abundance, wet weight and stomach content index (stomach content weight x 100/body weight) of no-marked and ALC marked juvenile chum salmon captured at the 1km, 4km and 7km off the Abashiri coast in the Okhotsk Sea.



they moved offshore where SST > 14 °C (Irie, 1990). The 2002 year migration event showed the same pattern. Kaeriyama (1986 and 1989) found different types of offshore migration patterns for juvenile Japanese chum salmon. Early migrating juveniles tended to remain in the coastal waters with favorable conditions for a relatively long time where they grew fast, moving offshore later. In contrast, late migrating juveniles tended to remain nearshore for a short time because of unsuitable conditions such as a lack of food and high SST, subsequently they moved passively offshore. Our results also suggested that juvenile chum salmon that migrated early from the Abashiri River remained near shore and grew fast under the preferable conditions of abundant food and optimal SST (8–13 °C), and actively moved offshore afterwards. Later migrating juveniles may experience unfavorable conditions because of increases in fish density, decreases in prey abundance and high water temperatures, often exceeding 13 °C.

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### Stock-Specific Distribution and Migration of Juvenile Chum Salmon along the Eastern Bering Sea Shelf

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Keywords: Genetic analyses, western Alaska, juvenile chum salmon, stock-specific migration

Genetic stock identification techniques were used to identify the origin and provide stock-specific migration and distribution patterns of juvenile chum (*Oncorhynchus keta*) salmon caught during annual fall surveys (2002) along the eastern Bering Sea (Fig. 1). Preliminary results indicate that: 1) Yukon River Fall chum salmon are widely distributed from offshore of the Yukon River, eastward to 62°N, 172°W, and as far south as Nunivak Island (60°N), suggesting a southwesterly migration pathway along the Bering Sea shelf; 2) juvenile chum salmon from the Kuskokwim River are narrowly distributed south of Nunivak Island from the mouth of the Kuskokwim River, south to 58°N, and as far west as 168°W, suggesting a westerly migration pathway along the Bering Sea shelf; and 3) northern Russia juvenile chum salmon stocks (mainly stocks from rivers draining into the Gulf of Anadyr) are distributed as far east as 62°N, 171°W (Fig. 2). These results are unique in that they represent the first attempt to identify early marine distribution and migration of juvenile chum salmon stocks on the eastern Bering Sea shelf.

**Fig. 1.** Station locations and juvenile chum salmon catch per unit effort (catch during a 30 minute trawl) during the Ocean Carrying Capacity August–October 2002 Bering-Aleutian Salmon International Survey (BASIS).

Fig. 2. Percent of Fall Yukon (FYukon), Northern Russia (NRussia), and other western Alaska juvenile chum salmon stocks during the Ocean Carrying Capacity, August–October 2002 Bering-Aleutian Salmon International Survey (BASIS).





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### Development of DNA Microarray for Rapid Detection of Mitochondrial DNA Haplotypes of Chum Salmon

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Keywords: Chum salmon, mitochondrial DNA, haplotypes, DNA microarray

Molecular techniques to assess the genetic variation of fish populations have been suggested as a promising means of genetic stock identification (GSI) of salmon (Ferguson et al. 1995). We recently have detected a greater variation in the mitochondrial DNA (mtDNA) control region of chum salmon (*Oncorhynchus keta*) by nucleotide sequence analysis than the variation detected by the analysis of restriction fragment length polymorphisms (Sato et al. 2001, in press). Base substitutions and indels observed in 20 sites of the 5' half of the mtDNA control region defined a total of 30 haplotypes in more than 2,000 individuals from 48 populations collected from Japan, Korea, Russia, and North America, serving as a useful tool for phylogeographic analysis of Pacific Rim populations (Sato et al. 2001, in press). However, nucleotide sequence analysis requires specialized, expensive laboratory equipment and expert skill. In addition, time-consuming sequence analysis may not be suitable for salmon stock identification that will need a large number of samples. We attempted to develop a rapid and accurate method to detect mtDNA haplotypes of chum salmon for GSI using DNA microarray on a slide glass, which immobilized synthetic oligonucleotides containing the reported polymorphic nucleotide sites in the control region (Sato et al. 2001, in press). The developed DNA microarray was evaluated for determination of mtDNA haplotypes of nearly 1000 chum salmon collected during the research cruise of R/V *Kaiyo maru* of the Fisheries Agency, Japan, in the Bering Sea September 2002.

The method includes; 1) immobilization of synthesized 17 to 20 mer oligonucleotides containing the variable nucleotide on slide glass pre-coated with Poly-carbodiimide resin (CarboStation), 2) PCR amplification of the 5' variable portion with biotinylated primer from mtDNA extracted as previously described (Sato et al. 2001, in press), 3) two-hour hybridization of biotinylated PCR fragments with DNA microarray and subsequent short washing, and 4) visualization of hybridization signals colored by conventional ABC method and comparison of scanner-taking signal image on a computer. All the process of hybridization and detection was completed within eight hours.

As shown in Fig.1, the oligomer spots with intense hybridization signals were thought to contain perfectly matched sequence with a single nucleotide variation in the PCR fragments, whereas those with faint or no signals were thought to have no such sequence identity in the fragments. Therefore, an intense hybridization signal at each variable nucleotide site was taken as positive, and those positive sites were aligned to determine a corresponding **Fig.1.** DNA microarray detection of polymorphic sites in the 5' half of the mtDNA control region sequence of chum salmon. The number of the variable sites indicates nucleotide position from the 5' end of the mtDNA control region. Polymorphic nucleotide in the right of each signal positive oligomer corresponds to those presented in Table 1. Alignment of the signal positive polymorphic sites indicates the haplotype to be A-3 (see also Table 1).

| Variable<br>site |   | Nucleotide    | Variable<br>site |   | Nucleotide  |           |
|------------------|---|---------------|------------------|---|-------------|-----------|
| 10               | 0 | T<br>C        | 194              | • | A<br>T      |           |
| 30               | 0 | T<br>C        | 231              | • | T<br>C      |           |
| 42               | e | A<br>G        | 242              | • | C<br>T      |           |
| 57               | • | A<br>T        | 250              | • | T<br>C      |           |
| 70               | 9 | T<br>C        | 260              | • | A<br>G      | A-3       |
| 79               | ð | T<br>C        | 339<br>8340      | 0 | T,C<br>A,C  | / <b></b> |
| 96               | • | -<br>A        | 386              | • | A,T<br>G,C  |           |
| 108              | • | A<br>T        | &395<br>401      | • | -,A<br>T    |           |
| 154              | ۰ | СС.<br>С<br>G | 471              | • | C<br>A<br>C |           |
|                  |   |               |                  |   |             |           |

haplotype (Fig. 1). Likewise, all the single nucleotide mutations defining a total of 30 haplotypes were detected by the obtained DNA microarray (Table 1). In fact, haplotype determination of about 40 chum salmon by the present DNA microarray was perfectly concordant with the results from direct sequence analysis, which further confirmed the accuracy of the DNA microarray method for detection of sequence variation.

Since the present method does not require any specialized laboratory equipment, capability of this technique to identify mtDNA haplotypes on ship for commercial fisheries was tested using 978 chum salmon collected from 17 stations in the Bering Sea (172°30'E–172°30'W 51°30'N–58°30'N) during the *Kaiyo maru* research cruise September 2002. Figure 2 shows the distribution of chum salmon mtDNA haplotypes per station that were identified immediately after catch on-board the ship. All the haplotype analysis using the DNA microarray was completed within one month. The observed distribution of mtDNA haplotypes was nonrandom, showing a predominance of groups A and C haplotypes in the central Bering Sea (180°–177°30'W 56°N–58°30'N). In other areas, group B haplotypes tended to predominate over the other two haplotype groups, although the occurrence of the haplotype B-3 was common in all the locations. These results strongly suggest the abundance of Japanese and Russian stocks in the Bering Sea, since the observed groups A and C haplotypes were specific to or predominant in Japan and Russia (Sato et al. in press). Specifically, the abundance of Japanese stocks in the central Bering Sea was inferred from the occurrence of the A-3, A-7, A-8, C-3, and C-4 haplotypes, all of which were limited to Japan (Sato et al. in press).

Thus, the present findings suggest that the DNA microarray method is accurate and time effective, and suitable for analyzing large numbers of samples for chum salmon GSI in the field or on-board ship.

| Variable<br>Hap site<br>lotype | 10 | 30 | 42 | 57 | 70 | 79 | 96 | 108 | 154 | 194 | 231 | 242 | 250 | 260 | 339,<br>340 | 386,<br>395 | 401 | 471 |
|--------------------------------|----|----|----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-------------|-------------|-----|-----|
| A-1                            | Т  | Т  | Α  | Α  | Т  | Т  | -  | Α   | С   | A   | Т   | С   | Т   | А   | T,C         | G,C         | Т   | A   |
| A-2                            | С  | Т  | Α  | Α  | Т  | Т  | -  | Α   | С   | А   | Т   | С   | Т   | Α   | T,C         | G,C         | Т   | Α   |
| A-3                            | Т  | Т  | G  | Α  | Т  | Т  | -  | Α   | С   | Α   | Т   | С   | Т   | Α   | T,C         | G,C         | Т   | Α   |
| A-4                            | Т  | Т  | Α  | Α  | Т  | Т  | -  | С   | С   | Α   | Т   | С   | Т   | Α   | T,C         | G,C         | Т   | Α   |
| A-5                            | Т  | Т  | Α  | Α  | Т  | Т  | -  | Α   | С   | Т   | Т   | С   | Т   | Α   | T,C         | G,C         | Т   | Α   |
| A-6                            | Т  | Т  | Α  | Α  | Т  | Т  | -  | Α   | С   | Α   | С   | С   | Т   | Α   | T,C         | G,C         | Т   | Α   |
| A-7                            | Т  | Т  | Α  | Α  | Т  | Т  | -  | А   | С   | Α   | Т   | С   | Т   | Α   | T,C         | G,C         | Т   | С   |
| A-8                            | Т  | Т  | Α  | Α  | Т  | Т  | Α  | А   | С   | Α   | Т   | С   | Т   | Α   | T,C         | G,C         | Т   | Α   |
| B-1                            | Т  | Т  | Α  | Α  | Т  | Т  | -  | Α   | С   | Α   | Т   | С   | Т   | Α   | T,C         | -,A         | Т   | Α   |
| B-2                            | Т  | С  | Α  | Α  | Т  | Т  | -  | Α   | С   | Α   | Т   | С   | Т   | Α   | T,C         | -,A         | Т   | Α   |
| B-3                            | Т  | Т  | Α  | Α  | Т  | Т  | -  | Α   | G   | Α   | Т   | С   | Т   | Α   | T,C         | -,A         | Т   | Α   |
| B-4                            | Т  | Т  | Α  | Α  | Т  | Т  | -  | А   | С   | Α   | С   | С   | Т   | Α   | T,C         | -,A         | Т   | Α   |
| B-5                            | С  | Т  | Α  | Α  | Т  | Т  | -  | А   | G   | Α   | Т   | С   | Т   | Α   | T,C         | -,A         | Т   | Α   |
| B-6                            | Т  | Т  | Α  | Α  | С  | Т  | -  | Α   | G   | А   | Т   | С   | Т   | Α   | T,C         | -,A         | Т   | Α   |
| B-7                            | Т  | Т  | Α  | Α  | Т  | С  | -  | Α   | G   | Α   | Т   | С   | Т   | Α   | T,C         | -,A         | Т   | Α   |
| B-8                            | Т  | Т  | Α  | Α  | Т  | Т  | -  | С   | G   | Α   | Т   | С   | Т   | Α   | T,C         | -,A         | Т   | Α   |
| B-9                            | Т  | Т  | Α  | Α  | Т  | Т  | -  | А   | G   | Α   | С   | С   | Т   | Α   | T,C         | -,A         | Т   | Α   |
| B-10                           | Т  | Т  | Α  | Α  | Т  | Т  | -  | А   | G   | Α   | Т   | Т   | Т   | Α   | T,C         | -,A         | Т   | Α   |
| B-11                           | Т  | Т  | Α  | Α  | Т  | Т  | -  | Α   | G   | Α   | Т   | С   | С   | Α   | T,C         | -,A         | Т   | Α   |
| B-12                           | Т  | Т  | Α  | Α  | Т  | Т  | -  | Α   | G   | Α   | Т   | С   | Т   | G   | T,C         | -,A         | Т   | Α   |
| B-13                           | Т  | Т  | Α  | Α  | Т  | Т  | -  | Α   | G   | Α   | Т   | С   | Т   | Α   | A,C         | -,A         | Т   | Α   |
| B-14                           | Т  | Т  | Α  | Α  | Т  | Т  | -  | Α   | G   | Α   | Т   | С   | Т   | Α   | T,C         | -,A         | С   | Α   |
| B-15                           | Т  | Т  | Α  | Α  | Т  | Т  | -  | Α   | G   | Α   | Т   | С   | Т   | Α   | T,C         | -,A         | Т   | С   |
| B-16                           | Т  | Т  | Α  | Α  | Т  | Т  | -  | Α   | G   | Α   | Т   | С   | Т   | Α   | A,T         | -,A         | Т   | Α   |
| B-17                           | Т  | Т  | Α  | Α  | Т  | Т  | -  | Α   | G   | Α   | Т   | С   | Т   | Α   | A,C         | -,A         | С   | Α   |
| C-1                            | Т  | С  | Α  | Α  | Т  | Т  | -  | Α   | С   | A   | Т   | С   | Т   | Α   | T,C         | G,C         | Т   | Α   |
| C-2                            | Т  | С  | Α  | Т  | Т  | Т  | -  | Α   | С   | Α   | Т   | С   | Т   | Α   | T,C         | G,C         | Т   | Α   |
| C-3                            | Т  | С  | Α  | Α  | С  | Т  | -  | Α   | С   | A   | Т   | С   | Т   | Α   | T,C         | G,C         | Т   | Α   |
| C-4                            | Т  | С  | Α  | Α  | Т  | Т  | -  | Т   | С   | A   | Т   | С   | Т   | Α   | T,C         | G,C         | Т   | Α   |
| C-5                            | Т  | С  | Α  | A  | Т  | Т  | -  | Α   | С   | Α   | С   | С   | Т   | Α   | T,C         | G,C         | Т   | Α   |

Table 1. Thirty haplotypes defined by 20 variable nucleotide sites.

Number at variable site shows nucleotide positions from the 5' end of the mtDNA control region of chum salmon.

**Fig.2.** Distribution of chum salmon mtDNA haplotypes in 17 stations in the Bering Sea (172°30'E–172°30'W 51°30'N–58°30'N) during the Kaiyo maru research cruise September 2002. The observed distribution of mtDNA haplotypes showed a predominance of groups A and C haplotypes in the central Bering Sea (180°–177°30'W 56 °N–58°30'N). In other areas, group B haplotypes tended to predominate over the other two haplotype groups, although the occurrence of the haplotype B-3 was common in all the locations.



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### Population Structure and Stock Identification of Chum Salmon (*Oncorhynchus keta*) Based upon Microsatellite Analysis

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Keywords: Chum salmon, microsatellites, population structure, stock identification

Stock identification of chum salmon (*Oncorhynchus keta*) migrating through particular locations on the high seas can be of scientific and management interest. Although allozyme-based methods of stock identification have proven useful in estimation of chum salmon stock composition in mixed-stock fisheries (Shaklee et al. 1999), and differentiation at allozyme loci occurs among chum salmon (Beacham et al. 1987; Seeb and Crane 1999), the level of discrimination available in some applications is not sufficient for fisheries management decisions. Variation in microsatellite loci has been applied in other species requiring discrimination among salmonid populations within watersheds (Small et al. 1998; Beacham and Wood 1999; Beacham et al. 2001), and has been shown to be useful in stock discrimination in chinook salmon (Banks et al. 2000). Variation at microsatellite loci has been particularly useful for population-specific estimates of stock composition of Fraser River chinook salmon (Beacham et al. 2003), and may work well for chum salmon. In the present study, we survey variation at 13 microsatellite loci in chum salmon, and evaluate the utility of using microsatellite variation for stock identification on a regional and local basis. This is accomplished by analysis of simulated mixtures containing chum salmon from different regions, and on a local basis by incorporation of specific chum salmon populations.

Tissue samples were collected from adult chum salmon from populations in Japan, the Yukon River, southeast Alaska, and British Columbia and DNA extracted from the samples as described by Withler et al. (2000). For the survey of baseline populations, PCR products at 13 microsatellite loci: Ots3 (Banks et al. 1999), Oke3 (Buchholz et al. 2001), Oki2 (Smith et al. 1998), Oki100 (Miller et al. unpub), One101, One102, One103, One104, One106, One108, One109, One111, and One114 (Olsen et al. 2000), Ssa419 (Cairney et al. 2000), and OtsG68 (Williamson et al. 2002) were size fractionated on denaturing polyacrylamide gels with the ABI 377 automated DNA sequencer. Allele sizes were determined with Genescan 3.1 and Genotyper 2.5 software (PE Biosystems, Foster City, CA). Cavalli-Sforza and Edwards (1967) chord distance was used to estimate distances among populations. An unrooted neighbor-joining tree was generated with PHYLIP (Felsenstein 1993).

Genotypic frequencies were determined at each locus in each population and the statistical package for the analysis of mixtures software program (SPAM) (Debevec et al. 2000) was used to estimate stock composition of each mixture. A recent version of SPAM (3.7) that incorporates a correction to baseline allele frequencies was used in the analysis in order to avoid the occurrence of fish in the mixed sample from a specific population having an allele not observed in the baseline samples from that population (SPAM software available at http://www.cf.adfg.state.ak.us/geninfo/research/genetics/software/spampage.htm). All loci were considered to be in Hardy-Weinberg equilibrium, and expected genotypic frequencies were determined from the observed allele frequencies.

Each baseline population was resampled with replacement in order to simulate random variation involved in the collection of the baseline samples before the estimation of stock composition of each simulated mixture. Simulated mixtures composed of chum salmon from different regional groups were examined in order to evaluate accuracy and precision of the estimated stock compositions. Simulated fishery samples of 150 fish were generated by randomly resampling with replacement the baseline populations in each drainage. Estimated stock composition of a simulated mixture was then determined, and the whole process was repeated 100 times to estimate the mean and standard deviation of the individual stock composition estimates. A baseline incorporating 90 populations ranging from Japan, the Yukon River, southeast Alaska, and British Columbia was used in analysis of the simulated mixtures.

The dendrogram analysis indicated that there was regional structure in the chum salmon populations analyzed. Regional structure was observed for chum salmon populations from Japan, the Yukon River, southeast Alaska, the Taku River, the east and west coast of the Queen Charlotte Islands in British Columbia, the central coast of British Columbia, the east and west coasts of Vancouver Island, the southern British Columbia mainland, and the Fraser River.

Analysis of simulated mixtures was conducted to evaluate the utility of microsatellites for estimation of stock composition in mixed-stock samples. Samples containing only Japanese chum salmon from four populations (Abashiri, Chitose, Horonai, Tokachi) were well estimated on both an individual population and region level. With

each of the four populations comprising from 10–40% of the mixture, mean errors of estimation for each of the 4 populations were generally within 3% of the actual values, and mean error of the regional estimate was 1.2% (estimate of 98.8% Japanese component versus 100% actual component). Similar results were observed for four Yukon River populations (Kantishna, Toklat, Fishing Branch, Kluane) comprising 10–40% of the mixtures, with mean population-specific errors generally < 3% of actual values, and with the error in the regional estimates 0.2%. For west coast Queen Charlotte Islands populations (Botany, Clapp Basin, Gold Harbour, Government), mean population-specific errors for populations comprising 10–40% of the mixtures was < 3%, and mean error of the regional estimate was 4%. Similar results were again observed for east coast of Vancouver Island populations (Cowichan, Goldstream, Nanaimo, Campbell River), with population specific errors < 6% of actual values, and the regional estimate in error by 7% (93.2% versus 100%). When multi-regional mixtures were evaluated, mean regional estimates were in error by < 2% for all regions.

Analysis of actual mixed-stock fishery samples from the vicinity of the Saanich Inlet on the south east coast of Vancouver Island provided a practical way to evalute fishing boundary locations designed to reduce exploitation of Cowichan River chum salmon. Microsatellites appear to be a very effective method to provide a regional estimate of stock composition in samples comprising populations from multiple regions, and may also provide population-specific estimates if the baseline survey of populations in the region has been adequate.

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### Recent Analyses of Chum Salmon Homing Migration from the Bering Sea to Japan

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Keywords: Chum salmon, homing migration, biotelemetry, swimming speed, hormone profiles, salmon gonadotropin-releasing hormone, olfactory discrimination, natal river

A number of studies have investigated the amazing ability of salmon to migrate long distances from the ocean to their natal river for spawning (Ueda and Shoji 2002). For a better understanding on the mechanisms of salmon homing migration, three different analyses have recently been applied using Japanese chum salmon (*Oncorhynchus keta*) migrating from the Bering Sea to Japan and then to their natal river. The first is behavioral analysis on swimming speeds of homing chum salmon by means of a micro-data logger with a propeller, the second is endocrinological analysis on hormone profiles in the brain-pituitary-gonadal axis, and the third is olfactory analysis on discriminating ability of the natal river.

Swimming speeds in the oceanic phase can be one of the keys to understand the mechanism of chum salmon homing migration. We tagged a maturing chum salmon (fork length = 685 mm) which was considered to be of Japanese origin with a data logger (sampling intervals: speed and depth = 5 sec; temperature = 60 sec) in the central Bering Sea on July 9, 2000 (Fig. 1). This salmon was retrieved by a set net along the eastern Hokkaido coast 67 days after the release, and 51-day data were recorded. The fish usually swam in the surface water column and rarely stayed deeper than 50 m. The average swimming speed was 60-70 cm per sec, and horizontal rate calculated by an





empirical relationship between the attack angle and vertical rate was 42.3–47.7 km per day. The estimated horizontal rate indicates that chum salmon traveled 2,763 km in 67 days, which is almost equivalent to the distance between points of release and retrieve. It implies that chum salmon moved to the coastal area near the spawning ground almost straightly from the Bering Sea, partly helped by currents. Vertical profiles of ambient temperature sampled by salmon suggest that the fish passed through the Okhotsk Sea around mid-August. All through the recording period, chum salmon showed a clear foraging period in the daytime, which consisted of repeated short diving from the surface water column to the depth beyond the thermocline. It indicates that chum salmon traveled searching a prey patch during their oceanic migration. These results suggest that homing chum salmon migrated along the continental shelf of Kuril Islands.

Gonadtropin-releasing hormone molecules produced in the various brain regions are considered to be involved in many physiological functions of teleost life cycle. In order to clarify GnRH roles on salmon homing migration, measurements of two molecular types of GnRH, salmon GnRH (sGnRH) and chicken GnRH-II (cGnRH-II) in different brain regions, as well as gonadotropin (GTH) and steroid hormones were conducted using specific timeresolved fluoroimmunoassay (TR-FIA) systems (Yamada et al., 2002; Leonard et al., 2002). Maturing chum salmon were caught in nine points from the Bering Sea to the Chitose River. After decapitation, the brain was divided into six regions; olfactory bulb (OB), telencepharon (TC), diencephalon (DC), optic tectum (OT), cerebellum (CB), and medulla oblongata (MO). During spawning migration of chum salmon, sGnRH levels in OB, TC, and pituitary of both sexes were increased at the coastal sea to the branch point of the Chitose River from the Ishikari River. Moreover, sGnRH levels in the pituitary tended to increase at the same time of elevation in female pituitary GTH II and ovarian GTH I levels, cGnRH-II level in MO was increased at the pre-spawning ground in both sexes, and levels of OT were also increased in male. Both GnRH levels in DC showed no significant changes during spawning migration. GTH II levels in gonads were not detected though the sampling period. Serum steroid hormone levels showed similar profiles as previous observations (Ueda 1999); estradiol-17 $\beta$  in females and 11-ketotestosterone in males increased during vitellogenesis and spermatogenesis, respectively, and  $17\alpha$ ,  $20\beta$ -dihydroxy-4-pregnen-3-one increased dramatically at the time of final gonadal maturation in both sexes. It is quite interesting to note that both sGnRH content in TC and serum testosterone level showed coincident peaks at the branch point of the Chitose River from the Ishikari River. Theses results confirm the previous findings that sGnRH plays a role on GTH secretion in the pituitary of chum salmon, and sGnRH and cGnRH-II might be involved in brain region-dependent roles on sexual maturation and behavior in salmonid fishes.

For upstream homing migration from the coastal area to the natal stream, the olfactory hypothesis which was proposed by Hasler and Wisby (1951) has been discussed in many behavioral and electrophysiological studies, but the odor substances of home stream are still unknown. We found that the response to artificial stream water based on the compositions of amino acids and salts closely resembled the response to the corresponding natural water (Shoji et al., 2000), and we carried out behavior experiments to test whether amino acids mixtures of the home stream have attractive effects on chum salmon upstream movement. Mature male chum salmon (mainly 4 year olds) were captured at the weir in the Osaru River, Hokkaido, Japan, in the late spawning season of 2002, transferred to the Toya Lake Station, Hokkaido University, and reared for several days before experiments. Behavior experiments were conduced in the two-choice test tank. The artificial home stream water was prepared by the amino acid and related substance composition of the Osaru River and dissolved in artificial freshwater. A total of 44 chum salmon was tested, and 28 fish (63.6%) showed upstream movement to one of the choice arm. Among those that moved, 24 fish (85.7%) were found in the arm running the artificial home stream water, and 4 fish (14.3%) were observed in the arm running the natural lake water. These results demonstrate clearly that the artificial home stream water reconstituted by the amino acid composition of home stream has attractive effects on the chum salmon upstream selective movement. We concluded that amino acids dissolved in the home stream water are home stream odorants, and the hypothesis that amino acids dissolved in stream waters are home stream substances for salmon homing is strongly supported by these results.

These different new approaches will help to understand mechanisms of salmon homing migration and eventually to evaluate the stock dynamics of salmon in the North Pacific Ocean and Bering Sea.

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### Identification of Stocks and Environmental Characteristics of North Pacific Chum Salmon, *Oncorhynchus keta*, by Chemical Analysis of Otolith

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Keywords: Chum salmon, otolith, stock identification, life history, stable isotope, Sr/Ca ratio

Since Reibisch's observations in 1899, otoliths have been used to identify the age and/or daily rings of fish. However, the relatively sudden development of otolith elemental analyses appeared in the 1980s (Campana and Thorrold 2001). The advent of otolith chemistry expanded the applications of otoliths to environmental recorders. Elemental composition analyses, called elemental tags or fingerprints, of the fish otoliths have been applied to study age, growth, stock identification, temperature and salinity histories, migration pathways, anadromy, pollution of habitat, and chemical mass marking (Edmonds et al. 1999; Gillanders and Kingsford 1996).

To investigate the stock identification and habitat characteristics of chum salmon (*Oncorhynchus keta*) in the North Pacific Ocean, the stable isotopes  $\delta^{18}$ O,  $\delta^{13}$ C and trace elements in otoliths were measured. Otoliths were collected from four sites (Canada, Japan, Korea and USA) during 1997–1999 spawning seasons except Japanese salmon in 1999. Otoliths of fry and immature salmon were collected from the Korean hatchery and off the Aleutian Islands, respectively. The  $\delta^{18}$ O and  $\delta^{13}$ C values from whole ground otoliths were measured using a mass spectrometer. The  $\delta^{18}$ O and  $\delta^{13}$ C values increased with age, showing especially high correlation with salmon size (Fig. 1). The mean  $\delta^{18}$ O and  $\delta^{13}$ C isotope ratios of fry showed –6.97‰ and –12.18‰, where the values of adult salmon were 0.62‰ and –4.86‰, respectively. The isotopic values for immature salmon collected off the Gulf of

salmon were 0.62‰ and –4.86‰, respectively. The isotopic values for immature salmon collected off the Gulf of Alaska appeared in between those of the fry and adult salmon.

The values of the two isotopes of adult salmon separated largely into two groups: Asian and North American chum salmon (Fig. 2). The  $\delta^{18}$ O isotope appeared in order of Japan, Korea, US, and Canada with mean differences of ca. 0.81‰ between Japan and Canada in three consecutive years. When assuming  $\delta^{18}$ O values are indicative of ocean temperature of the salmon habitat, Asian salmon seemed to reside in lower temperature than North American stocks. Carbon stable isotopes showed the opposite pattern; higher values from North American salmon, and lower from Asian salmon.



**Fig.1.** Comparison of isotopic composition with respect to life history difference.

Fig.2. Envelopes of scatter plots of  $\delta^{13}$ C and  $\delta^{18}$ O in otoliths of adult chum salmon, which were collected in 1997.

For the trace element analysis, two methods were used: line scanning and spot analysis methods. Concentrations of Ca and Sr in otoliths were measured using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) for the line scanning method. Sr/Ca ratios, known as an indicator of salinity, were elevated in the core of the otoliths, decreased during the freshwater stage, increased suddenly at a certain point and oscillated periodically to the margin corresponding to year-ring (Fig. 3). Peaks of Sr/Ca at nucleus may indicate marine-origin Sr contributed to the egg. During the marine life stages, the Sr peaks of otolith correspond to the translucent (winter) part of the annuli. We speculate that chum salmon might move onshore/offshore or north/south. On the spot analysis of core part of otolith, the result of discriminant analysis with 23 elements (<sup>7</sup>Li, <sup>55</sup>Mn, <sup>65</sup>Cu, <sup>66</sup>Zn, <sup>86</sup>Sr, <sup>89</sup>Y, <sup>135</sup>Ba, <sup>139</sup>La, <sup>140</sup>Ce, <sup>141</sup>Pr, <sup>146</sup>Nd, <sup>147</sup>Sm, <sup>151</sup>Eu, <sup>157</sup>Gd, <sup>159</sup>Tb, <sup>162</sup>Dy, <sup>165</sup>Ho, <sup>166</sup>Er, <sup>169</sup>Tm, <sup>174</sup>Yb, <sup>175</sup>Lu, <sup>208</sup>Pb, and <sup>238</sup>U) represents distinct separation in accordance with natal areas of stocks (Fig. 4). Therefore, otolith chemistry is a very effective technique for stock separation and for revealing the effects of environment of salmon populations.

**Fig. 3.** Combined photographic view of annual rings in otolith with line scan image from Japanese chum salmon. Small box contains the profile of strontium concentration scanned from nucleus to edge across a chum salmon otolith. Four yearly oscillations in strontium concentration matched with annuli in otolith photo.





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## Which Genetic Markers and GSI Methods are More Appropriate for Defining Marine Distribution and Migration of Salmon?

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Keywords: Genetic marker choice, GSI, statistical power

Potential for increased statistical power in stock discrimination gained through employing more polymorphic marker types such as microsatellites is attractive for applications in determining the distribution and migration of salmon in the high seas. Increased sampling requirements owing to the many character types (alleles) that typify microsatellites, however, raise question about accuracy. While increasing the number of individuals characterized for baseline data addresses this issue, it is seldom feasible to characterize all likely contributing stocks. Furthermore, increasing population sample size (minimum n) requirements per stock inevitably will result in a reduction in the total number of potential contributing stocks that can be characterized in baselines, as funding for research is always finite. This approach thus results in diminishing returns because it increases the potential for more individuals in any study that may have originated from stocks other than those characterized in baselines. Methods invoking Baye's rule and a number of new approaches have shown promise in overcoming this enigma. This study reviews empirical findings from working with some of these new approaches (SPAM, Debevec et al. 2000; WHICHLOCI, Banks et al. 2003; and GMA, Kalinowski 2003). Primary goals were to determine how alternate genetic markers, sample sizes and population compositions effect precision and accuracy of results for known mixtures. Data includes microsatellites, major histocompatibility complex (MHC), and D-Loop mtDNA sequence variance (Table 1) from five alternate Chinook life history types of the Sacramento and San Joaquin rivers of central California, USA.

Our primary finding is that the statistical power of genetic stock identification is critically dependent on the information content of genetic markers (loci) employed. Power is maximized through ranking loci according to their discriminatory ability among populations under consideration and using a resource such as WHICHLOCI (Banks et al. 2003) to evaluate what minimum numbers of high ranking loci are necessary in order to achieve desired assignment accuracy and precision criteria (Fig. 1). Earlier studies have suggested that a modest number of loci, each with a modest number of alleles provides better assignment accuracy (Smouse and Chevillon 1998; Bernatchez and Duchesne 2000) and Cornuet et al. (1999) noted a converse relationship between number of loci (8-30) and number of individuals required (30-12) in order to achieve 100% assignment. Our studies, however, reveal that no simple predictor such as number of alleles or heterozygositiy of loci provides consistent prediction of individual based assignment performance. While more polymorphic and heterozygous loci generally rank higher, on occasion specific loci with as few as four or five alleles may rank high because of unique frequency and distribution of genotypes among populations under consideration. It is thus gainful to apply methods to assess information content of alternate loci and evaluate what minimum number of combined loci is necessary in order to achieve sufficient stock assignment power. For example, iteration over 10 data sets with a simulated sample size of 10,000 using the 16 best ranking loci among those employed in this study achieved 99.91% accuracy with a variance of 0.023, yet 99.18 accuracy is attained with just nine of these highest ranking loci with minimal variance increase (to 0.61).

Interestingly, when comparing the performance criteria of alternate loci choices for individual based population assignment (discussed above) with those attained for population based genetic stock identification methods such as GMA (Kalinowski 2003) or SPAM (Debevec 2000) both number of alleles and heterozygosity were better predictors of top performing loci than WHICHLOCI (Fig. 2). Note that sample size for data used in this study was only 19 for each life history type; a value well below the number (150–200) assessed as necessary to ensure reliable microsatellite allele frequencies (Banks et al. 2000). Ongoing research will test if these findings hold true for population based genetic stock identification methods using greater sample sizes per life history type.

Current advances in genotyping technology are facilitating the application of single nucleotide polymorphisms (SNPs) in genetic stock identification (GSI). At first glance, SNPs do not appear suited to GSI because most SNPs have only two alleles and can never have more than 4 alleles (because there are only four different bases in DNA), however, technology advances may allow effective characterization of several orders of magnitude more SNPs than the number of microsatellites that can be characterized for the same cost. These methods could thus harness similar

numbers of characters but from a broader component of the genome, with potential to provide even better stock discrimination performance, although linkage rears as a likely confounding factor. MHC is the only 'SNP' locus characterized among loci considered in this study, however, Rosenberg et al. (2003) provides insightful findings from their study of informativeness of alternate genetic markers for inferring ancestry among human populations. This study compares findings from 377 microsatellites and 8,714 SNPs and finds that random dinucelotide microsatellites are from five to eight times more informative than random SNPs, but also that 2–12% of SNPs have higher information content than the median for dinucleotides. We are only just beginning to accumulate sufficient data to allow similar comparison between microsatellites and SNPs for fisheries contexts, but the field is advancing fast, presenting exciting potential for high resolution stock identification that should provide enlightening perspective for marine distribution and migration of stocks in the near future.

| Locus name | Number of alleles | Heterozygosity | WHICHLOCI rank | Reference                    |
|------------|-------------------|----------------|----------------|------------------------------|
| OtsG311    | 31                | 0.72026        | 1              | Williamson et al. (2002)     |
| Ots-107    | 25                | 0.76586        | 2              | Nelsen & Beacham (1999)      |
| OtsG409    | 41                | 0.87952        | 3              | Williamson et al. (2002)     |
| OtsG422    | 41                | 0.83782        | 4              | Williamson et al. (2002)     |
| Ots-209    | 31                | 0.8762         | 5              | Greig et al. (2003)          |
| OtsG253    | 25                | 0.83838        | 6              | Williamson et al. (2002)     |
| Ots-204    | 34                | 0.67852        | 7              | Greig et al. (2003)          |
| Ots-104    | 23                | 0.86344        | 8              | Nelsen & Beacham (1999)      |
| OtsG249    | 41                | 0.87342        | 9              | Williamson et al. (2002)     |
| Ots-211    | 33                | 0.7877         | 10             | Greig et al. (2003)          |
| OtsG83b    | 32                | 0.79526        | 11             | Williamson et al. (2002)     |
| Ots-213    | 27                | 0.70234        | 12             | Greig et al. (2003)          |
| Ots-201    | 20                | 0.8614         | 13             | Banks et al. (pers. commun.) |
| Ots-215    | 20                | 0.78456        | 14             | Banks et al. (pers. commun.) |
| OtsG78b    | 36                | 0.79196        | 15             | Williamson et al. (2002)     |
| Ots-208    | 27                | 0.83854        | 16             | Greig et al. (2003)          |
| Ots-2      | 13                | 0.63838        | 17             | Banks et al. (1999)          |
| One-13     | 10                | 0.72378        | 18             | Scribner et al. (1996)       |
| Ots-212    | 25                | 0.92508        | 19             | Greig et al. (2003)          |
| Ots-515    | 17                | 0.78348        | 20             | Naish & Park (2002)          |
| Ots-503    | 16                | 0.8062         | 21             | Naish & Park (2002)          |
| OtsG474    | 10                | 0.72574        | 22             | Williamson et al. (2002)     |
| OtsG432    | 24                | 0.8721         | 23             | Williamson et al. (2002)     |
| D-Loop     | 5                 | -              | 24             | Nielsen et al. (1994)        |
| Ots-3      | 10                | 0.76222        | 25             | Banks et al. (1999)          |
| Ots-501    | 11                | 0.68698        | 26             | Naish & Park (2002)          |
| Ots-528    | 9                 | 0.64108        | 27             | Naish & Park (2002)          |
| MHC        | 4                 | 0.59988        | 28             | Kim et al (1999)             |
| Ots-519    | 8                 | 0.56574        | 29             | Naish & Park (2002)          |
| Ots-504    | 5                 | 0.5448         | 30             | Naish & Park (2002)          |
| Ots-10     | 7                 | 0.52104        | 31             | Banks et al. (1999)          |
| Ots-514    | 10                | 0.64692        | 32             | Naish & Park (2002)          |

 Table 1. Genetic markers evaluated for information context in the context of genetic stock identification

**Fig. 1.** Factorial correspondence analysis using data of 500 samples per Chinook life history type, simulated using WHICHLOCI (Banks et al. 2003) and analyzed using GENETIX (Belkhir et al. 2002). Square plots represent individual salmon samples colored in accord with their life-history type and positioned on the graph in relation to their genetic relationship. This is approximated from either: **A**. nine loci from the bottom of the rank depicted in Table 1 or **B**. nine loci from the top of this same rank. Tighter clustering of life-history types and zero overlap in B demonstrates the power gained through using loci with greater information content.



**Fig. 2.** Genetic stock Identification results using the population based GMA method of Kalinowski (2003). One thousand simulated fishery samples had N = 500. For fair comparison with individual based methods, relative component estimates were set with the two spring life history types and late fall each at a frequency equivalent to just one fish in the 500 fishery sample (0.002), setting the remainder for the components made up almost equal proportions of fall (0.442) and winter (0.522). Bars shown in black depict the absolute sum of the differences between observed and expected frequencies across all life history types and bars shown in white depict average standard deviation. Paired columns are displayed according to alternate loci combinations used: From left to right, all 33 loci together, 12 loci that ranked highest using WHICHLOCI, 12 loci with highest heterozygosities, 12 loci with the greatest number of alleles and lastly 9 loci that ranked highest using WHICHLOCI.



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## Determining Accuracy of a Bayesian Approach to Estimate Individual Identification to Stock-of-Origin for Pacific Salmon in Marine Fisheries Using Microsatellite and MHC Loci

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Keywords: Stock identification, sockeye salmon

Successful identification of individual sockeye to stock-of-origin remains one of the most useful and most challenging problems for mixed stock analysis. Capability to correctly identify individuals on the high-seas provides new research opportunities to study numerous biological and behavioural traits of Pacific salmon. A number of applications which employ Bayesian analysis for individual identification are available and work with varying degrees of success. The accuracy of individual identification is best evaluated using known mixture samples. The most convincing evidence for the success of individual identification requires "blind sample testing" where the laboratory provides estimates without knowing the true stock-of-origin for the sample.

Genomic DNA was extracted from liver, scales, operculum punches, muscle tissue, or fin clips from over 46,000 sockeye salmon collected between 1983 and 2002 representing 261 Pacific Rim populations spanning Washington, British Columbia, Alaska, Russia, and Japan. PCR products at 14 microsatellite loci: Ots2, Ots3 (Banks et al. 1999), Ots100, Ots103, Ots107, and Ots108 (Small et al. 1998; Nelson and Beacham 1999), Oki1a, Oki1b, Oki6, Oki10, Oki16, and Oki29 (Smith et al. 1998 and unpub.), One8 (Scribner et al. 1996), and Omy77 (Morris et al. 1996) were size fractionated on denaturing polyacrylamide gels and allele sizes determined with the ABI 377 automated DNA sequencer. Genetic variation at the MHC class II DAB-B1 locus was surveyed by denaturing gradient gel electrophoresis (DGGE) using methods of Miller et al. (2000, 2001). Four different "blind" mixture samples were collected from locations near or on the spawning grounds in the Fraser River, Nass/Skeena rivers, and populations from the West Coast of Vancouver Island and S.E. Alaska. The mixture samples were screened for the same 14 microsatellite and 1 MHC loci used for the baseline. Individual multi-locus genotypes in the mixture were assigned to population using the Pacific Rim baseline allele frequencies and a Bayesian mixture model (Pella and Masuda 2001). Stock assignments of the mixture individuals were determined by the greatest posterior probability. Once assignments were made, the estimated stock-of-origin was verified against the known sample location determining if individuals were correctly assigned to population, run-timing (where applicable), and region.

The first sample was a mixture of 140 fish consisting of 10 Fraser River populations collected in 1999. Accuracy of individual identification was 82% correct to population, 91% correct to run timing (Early Stuart, Early Summer, Summer, Late) and 100% correct to the Fraser River (Table. 1). The second sample was collected from three geographically proximate populations in Barkley Sound (Henderson, Great Central, and Sproat), on the west coast of Vancouver Island. For the 91 fish sample, individual identification was 90% correct to population and 100% correct to Barkley Sound, again using the Pacific Rim baseline. In the third sample a blind mixture was provided by the Alaska Department of Fish and Game (ADFG) composed of SE Alaska, Skeena, and Nass populations. Since population specific population specific accuracy was only available for S.E. Alaska (Table 1). However, individual identification to region was 93% accurate. In the fourth sample, approximately 800 sockeye salmon returning to the Fraser River were radio-tagged in near-shore areas. Prior to reaching the spawning grounds, each tagged fish was individually identified. Of the 275 fish that were tracked to the spawning grounds 85% were correctly identified to population, 92% were identified to run time, and 100% were correctly identified to the Fraser River.

Analysis of these known samples highlights the high level of accuracy to stock-of-origin obtained from Bayesian analysis for individual assignments using a microsatellite/MHC genetic markers and extensive Pacific Rim baseline. Levels of accuracy expected from any high-seas collections of sockeye salmon should be ~90% correct to population and ~100% correct to region given an extensive Pacific Rim baseline.

| Mixture Sample    | Ν   | %toPopulation | %toRun Time | %toRegion |
|-------------------|-----|---------------|-------------|-----------|
| 99PSCBlind        | 140 | 86%           | 91%         | 100%      |
| 02BarkleySndBlind | 91  | 90%           | n/a         | 100%      |
| 02AlaskaBlind     | 525 | 91%*          | n/a         | 93%       |
| 02LGLTagging      | 275 | 85%           | 92%         | 100%      |

**Table 1.** Accuracy of known sockeye salmon mixture samples analyzed with a Pacific Rim baseline consisting of 261 populations ranging from Japan to Washington State. \*indicates accuracy to S.E. Alaska where mixture sample contains only those Alaskan populations in the baseline.

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## Development of 5'-Nuclease Reactions for High-Throughput SNP Genotyping in Salmon

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Keywords: 5'-nuclease, SNP, salmon

Information on the oceanic migration patterns and relative marine survival of individual stocks is critical to our understanding of fluctuations of salmonid populations under changing climatic conditions. Migrations following stock-specific corridors may lead to differing marine survival and varying rates of return among stocks during periods of changing marine conditions. By comparing genetic attributes of collections of fish taken in high-seas and near-shore areas with those characteristic of potentially contributing stocks one can infer the origin of the collection and thus a point on the migratory route of that stock.

Single nucleotide polymorphisms (SNPs) are a class of genetic markers consisting of differences in DNA bases between individuals or individual chromosomes. These polymorphisms have been assayed in salmonids and other taxa using a wide variety of technologies over the past couple of decades. Although many of these SNPs provided powerful information for fisheries management, the technologies used to collect genotype data for them were slow and expensive relative to those for alternative marker classes (e.g. allozymes and microsatellites). In recent years

several high-throughput, low-cost SNP genotyping techniques have been developed (several are described in Kwok 2003). We are developing markers based on one of these techniques known as the 5'-nuclease reaction (Fig. 1) and are using these markers to genotype SNPs in large numbers of chinook, sockeye and chum salmon. These SNP genotyping assays are being developed in order to utilize the wealth of previously described polymorphisms that have not been widely applied to migration or mixture studies due to throughput constraints of older technologies.

Using two thermal cycler blocks four times per day, we observed that a single technician could genotype over 3,000 individuals. Since thermal cycling was the limiting step, the use of additional cycler units could multiply this throughput rate several times. The relative simplicity of the raw data analysis (Fig. 2) and the lack of an electrophoresis component rendered genotyping based on the 5'nuclease reaction faster and thus less expensive than genotyping using the techniques originally published for each SNP. This greatly increases the potential utility of SNPs for fishery management applications.

**Fig. 1.** Genotyping via the 5'-nuclease reaction involves adding allele-specific fluorescent oligonucleotide probes to a typical polymerase chain reaction. During the reaction the probes anneal to the template DNA either (1a) perfectly or (1b) imperfectly. In the former case, the probe is cleaved causing fluorescence. In the latter case, the probe is simply knocked free and no color change is observed.



The number of SNP loci required for migration and mixture studies will depend on the questions being addressed, but will likely be larger than the number of markers with greater allele numbers (such as AFLPs or microsatellites). This will impact the relative economy of applying SNPs. In the absence of compelling evidence for any simple relationship between number of alleles and utility of a marker for migratory studies, we agree with Banks et al. (2003) that an empirical approach is an appropriate way to find the optimal combination of markers with which to address each question.

By facilitating the detection of only one amplification product per reaction and by eliminating the multitude of potential inconsistencies associated with electrophoresis, SNP genotyping assays should be much easier to transfer among laboratories than other markers. Furthermore, SNP data collected using the 5'-nuclease reaction are readily combined with SNP data collected using any other method. This portability, in combination with the relatively low time and monetary requirements for running SNP genotyping assays and the wealth of **Fig. 2.** Analysis of data produced by genotyping 380 fish using the 5'nuclease reaction. Sequence Detection Software V. 2.0 (Applied Biosystems Inc.) was used to generate the chart below in which the two axes represent the amounts of signal from each of the allelespecific probes. Each dot on the chart below represents an individual fish, whose genotype is determined by where its dot falls on the axis. The circles indicate clusters of a single genotype each. Mitochondrial DNA assays produce two clusters while nuclear DNA assays (such as the example below) produce three clusters. The "x"s represent unreadable samples (air-bubbles, failed PCRs etc.) and the dots in the bottom left corner are the negative controls (no template DNA added).



previously described polymorphisms that may be accessed using these new technologies, suggest that SNPs will become an increasingly important tool for migration and mixture studies of salmonids on the high seas where large sample sizes and exhaustive baselines are the norm.

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## Genetic Mixed Stock Analysis of Juvenile Chinook Salmon in Coastal Areas of Western North America

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Keywords: Chinook salmon, mixed stock analysis, allozymes, juvenile migration

Genetic mixed-stock analysis has been used routinely for over two decades to estimate the origins of oceancaught Pacific salmon. However, nearly all of these applications have focused on the study of maturing adult fish taken in fisheries. Recently, genetic stock identification efforts are also being directed at the study of juvenile mixtures (e.g., Teel et al. 2003; Brodeur et al. 2004). This shift is due in part to the recent availability of samples taken in surveys along the coast of North America in large-scale programs to study juvenile salmonids during their ocean phase (e.g., Brodeur et al. 2000). The genetic analysis of juveniles is also motivated by a lack of available information on the initial marine movements of many stocks, particularly of untagged natural spawning populations. The purpose of this paper is to use results from allozyme analyses of juvenile chinook salmon (*Oncorhynchus tshawytscha*) sampled in nearshore areas of the U.S. Pacific Northwest to illustrate examples of genetically distinct stocks with contrasting migration patterns.

Fish were captured during the summers of 1998–2001 in surveys of nearshore coastal areas ranging from central Oregon to northern Washington (Emmett and Brodeur, 2000). Trawls consisted of one-half-hour long surface tows with a 264 Nordic rope trawl along nine transects perpendicular to shore ranging from La Push, Washington (47°55'N) to Cape Perpetua, Oregon (44°15'N). Sampling stations began 1–5 nautical miles offshore and continued, in about 5 nautical-mile increments, to about 30 nautical miles offshore. Cruises, of about one week duration, were conducted in May, June, and September each year. Tissues were dissected from approximately 2,600 juveniles which were genotyped at 32 allozyme loci. Allele frequencies from 150 chinook salmon spawning populations in California and the Pacific Northwest were used as baseline data (Teel et al. 1999). Estimates of stock composition were made using maximum likelihood procedures and weighted by catch to estimate the numbers of fish sampled from a particular stock group. Additional details on methods are given in Teel et al. (2003).

Stock compositions of marine samples varied greatly by month and location reflecting the juvenile movements of genetically distinct populations of chinook salmon. Spring-run (season of adult migration to freshwater) fish from the interior Columbia River basin were the most abundant population group off northern Oregon and Washington throughout the early summer (58% of the marine catches) and were nearly absent in September. The

estimated number of interior spring-run fish caught in May and June cruises illustrated the rapid northward movement of these juveniles (Fig. 1). In May, most of the fish captured near the mouth of the Columbia River (Columbia region) were spring-run chinook salmon from upstream sources. In June, nearly all of the interior spring-run individuals were sampled further north (Washington region).

In contrast to interior spring-run juveniles, Columbia Basin fall-run chinook salmon were evident throughout summer sampling, and predominate in September (45% to 89%). Two ecotypes of fall-run populations identified by genetic and life history differences ("brights" and "tules") showed distinct juvenile migration patterns after sea entry (Fig. 2). Brights were mostly caught in southern sampling areas (Oregon and Columbia regions) and tules were more abundant further north (Washington region). **Fig. 1.** Estimated number of juvenile chinook salmon from three stock groups captured in two regions along the Oregon and Washington coasts during early summer trawls during 1998–2001. Regions are Columbia River (CR) and Washington Coast (WA).



**Fig. 2.** Estimated number of juvenile chinook salmon from two stock groups captured in three regions along the Oregon and Washington coasts during September trawls during 1998–2001. Regions are Oregon coast (OR), Columbia River (CR), and Washington Coast (WA).



The contrasting juvenile migration patterns of interior Columbia Basin spring- and fall-run populations are consistent with the chinook salmon population structure recently described by Waples et al. (in press.) That study jointly considered genetic and life-history variation, including patterns of adult ocean migration, and revealed distinct evolutionary lineages within the Columbia Basin. The genetic analysis presented here suggests that differing initial juvenile marine migrations also provide an important source of diversity among these populations of chinook salmon.

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### Parasite Community Composition: Insights on the Ecology and Migration of Juvenile Salmon

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#### Keywords: Parasites, salmon, migration

Numerous studies have used parasites as biological tags for understanding the origins and migrations of marine fishes. Reviews by Lester (1990), Moser (1991), and MacKenzie and Abaunza (1998) describe many of these studies and summarize the guidelines for using parasites as biological tags. In principal, fish become infected with a parasite only if they come within an endemic area of the parasite. If infected fish are sampled outside of the endemic area we can conclude that they migrated through that habitat (MacKenzie and Abaunza 1998). By following these guidelines researchers have successfully used parasites to determine differences in recruitment and migration of anadromous and marine fishes.

Studies have used single parasite species to identify stocks of sockeye salmon (*Oncorhynchus nerka*) (Margolis 1963; reviewed in Konovalov 1995) and chinook salmon (*O. tshawytscha*) (Urawa et al. 1998) in the North Pacific Ocean and the Bering Sea. Parasite communities have also been used to determine migratory patterns of sockeye salmon in the Strait of Georgia, British Columbia (Groot et al. 1988). We are exploring both approaches for juvenile chinook salmon and coho salmon (*O. kisutch*) populations in the Northern California Current (NCC).

Our parasite analyses are components of two ecosystem projects in the NCC designed to examine influences on early-ocean growth and survival of juvenile chinook salmon and coho salmon. A project funded by the Bonneville Power Administration focuses on the potential effect of the Columbia River plume habitat on the ocean ecology of juvenile salmonids. A second project funded by the U.S. Global Oceans Ecosystems Dynamics (U.S. GLOBEC) focuses on how wind-driven processes and the physical features near Cape Blanco in Oregon might affect oceanographic conditions, local productivity, zooplankton populations, juvenile salmonid populations, and their interactions.

Although it is a critical period for growth and survival, information on the early marine ecology of juvenile salmon off of Oregon and Washington is limited (Pearcy 1992). We study parasite communities to help characterize

trophic interactions, habitat, migration, and salmon population origins during early marine residence. As parasitologists, our interests in juvenile salmon origins are two-fold. Salmon pathogens acquired in freshwater, such as *Nanophyetus salmincola*, *Renibacterium salmoninarum*, and *Ceratomyxa shasta*, could continue to affect juvenile salmon upon and after entering the ocean. Differences in pathogen prevalences from different freshwater systems could result in differential growth or survival. However without known stock origins this cannot be confirmed. Also, we are attempting to use nonpathogenic parasites to help delineate stocks or elucidate movement and migration of juvenile salmonids within and through Oregon and Washington coastal waters.

Juvenile salmon were caught along transects between Crescent City, California and La Push, Washington with a 30m – 20m rope trawl fished near the surface. Cruises were conducted between May and September of 1998– 2000 (Fig. 1). Lengths and initial identifications were done at sea and fish were immediately frozen. Muscle, Fig. 1. Map of the two study areas in the Northern California Current showing sampling transects that run from approximately 2 to 55 km offshore.  $124^{\circ}$ 



stomachs, intestines, and body cavities were examined for parasites. Multivariate community analyses were performed with the PRIMER computer software package (PRIMER-E Ltd, Plymouth).

Our analyses on habitat and migration studies focused on parasites acquired through trophic interactions. We identified 17 different parasites from juvenile chinook salmon and coho salmon: 4 nematodes; 7 trematodes; 3 acanthocephalans; and 3 cestodes. Prevalences and intensities varied between the two salmonids, but these parasites were found in both species. We chose the most prevalent parasites for community analyses.

Spatial comparisons within the NCC suggested geographic differences in the distribution of two common trematodes: *Brachyphallus* sp. and another hemiurid trematode (Fig. 2). *Brachyphallus* sp. was most prevalent in salmon caught off of Northern Washington and not found in juvenile salmon south of Newport, Oregon. The other hemiurid trematode was most prevalent south of Newport. The lifespan of these parasites within salmon is unknown but based on other marine hemiurids is a minimum of several months (Rhode 1984). These trematodes may have potential as migration markers for juvenile salmon off of Oregon and Washington.

Examining the temporal patterns of parasitic infections within the same region provided insights into juvenile salmon habitat use and migration off of southern Oregon and northern California. During our 2000 GLOBEC studies we found that the prevalences of most parasite species were different in chinook salmon caught in August compared to June (Fig. 3). For example, the prevalence of *Anisakis* sp. was 83% in August and 33% in June, although intensities did not differ. Most of the other parasite species declined in prevalence. The known longevity of these parasites suggested that the June and August fish were not from the same populations. Multivariate analyses of these parasite communities also suggested different fish populations **Fig. 2.** Prevalences of two trematodes isolated from the stomachs of juvenile salmon sampled off of Washington and Oregon. Data were combined from Chinook salmon and coho salmon sampled from summer cruises conducted in 1998–2000. Transects span from northern Washington (La Push) to northern California (Crescent City).



Fig. 3. Prevalences of nematodes and trematodes of yearling Chinook salmon caught in the GLOBEC study area in 2000.



based largely on the increase in the prevalence of anisakid nematodes and declines in prevalence of the trematode *Podocotyle* sp. (Fig. 4). Our interpretations were supported by genetic mixed stock analysis, which indicated that during June, most of the chinook salmon sampled south of Newport, Oregon originated from rivers along the central Oregon coast. In August, chinook salmon sampled south of Cape Blanco, Oregon were largely from southern Oregon and northern California coastal streams, while north of Cape Blanco most were from the California Central Valley (Brodeur et al. 2004).

In conclusion, we found different geographical distributions in the Northern California Current of two trematode species in juvenile salmon. We used parasite communities to support temporal shifts in salmon habitat use. Further research and analyses are needed to determine how parasites in juvenile salmon will compliment other tagging efforts. Possibilities include the addition of other parasites to the analyses, genetic analyses of key parasite populations, increased temporal sampling, and further statistical testing. We are also currently analyzing our results with microsatellite information on a subset of juvenile chinook salmon from the GLOBEC study area. Due to the different time scales addressed by genetics, parasites, and conventional tagging, the conclusions drawn from each method may differ, but together they provide a more robust study of fish stocks (Lester 1990).

**Fig. 4.** A) Multi-dimensional scaling (MDS) of Bray-Curtis similarities between parasite communities of yearling chinook salmon sampled in the GLOBEC study area in 2000. Data were averaged by station resulting in mean abundance per station. Bubble plots on MDS show mean parasite abundance per station, B) *Anisakis* sp. C) *Podocotyle* sp.



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# Using Genetic Markers to Understand the Coastal Migration of Juvenile Coho (*Oncorhynchus kisutch*) and Chinook Salmon (*O. tshawytscha*)

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Keywords: Salmon, migration, microsatellite, DNA, Oncorhynchus tshawytscha, Oncorhynchus kisutch

The production of salmon has been shown to vary in relation to climate and ocean conditions at small and large spatial scales (Beamish et al. 1999; Hare et al. 1999; Welch et al. 2000; Mueter et al. 2002). Hence, the fate of individual salmon stocks may depend on where they migrate to in the ocean, and the amount of time they spend in different regions of the ocean.

The ocean migration of juvenile salmon has generally been studied using coded-wire tags (CWT) (Percy and Fisher 1988; Fisher and Pearcy 1995; Orsi and Jaenicke 1996; Orsi et al. 2000) and thermally marked otoliths (Carlson et al. 2000). Although the determination of the origin of CWT and thermally marked fish is unequivocal, these approaches may not reflect the stock composition of salmon in a given area as only a fraction of the fish are marked. The development of molecular techniques has enabled fisheries scientists to accurately assess the origin of ocean caught fish (Beacham et al. 2003a; Hallerman 2003). Hence, genetic markers could be a useful tool for assessing the migration routes of juvenile salmon at sea (Teel et al. 2003).

The objectives of this study were to determine the ocean distribution and migration of juvenile chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) along the west coast of British Columbia (BC) and Southeastern Alaska (SEA) using genetic markers. We also compared stock composition estimates derived using CWT and genetic markers.

Juvenile chinook and coho salmon were collected on the west coast of BC and SE Alaska during the summer and fall of 1998–2002 and winter 2001–2002. A mid-water rope trawl (ca. 90 m long X 30 m wide X 15 m deep; cod-end mesh 0.6 cm; Cantrawl Pacific Ltd., Richmond, BC) was hauled at the surface (0–20 m) for 15–30 minutes at 5 knots using the R/V *W.E. Ricker*. Sampling was conducted between 06:00 and 18:00 (Pacific Time). Up to 30 juvenile salmon of each species were randomly selected from each net tow. Fork length and mass were determined on board the research vessel using a ruler and an electronic scale equipped with a counterweight to correct for ship motion. Otoliths and scales were removed from these fish for age determination. A tissue sample was also taken from the operculum using a hole punch and preserved in 70% ethanol for stock identification. All the fish were scanned with a metal detector to determine the presence of CWT.

Juvenile chinook salmon were surveyed for 13 microsatellite loci (MSL) and coho were surveyed for 2 major histocompatibility complex (MHC) and 4–8 MSL (some of the early data in 1998 and 1999 were surveyed for 2 MHC and 4 MSL) (Small et al. 1998a, b; Beacham et al. 2003a, b). Using a maximum likelihood method (MLE), individuals of unknown origin were compared to coast-wide genetic baselines consisting of ca. 44,000 individuals representing over 240 populations from California to Alaska. The MLE model assigned the mixture composition to individual populations which were then summed into regional groupings for three seasons: summer (May–August), fall (September–November), and end-of-winter (February–March). Further details on the mixed-stock analyses used in this study can be obtained from Small et al. (1998a, b) and Beacham et al. (2003a, b).

A total of 2,529 juvenile salmon was analysed in this study for MSL and MHC. Columbia River (CR) chinook salmon represented approximately 50% of the juvenile chinook caught in BC and Alaska shelf waters during summer, but constituted less than 10% of the samples caught during fall and end-of-winter (Table 1). Spring chinook from the Upper CR and Snake River represented 31% and 39%, respectively, of the CR chinook. In the fall and end-of-winter samples, juvenile chinook salmon collected in Southern BC and SEA originated mostly from the west coast of Vancouver Island (WCVI) and central coast region of BC, respectively (Table 1). Stock composition of chinook had established stable residence in these areas. One notable difference, however, was that the proportion of Strait of Georgia or Puget Sound stocks consistently increased off the WCVI while WCVI stocks decreased, suggesting an influx of Georgia Basin to the west coast of Vancouver Island over the winter months.

CR coho salmon represented up to 25% of the juvenile coho caught in southern BC during summer (Table 2). They constituted less than 5% of the coho salmon caught during fall and winter and negligible proportions of the coho in the northern region (Table 2). Overwinter stock composition of coho salmon in southern BC coastal waters was also reasonably stable, suggesting that most stocks of coho established stable residence in this area, but that

there was an influx of coastal BC coho and a decline in WCVI coho abundance (Table 2). It is unclear where northern stocks moved to over the winter months. Juvenile coho salmon were absent from northern BC and SEA coastal waters by the end of winter. Coho resident in this area likely migrated north on the continental shelf or due west directly into the open waters of the Gulf of Alaska, as the proportion of the northern stocks did not increase on the west coast of Vancouver Island during winter (Table 2).

Ten percent of the juvenile salmon examined in this study were marked with CWT. Although the presence of CR chinook and coho in BC and SEA was confirmed by CWT, CWT stock composition did not agree with DNAderived estimates. CWT indicated that Alaska and CR chinook represented 65% and 27% of the juvenile chinook caught in SEA. In contrast, DNA analyses indicated that Alaska chinook represented less than 10% of the chinook caught in SEA. Similarly, CWT indicated that 78% and 16% of the juvenile chinook salmon originated from the CR

**Table 1.** Stock composition (%) of juvenile chinook salmon (< 350 mm FL) caught in southern British Columbia (49–51°N) and southeastern Alaska (54–58°N) in 1998–2002 determined using microsatellite DNA. SE Alaska: Southeastern Alaska; Coastal B.C. Coastal British Columbia; ECVI: East coast of Vancouver Island; WVCI: West coast of Vancouver Island.

|              |        | Southern BC |        | Ν      | Northern BC – SE Alaska |        |  |  |
|--------------|--------|-------------|--------|--------|-------------------------|--------|--|--|
| _            | Summer | Fall        | Winter | Summer | Fall                    | Winter |  |  |
|              | n=350  | n=345       | n=255  | n=113  | n=176                   | n=165  |  |  |
| SE Alaska    | 0.8    | 0.3         | 0.0    | 1.7    | 5.8                     | 5.4    |  |  |
| Coastal B.C. | 5.2    | 11.8        | 4.0    | 11.9   | 84.2                    | 82.5   |  |  |
| Skeena/Nass  | 1.8    | 0.2         | 0.0    | 5.8    | 6.5                     | 10.8   |  |  |
| Fraser       | 17.8   | 3.4         | 11.2   | 11.0   | 0.5                     | 1.1    |  |  |
| ECVI         | 2.2    | 0.2         | 2.3    | 7.0    | 0.7                     | 0.0    |  |  |
| WCVI         | 5.3    | 77.1        | 65.1   | 7.4    | 0.5                     | 0.6    |  |  |
| Puget Sound  | 8.0    | 0.7         | 9.0    | 4.6    | 0.0                     | 0.0    |  |  |
| Coastal Wash | 4.2    | 0.0         | 3.3    | 5.6    | 0.2                     | 0.0    |  |  |
| Columbia     | 53.1   | 4.9         | 4.9    | 40.8   | 1.8                     | 0.0    |  |  |
| Oregon       | 0.8    | 0.6         | 0.0    | 4.3    | 0.0                     | 0.0    |  |  |
| California   | 0.8    | 0.3         | 0.0    | 0.0    | 0.0                     | 0.0    |  |  |

**Table 2.** Stock composition (%) of juvenile coho salmon caught in southern British Columbia (49–51°N) and southeastern Alaska (54–58°N) in 1998–2002 determined using microsatellite DNA and major histocompatibility complex. SE Alaska: Southeastern Alaska; Coastal B.C. Coastal British Columbia; ECVI: East coast of Vancouver Island; WVCI: West coast of Vancouver Island.

|              |        | Southern BC |        | Nor    | Northern BC – SE Alaska |        |  |  |  |
|--------------|--------|-------------|--------|--------|-------------------------|--------|--|--|--|
|              | Summer | Fall        | Winter | Summer | Fall                    | Winter |  |  |  |
|              | n=440  | n=169       | n=227  | n=113  | n=176                   | n=0    |  |  |  |
| SE Alaska    | 1.8    | 3.4         | 0.6    | 20.7   | 12.2                    |        |  |  |  |
| Coastal B.C. | 12.8   | 22.4        | 37.6   | 30.3   | 26.1                    |        |  |  |  |
| Skeena/Nass  | 2.0    | 7.4         | 6.3    | 17.5   | 31.8                    |        |  |  |  |
| Fraser       | 4.8    | 5.8         | 12.5   | 3.5    | 6.9                     |        |  |  |  |
| ECVI         | 13.0   | 23.2        | 18.8   | 5.6    | 6.7                     |        |  |  |  |
| WCVI         | 11.8   | 23.5        | 10.2   | 8.1    | 8.9                     |        |  |  |  |
| Puget Sound  | 9.7    | 3.8         | 9.2    | 3.9    | 3.9                     |        |  |  |  |
| Coastal US   | 18.0   | 8.5         | 7.9    | 7.3    | 2.8                     |        |  |  |  |
| Columbia     | 25.7   | 2.0         | 1.9    | 1.9    | 0.8                     |        |  |  |  |
| California   | 0.5    | 0.0         | 0.0    | 1.3    | 0.4                     |        |  |  |  |

and BC in southern BC. DNA analyses indicated that CR chinook represented at most 40% of the chinook caught in southern BC during summer, but then declined to less than 5% thereafter, while BC chinook represented over 70% of the chinook caught during fall and winter in this region. Similar results were also observed for juvenile coho salmon. The discrepancy between stock composition estimates obtained with DNA and CWT may be attributed to the low recoveries of CWT in our samples, and to the limited number of populations of salmon are marked with CWT.

In summary, our analyses indicate that there are clear stock-specific differences in the migratory behaviour of chinook and coho salmon. Thus, individual stocks will be affected differently by climate depending on the biological and physical conditions encountered during their ocean life.

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### Patterns of Genetic Diversity in Alaskan Coho Salmon

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Genetic baselines can be used to reveal population structure, perform mixed-stock analysis (MSA), infer the population origin of individuals and characterize patterns of ocean migration in North American and Asian salmon. Extensive genetic baselines have been developed for chinook (*Oncorhynchus tshawytscha*), chum (*O. keta*), and sockeye salmon (*O. nerka*) using allozyme loci. Unfortunately, allozymes show little variation in coho salmon (*O. keta*), particularly in Alaska. In this study, we used nine microsatellite loci to genotype 32 putative coho salmon populations (N = 2,581) from seven regions of Alaska. The objectives were to: 1) estimate the degree and spatial distribution of neutral genetic diversity in Alaskan coho salmon; 2) evaluate the potential for regional-level mixed-stocked analysis and individual assignment.

Fin tissue samples for microsatellite analysis were obtained from 32 collections of coho salmon representing seven major regions of Alaska (Fig. 1): Northwest Alaska (N = 4); Southwest Alaska (N = 3); Aleutian Islands/Alaska peninsula (N = 3); Kodiak Archipelago (N = 8); Cook Inlet (N = 8); Prince William Sound (N = 3); Southeast Alaska (N = 3). Nine microsatellite loci were used to estimate genetic variation in each population (*Oke2*, *Oke3*, and *Oke4*, Buchholz et al. 2001; *Oki1*, *Oki3*, and *Oki11*, Smith et al. 1998; *One3*, Scribner et al. 1996; *Ots3.1*, Banks et al. 1999; *Ots105*, Nelson and Beacham 1999). Two of these loci (*Oki1*, *Ots3.1*,) have also been used for MSA of coho salmon in British Columbia (Beacham et al. 2001). Total genomic DNA was isolated from approximately 50–100 mg of fin tissue for use in PCR. A description of the microsatellite loci, PCR chemistry, gel electrophoresis and microsatellite allele scoring is given in Olsen et al. (2003).

Three analyses were performed. First, an analysis of molecular variation for diploid data (AMOVA, Michalakis and Excoffier 1996) was used to test for genetic structure within and among the seven regions. AMOVA was performed using ARLEQUIN version 2.0 (Schneider et al. 2000) and the degree of population divergence,  $F_{ST}$ , was estimated according to Weir and Cockerham (1984). The estimate of  $F_{ST}$  was partitioned into within-region ( $F_{SR}$ ) and between-region ( $F_{RT}$ ) genetic variation. Randomization tests were used to test if the estimates of  $F_{ST}$ ,  $F_{SR}$ , and  $F_{RT}$  were significantly greater than zero.

Second, MSA simulations were performed using the direct maximum likelihood method implemented in the program SPAM 3.7 (Debevec et al. 2000). Parametric bootstrap resampling of both the baseline and mixture was

carried out 1,000 times to derive the mean allocation estimate and to evaluate precision. Artificially simulated mixtures (N = 400) representing 100% of each individual population were subjected to MSA as a test of baseline performance. Mean allocations to individual populations were then summed for geographically defined regions. The allele frequencies for the baseline samples were estimated using the Bayesian modeling approach of Rannala and Mountain (1997).

Finally, the program GENECLASS version 1.0.02 (Cornuet et al. 1999) was used to conduct a (re)assignment test by the partial Bayesian method with leave-one-out classification.





A chi-square test was used to determine if the number of successful classifications was significantly different than expected when individuals were randomly assigned to region.

The AMOVA results indicated that the degree of differentiation ( $F_{ST} = 0.093$ ) among populations of Alaskan coho salmon was significantly greater than zero (P < 0.001) and was as large or larger than that reported for other Pacific salmon species in Alaska (Table 1). AMOVA also showed that Alaskan coho salmon exhibit significant inter- and intra-regional population structure. Estimates of among-region ( $F_{RT} = 0.036$ ) and within-region ( $F_{SR} =$ 0.057) genetic differentiation were significantly greater than zero (P < 0.001).  $F_{SR}$  was greater than  $F_{RT}$ , indicating that intra-regional variation is a large component of the overall genetic population structure in Alaskan coho salmon. The relatively high degree of intra-regional genetic variation suggests that population structure in Alaskan coho salmon is organized on a relatively small geographic scale and these populations should be managed to conserve this fine-scale genetic diversity.

The potential for accurate regional-level MSA is good. The mixture estimates ranged from 87.2% (SE) to 95.9% (PW) for simulated mixtures containing only fish from a single region (100% simulations, Table 2). The 95% confidence intervals were above 80% for all but the SE region (79.4%). The results suggest MSA could be a useful tool to estimate stock proportions in a regionally heterogeneous sample of coho salmon.

The potential for accurate regional-level individual assignment varies by region but is generally low. Of the 2,581 coho, 1,396 (54.1%) were correctly assigned to their true region. The percent of correct assignments ranged from 34.5% (AP) to 80.6% (PW) and was greater than 50% in four of the seven regions (Table 3). The percentage of individuals correctly assigned to region varied greatly among regions. The number of individual assignments was always greatest for the region of origin and was significantly different than expected when individuals were randomly assigned to region (P < 0.001, Table 3). Although the percentage of individuals (re)assigned to their true region is significantly larger than expected based on random assignment, a large percentage (mean = 45.9%) were assigned to the wrong region. Moreover, the number of miss-classified individuals assigned to the seven regions was not consistent with spatial distributions. For example, 20 individuals from Cook Inlet are misclassified to the adjacent Prince William Sound but 35 individuals are misclassified to the more distant Southwest Alaska region.

In summary, the microsatellite loci used in this study have high potential for accurate regional-level MSA of Alaskan coho salmon, but relatively low potential for accurate regional-level individual assignment. This is most likely because MSA makes better use of the genetic data by computing a single likelihood for all the mixed-sample genotypes and is therefore a more powerful method (Millar 1990). The accuracy of both methods will likely be improved by adding loci and increasing the sample size of the baseline populations (Cornuet et al. 1999). Simulation results by Cornuet et al. (1999) suggest 20 or more loci may be required to achieve near 100% assignment accuracy given the relatively low inter-regional genetic differentiation ( $F_{RT} = 0.036$ ) in Alaskan coho salmon.

| Grouping<br>Strategy       | Source of variation                | $\sigma^2$ | Percent<br>of total | F <sub>ST</sub> | F <sub>RT</sub> | $F_{SR}$ |
|----------------------------|------------------------------------|------------|---------------------|-----------------|-----------------|----------|
| Seven regions <sup>a</sup> | Total                              | 1.711      | 100.00              |                 |                 |          |
|                            | Within populations                 | 1.555      | 90.88               |                 |                 |          |
|                            | Between populations                | 0.156      | 9.12                | 0.093*          |                 |          |
|                            | Between regions                    | 0.062      | 3.64                |                 | 0.036*          |          |
|                            | Between populations within regions | 0.094      | 5.48                |                 |                 | 0.057*   |

**Table 1.** Hierarchical gene diversity analysis of 32 coho salmon population samples from Alaska. An asterisk (\*) denotes P < 0.001the value is not greater than zero; RT = among regions; SR = within regions.

**Table 2.** Results of mixed-stock analysis simulations for 100% contribution by region. Mean% is the average percent contribution over 1000 simulated mixtures. LCI and UCI are the bounds for the lower and upper 95% confidence intervals. Region abbreviations are as indicated in Fig. 1.

| Region | Mean% | LCI  | UCI  |
|--------|-------|------|------|
| NW     | 95.5  | 91.2 | 98.7 |
| SW     | 90.3  | 82.6 | 96.0 |
| AP     | 92.9  | 86.9 | 97.4 |
| КО     | 90.0  | 81.6 | 96.0 |
| CI     | 91.4  | 85.5 | 96.8 |
| PW     | 95.9  | 92.8 | 98.5 |
| SE     | 87.2  | 79.4 | 94.5 |

**Table 3.** Assignment test results for 2,581 coho salmon from seven regions of Alaska. The number of correct assignments (bold print), sample size (*n*), percent correct assignments (%CA), percent expected assignments by chance (%EA), number of expected assignments by chance (EA), and the chi-square test *P*-value are shown for each region. Region abbreviations are as indicated in Fig. 1.

| -      |     |     |     |     |     |     |    |     |      |      |       |         |
|--------|-----|-----|-----|-----|-----|-----|----|-----|------|------|-------|---------|
| Region | NW  | SW  | AP  | KO  | CI  | PW  | SE | Ν   | %CA  | %EA  | EA    | P-value |
| NW     | 209 | 33  | 4   | 28  | 23  | 2   | 3  | 302 | 69.2 | 12.5 | 37.8  | <0.001  |
| SW     | 46  | 101 | 12  | 44  | 57  | 7   | 13 | 280 | 36.1 | 9.4  | 26.3  | <0.001  |
| AP     | 14  | 19  | 131 | 92  | 75  | 17  | 32 | 380 | 34.5 | 9.4  | 35.6  | <0.001  |
| КО     | 17  | 29  | 33  | 340 | 90  | 33  | 45 | 587 | 57.9 | 25.0 | 146.8 | <0.001  |
| CI     | 21  | 35  | 42  | 98  | 346 | 20  | 21 | 583 | 59.3 | 25.0 | 145.8 | <0.001  |
| PW     | 1   | 2   | 4   | 15  | 16  | 191 | 8  | 237 | 80.6 | 9.4  | 22.2  | <0.001  |
| SE     | 11  | 7   | 18  | 43  | 39  | 16  | 78 | 212 | 36.8 | 9.4  | 19.9  | <0.001  |

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# Identification of Local Stocks of Sockeye Salmon (*Oncorhynchus nerka*) by Scale Pattern Analysis in the Russian Economic Zone

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Keywords: Sockeye salmon, identification, scale pattern analysis, scale baselines, age groups, dynamic abundance, economic zone of Russia

Identification of local stocks of sockeye salmon in the Russian economic zone were made on the basis of scales collected in 1995-2001. Samples of sockeye salmon from different local stocks of Asia and North America of age groups 1.3 and 2.3 were used for the formation of the scale baseline. These age groups of adult sockeye salmon are dominant in the coastal and driftnet catches in the Asian region. The baseline consisted of 8,653 fish: (Eastern Kamchatka – 3,675, Western Kamchatka – 3,294,, Kommander Islands – 250, Kuril Islands – 34, and Bristol Bay (Port Moller) – 1,400 fish) (Fig. 1).

Scales were taken from sockeye salmon that were collected from driftnet catches in the southwestern part of Bering Sea (Karaginskaya subzone – 61.02.1) and adjacent waters of the northwestern part of Pacific Ocean (Petropavlovsk-Kommander subzone – 61.02.2 and Pacific subzone – 61.03.1). The total sample size consisted of scales from 17,975 fish (61.02.1 - 5216 fish, 61.02.2 - 7630 fish, 61.03.1 - 5129 fish) (Fig. 2). Only mature sockeye salmon (about 90% from the total driftnet catches in economic zone of Russia) were included in analysis.

Scale pattern analysis was based on standard methods used by NPAFC (Davis et al. 1990). Analyses of the scale structure included measurements of the radius of the total freshwater zone and size of the first ocean annual zone (Fig. 3). The numbers of circuli and inter-circuli distances were measured in the first ocean zone. Fifteen scale criteria were used for identification of local stocks of sockeye salmon.

Results of the cluster analysis showed that significant differences were observed only between the stock complexes from Eastern Kamchatka - Western Kamchatka and Bristol Bay – Western Kamchatka. The stocks from Eastern Kamchatka and Bristol Bay formed a cluster using the scale baseline among age groups 1.3 and 2.3 in all cases. The most similarities in scale structure were observed between stocks from Northeastern Kamchatka (Navarinsky Bay and Olutorsky Bay) and Bristol Bay. Analogous results were found using the nonparametric Wilcoxon test that demonstrated a reliable difference (p < 0.01-0.05) only between designated stock complexes. This could be the result of similar environments for freshwater and early marine life history.

Thus, reliable identification by scale pattern analysis for complexes of sockeye salmon stocks from Eastern Kamchatka and Bristol Bay is impossible. Additional biological conditions will have to be used in this situation. For example, the dynamics of stock abundance or known tendency of distribution of major complexes of local stocks may be useful. We have to take into consideration other factors in our actual situation. Considering the time of the active commercial driftnet fishery and mass prespawning migrations of Asian and American origin sockeye salmon in Russian economic zone (June-July), and the geographic distances between continents (more than 2,000 km), it is logical to assume that the total catch of mature sockeye salmon in the Russian economic zone is predominantly of Asia origin.

**Fig. 1.** Map of the areas of scale collected for identification of local stocks of sockeye salmon in the Russian economic zone in 1995–2001: 1 – r. Palana; 2 – r. Icha; 3 – r. Oblukovina; 4 – r. Krutogorova; 5 – r. Vorovskaya; 6 – r. Kikhchik; 7 - r. Utka; 8 – r. Bolshaya; 9 – r. Opala; 10 – r. Ozernaya; 11 – r. Kambalnaya; 12 – r. Listvenichnaya; 13 – r. Paratunka; 14 – r. Avacha; 15 – r. Jupanova; 16 – r. Kamchatka; 17 – r. Khailulya; 18 – r. Rusakova; 19 – r. Dranka; 20 – r. Kulushnaya; 21 – r. Pakhacha; 22 – r. Impuka; 23 – r. Apuka; 24 – r. Anana; 25 – r. Severnaya; 30 – r. Gavanka; 31 – r. Ladyginka; 32 – r. Bettobu; 33 – Port Moller (Bristol Bay).





**Fig. 2.** Map of the fisheries areas within the far eastern part of the Russian economic zone (region 61.)

Therefore, the scale baseline is only useful for the identification of Western and Eastern Kamchatka stocks of sockeye salmon which account for more than 95% of the total Asian stocks. Stock identification error is not appreciable in this case. Note that in this situation, it does not pertain to immature fish. The mean accuracy for homogenous-mixture baseline-dependent simulation results for the cluster-based analysis are about 92–98%.

Results of the stock identification analyses of mature sockeye salmon in the driftnet catches are based on the maximum likelihood estimate (Millar 1987, 1988, 1990). Specific distribution of local stocks of sockeye salmon in the spring-summer period was determined for each fishery area. Stocks of Eastern Kamchatka were dominate in the southwestern part of the Bering Sea (Karaginskaya subzone - 61.02.1) in May–July at 86% (77–93%) (Fig. 4). The average fluctuations in the intensity of the run were about 1-3 fish / tan. The peak run of sockeye salmon was observed in the Karaginskaya subzone in mid-June. The proportions of Eastern and Western Kamchatka stocks were approximately equal (1: 1) in the northwestern part of the Pacific Ocean (Petropavlovsk-Kommander subzone -61.02.2) (Fig. 5). These complexes of local stocks are maximally mixed in this subzone. In May and June the stocks of Eastern Kamchatka are dominate at 71% (48-85%) and 54% (39-68%), respectively. The peak run is observed in June–July at about 3–5 fish / tan. In July and August the percentage of Western Kamchatka stocks were above 77% (61–88%) and 88% (81–98%) respectively. The percentage of the Western Kamchatka stocks was still increasing reaching approximately 83% (66-94%) in the June-July period in the waters of Kuril Islands (Pacific subzone - 61.03.1) (Fig. 6). A large run is

Fig. 3. Image of a scale and the criteria used for identification of local stocks of sockeye salmon in the Russian economic zone: FW – total distance in the freshwater growth zone; O1 – total distance in the first annual ocean growth zone; tr1-tr6 – triplets circulus distance from first circuli in the first annual ocean growth zone (sixth triplets); tr1-tr6 – revers-triplets circulus distance from last circuli in the first annual ocean growth zone (sixth revers-triplets).



**Fig. 4.** Frequency dynamics of complexes of local stocks of mature sockeye salmon by main age groups from driftnet catches in the Karaginskaya subzone – 61.02.1





Fig. 5. Frequency dynamics of complexes of local stocks of mature sockeye salmon by main age groups from driftnet catches in the Petropavlovsk-Kommander subzone – 61.02.2

observed in late June–July at the time of active migration of stocks from Ozernaya River. The mean catch was about 2-5 fish / tan in this period. Catches sometimes amounted to 6-8 fish / tan in the years of high abundance of sockeye salmon from the Ozernava River. The latitudinal distribution of sockeye salmon in the period of pre-spawning migration in the southwestern part of Bering Sea and adjacent waters of the northwestern part of Pacific Ocean depend upon the geographical location of reproduction areas and on the timing of spawning of stocks from Eastern and Western Kamchatka. It is seen from generalized scheme of distribution, that complexes of local stocks of mature sockeye salmon are found in the Russian economic zone in the spring-summer period (Fig. 7). Interannual fluctuations in the proportions of stock compositions in driftnet catches have coincided with the dynamics of abundance of these stock complexes.

The generalized model of pre-spawning migrations of Asian sockeye salmon was made on the basis of the data from identification of local stocks of sockeye salmon, and observations of the distribution and dynamics of driftnet catches in the Russian economic zone in the May–August period (Fig. 8). Only the distribution and intensity of migration of major Asian stocks complexes are demonstrated in this scheme. This process depends mainly on the dynamics of





Fig. 7. Generalized scheme of distribution of complexes of local stocks of mature sockeye salmon from driftnet catches in spring-summer





**Fig. 8.** Generalized model of pre-spawning migration of Asian sockeye salmon for all age groups in the Russian economic zone: A – stocks of Eastern Kamchatka; B – stocks of Western Kamchatka

abundance of stocks from the Kamchatka and Ozernaya Rivers. Sockeye salmon do not form well-defined stock complexes at the time of pre-spawning migrations, and only the broad migration front of stocks from Eastern and Western Kamchatka is shown in this scheme. A decrease in the intensity of driftnet catches of sockeye salmon at about 0.01-1.00 fish / tan is noted at the approach to the northern or southern boundary of migration fronts. The intensity of the catches average about 3.00-5.00 fish / tan in the center of the migration front. The size of the active migration front of sockeye salmon depends on stock abundance and the environment.

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- Millar, R.M. 1988. Statistical methodology for estimating composition of high seas salmonid mixtures using scale analysis. (INPFC Doc. 3284) 18p. Fishery Research Institute, University of Washington, Seattle, USA.
- Millar, R.M. 1990. Comparison of methods for estimating mixed stock fishery composition. Can. J. Fish. Aquat. Sci. 47: 2235–2241.
# Microsatellites, Allozymes, and SNPs Describe the Population Structure and Identify Spatial Distribution of Mixture Components of Sockeye Salmon in the Bering Sea

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Keywords: Marine migration, sockeye salmon, *Oncorhynchus nerka*, Bering Sea, stock, allozyme, microsatellite, single nucleotide polymorphism, Ocean Carrying Capacity, population, genetic structure

Knowledge of stock-specific marine migration patterns of sockeye salmon *Oncorhynchus nerka* in the Bering Sea could provide insights into stock composition of international harvests and into factors affecting stock-specific early marine survival. Alaska Department of Fish and Game Gene Conservation Laboratory and National Marine Fisheries Service Auke Bay Laboratory (ABL) are collaborating to develop a Bering Sea baseline of genetic markers to be used to determine composition of stock mixtures caught on the high seas.

The baseline is composed of 166 collections extending from Northern Alaska Peninsula (8 collections), through Bristol Bay (136; Fig. 1), north to Norton Sound (1) and west to the Kamchatka River and Kuril Lake drainages in Russia (23). Genotypes from 42 allozyme, eight microsatellite, and one single nucleotide polymorphism (SNP) loci were collected on approximately 100 fish per collection. Simulations using the Statistical Program to Analyze Mixtures (SPAM; Debevec et al. 2000) were used to identify reporting groups. High-seas collections from ABL Ocean Carrying Capacity (OCC) surveys were pooled regionally within years to attain approximately 400-fish samples. Initial stock composition estimates were made on these samples using allozymes in the 1999 and 2001 collections and using microsatellites and a SNP in some of the 2002 collections to identify patterns of stock distributions.

Simulation results produced 11 reporting groups that demonstrated high precision in estimating continent-oforigin, and distinguishing among North Alaska Peninsula, Bristol Bay and Norton Sound collections. Within Bristol Bay, collections from Kvichak Bay drainages south to Meshik River (eastern Bristol Bay) clustered together and separately from those from Nushagak Bay drainages east to the Kuskokwim Bay drainage (western Bristol Bay).

Within eastern Bristol Bay, each group of collections from drainages above obstacles to migration, such as rapids of falls, was a reporting group, but collections made from the remaining areas where unexpectedly homogeneous and produced a single reporting group (Fig. 2). Within western Bristol Bay collections, each major drainage was a reporting group (Fig. 3).

Results from 1999 surveys, which had the largest number of fish, are consistent with sequential migration along the North Alaska Peninsula whereby Bristol Bay drainage stocks enter the ocean at about the same time and then migrate south and east along the Alaska Peninsula (Figs. 4 and 5; Farley et al. 1999). Sampling. Design changed in 2000, so the pattern detected in 1999 was hard to compare with later years (Farley et al. 2000, 2001 and 2002). In 2001, only **Fig. 1.** Locations of sites within nursery lake drainages where adult sockeye salmon were captured and tissues taken to resolve microsatellite, SNP and allozyme loci used to examine genetic relationships among populations spawning in drainages of Bristol Bay, Alaska.



**Fig. 2.** Multidimensional scaling analysis of microsatellite and SNP data from of sockeye salmon captured at 38 sites in Eastern Bristol Bay, Alaska. In 100% simulations, at least 89% were correctly allocated to each of the circled groupings using microsatellites and SNP loci, and all circled groupings except Six Mile Lake using allozymes.

**Fig. 3.** Multidimensional scaling analysis of microsatellite and SNP data from sockeye salmon captured at 17 sites in Western Bristol Bay, Alaska. In 100% simulations, at least 89% were correctly allocated to each of the circled groupings using either microsatellite and SNP loci or allozymes.



Fig. 4. Stock composition, using allozymes, of juvenile sockeye salmon captured in the Ocean Carrying Capacity project in July, 1999 from four transects. 'N" equals the number of fish included in the mixture sample.



Fig. 5. Stock composition, using allozymes, of juvenile sockeye salmon captured in the Ocean Carrying Capacity project in September, 1999 from four transect groups. 'N' equals the number of fish included in the mixture sample.



enough samples were taken to produce two estimations of stock composition: 1) near shore and 2) offshore (Farley et al. 2001). Near shore estimates indicated relatively higher numbers of North Alaska Peninsula stocks, while offshore estimates indicated relatively more eastern Bristol Bay stocks (Fig. 6). Only enough samples were analyzed in 2002 to produce a single stock composition estimate, so no patterns could be identified (data not shown).

We plan to collect genotypes for allozyme, microsatellite and SNP loci on all the OCC samples from 1999 to 2002. We plan to collect genotypes from microsatellite and SNP loci from the 2003 OCC samples and the Bering Aleutian Salmon International Survey samples from 2003 and 2004. Adding the DNA markers will produce more precise estimates of stock composition and completing the remaining **Fig. 6.** Stock composition, using allozymes, of juvenile sockeye salmon captured in the Ocean Carrying Capacity project in August, 2001 for two sets of collections, those north and south of 56.50N. 'N' equals the number of fish included in the mixture sample.



samples will allow for better interpretation of migration patterns. In addition, we are screening an additional five microsatellite loci in the full baseline to determine if eastern Bristol Bay drainage stocks below obstacles to migration can be separated into more reporting groups.

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# DNA-Based Stock Identification of Coastal Sockeye Salmon: Evidence for Stock-Specific Migration Behaviour of Central Coast (Rivers Inlet) Sockeye Salmon

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Keywords: Migration, sockeye, salmon, Oncorhynchus nerka, DNA, Rivers Inlet

Sockeye salmon (*Oncorhynchus nerka*) stocks from the Central Coast of British Columbia (Rivers and Smith Inlet) formed the second most valuable salmon fishery in British Columbia until the late 1970s. Over a 20 year period these stocks then collapsed, with the Rivers Inlet stock falling to approximately 1/1,000th of its initial abundance by the late 1990s (McKinnell et al. 2001). Although the specific cause of the collapse has not been established, a review panel concluded that "*The drastic declines in abundance appear to be due to an extended period of poor marine survival*" and that the parallel collapses in both nearly pristine and logged watersheds "...*is convincing evidence that the cause of the declines does not lie in freshwater habitat disturbance*" (Holtby et al. 2000).

The collapse of central coast sockeye stocks is particularly puzzling because Fraser sockeye migrate up the coast directly past Rivers and Smith Inlets, but do not show the long-term decline in productivity since the 1970s that is evident for the Rivers-Smith Inlet sockeye. It would generally be expected that the poor marine survival affecting Rivers or Smith Inlet sockeye would therefore also influence Fraser River sockeye. This difference could be explained in three possible ways:

- (1) Fraser sockeye do not initially migrate north into the central coast region and remain sheltered within the Strait of Georgia for extended periods.
- (2) Fraser and Rivers Inlet sockeye migrate north along the shelf at equivalent speeds, thereby forming spatially separated discrete groups as they migrate.
- (3) Rivers Inlet sockeye remain as local residents of some coastal region while Fraser River sockeye migrate past them in on their northern migration.

We examined the changes in stock composition of juvenile sockeye caught in DFO trawl surveys using DNA stock identification techniques. All juvenile sockeye were caught in coastal waters. We define juvenile salmon as those caught during their first year of marine life. The sampling survey extended from 1997 to 2003, and was conducted in various months from May through February, thus allowing us to reconstruct changes in stock composition for a number of regions along the shelf for different seasons of the year. For convenience, we refer below to Rivers Inlet sockeye to also include the Smith Inlet sockeye except where explicitly identified.

We found that in early summer (May–June) catches in the various regions are dominated by sockeye from nearby river systems, as expected. For example, Nass and Skeena River sockeye (northern British Columbia stocks) dominated the catches made in the Dixon Entrance-Southeast Alaska area, and Vancouver Island sockeye initially predominated in the catches made off Vancouver Island and Queen Charlotte Strait. However, the stock composition changed substantially as the season progresses, indicating rapid migration northward along the coast of at least some stocks.

By mid-summer (June–July) Fraser River sockeye made up 50–77% of the trawl-caught sockeye in central and northern coastal regions of British Columbia, and 27–52% of sockeye caught off Southeast Alaska. In October, Fraser River juveniles formed 50% of the sockeye catch off Kodiak Island and 82% of the sockeye catch off Southeast Alaska, but still formed only a small proportion of the sockeye caught off the Alaskan Peninsula in November, where Nass and Skeena River stocks still predominated. There is thus evidence for some stocks remaining as spatially segregated units during their migration, although some mixing is also evident. Thus the Fraser River sockeye rapidly moved north along the continental shelf. In contrast, the Rivers and Smith Inlet sockeye formed only small and statistically insignificant proportions of the sockeye catch and 97% of the Queen Charlotte Sound sockeye catch.

Although Fraser sockeye migrate up the coast directly past the ocean-entry point of Rivers and Smith Inlets, they do not show as serious a decline in productivity as is evident for Rivers-Smith Inlet (their productivity has, however, been reduced in the 1990s as well). This difference can be explained if Rivers Inlet sockeye remain

resident in southern areas of the continental shelf for prolonged periods, while Fraser River sockeye migrate quickly through the region of poor growth and survival.

Overall autumn catch rates for sockeye dropped sharply relative to the summer, while the proportional abundance of Rivers and Smith Inlet sockeye increased. We therefore conclude that Fraser River sockeye migrated to the north along the coast while the central coast sockeye remained resident in the coastal region off their rivers of origin. We thus reject our first two hypotheses and accept the third. This difference in marine migration behaviour thus leaves Rivers-Smith Inlet sockeye particularly vulnerable to poor ocean conditions that developed in the south-central British Columbia coastal region for extended periods of time. In contrast, Fraser River sockeye that migrate through this region are not exposed to poor marine conditions for extended periods. Thus stock-specific differences in marine migration pathways coupled with regional variation in ocean conditions apparently led to the collapse of central coast sockeye stocks but not that of sockeye stocks to the south.

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# Three Genetic Stocks of Upriver Bright Fall Chinook Salmon Detected in the Columbia River Basin, USA

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Keywords: Genetic stock identification, microsatellite, chinook salmon, Columbia River

In order to detect stock structure in the Columbia River basin, we analyzed 694 upriver bright fall chinook salmon samples from seven locations at seven microsatellite loci. Results indicate three main stocks of upriver bright fall chinook salmon in the Columbia basin above Bonneville Dam. These three stocks are Deschutes River, Snake River (natural origin), and Columbia River mainstem (plus Lyons Ferry Hatchery). Samples from 424 unknown origin upriver bright fall chinook salmon passing Bonneville Dam in 1999 were assigned to one of the three genetic stocks detected in this study.

Columbia River Basin upriver bright fall chinook salmon are heavily harvested in the Alaskan, Canadian, coastal, and the Columbia River in-river fisheries. Ocean harvest of Columbia River salmon in mixed stock fisheries has been difficult to quantify due to the large number of wild fish, and incomplete coded wire tagging of hatchery fish. Current genetic methods may prove to be a strong indicator of stock composition in mixed fisheries by utilizing genetic markers inherent in all salmon (Beacham et al. 2003). In order to detect stock structure in the Columbia River basin, we analyzed 694 upriver bright fall chinook samples from seven locations at seven microsatellite loci, and assigned unknown individuals to stocks of origin based on these data.

Sample collections represent the major upriver bright fall chinook salmon producing locations in the Columbia Basin. These sample locations (and year collected) include the Grande Ronde River 1998 (n = 38), Clearwater River 1998 (n = 66), Hanford Reach 1998 (n = 54) & 1999 (n = 81), Lyons Ferry Hatchery 2000 (n = 85), Priest Rapids Hatchery 1998 (n = 36) & 1999 (n = 106), upper Deschutes River 1998 (n = 95) & 1999 (n = 91), and lower Deschutes River 1999 (n = 42). Further, unknown samples of upriver bright fall Chinook passing Bonneville Dam were collected from August 2 to October 28 in 1999 (n = 424).

Fin clips were digested and DNA extracted using standard manufacture's protocols from Qiagen® DNeasy<sup>™</sup> in conjunction with a Qiagen® 3000 robot. Genomic DNA was quantified and arrayed into 96 well plates for high throughput genotyping. Polymerase chain reaction (PCR) was used to amplify seven microsatellite loci (Table 1; also see Table 1 for PCR conditions). Forward primers were fluorescently labeled (Applied Biosystems®), and PCR products were genotyped using manufacture's protocols with an Applied Biosystems® model 3100 genetic analyzer.

| Locus   | Reference              | Annealing Temp. | Total A |
|---------|------------------------|-----------------|---------|
| OtsG68  | Williamson et al. 2002 | 52              | 43      |
| OtsG78  | Williamson et al. 2002 | 52              | 46      |
| OtsG249 | Williamson et al. 2002 | 52              | 44      |
| Ots311  | Williamson et al. 2002 | 52              | 52      |
| OtsG432 | Williamson et al. 2002 | 52              | 30      |
| Ots4    | Banks et al. 1999      | 58              | 13      |
| Ogo4    | Olsen et al. 1998      | 58              | 18      |

Table 1. Microsatellite loci and total number of alleles (A) in all samples. Annealing temperatures (°C) for PCR are shown as performed with the AmpliTaq Reagent System (Applied Biosystems) and 25ng genomic DNA in 15ul total volume.

To estimate the level of within-population genetic diversity, observed unbiased gene diversity and allelic richness (average alleles per locus corrected for sample size) were calculated for all microsatellite loci (FSTAT; Goudet 1995). Genetic variance was calculated from allele frequencies ( $F_{ST}$ ; Weir and Cockerham 1984) using

GENEPOP v. 3.3 (Raymond and Rousset 1995) to estimate pairwise genetic divergence among collections from the Columbia River Basin. Exact-significance testing methods were used to evaluate conformance to Hardy-Weinberg, linkage equilibria, and differences in allele frequency distributions (temporally and geographically). Unbiased estimators of exact significance probabilities were obtained using the Markov-Chain algorithm described in Guo and Thompson (1993), as implemented in GENEPOP v. 3.3 (Raymond and Rousset 1995), using 500,000 steps. Corrections were made against Type I error in exact tests using the sequential Bonferroni method (Rice 1989). Assignment tests were performed using the Bayesian method in GeneClass (Cornuet et al. 1999) to assign unknown individuals to populations of origin. In order to infer the degree of relatedness between sample collections, pairwise genetic distances (Cavalli-Sforza and Edwards 1967) were calculated between all populations and were used to construct a neighbor joining (NJ) tree with 1000 iterations (PHYLIP v. 3.5; Felsenstein 1993).

A high number of alleles were detected in all samples with an average of 36 alleles per locus (average allelic richness = 21.6; Table 2). Gene diversity was also high with an average of 0.897 (Table 2). Results from the NJ tree indicate three main stocks of fall chinook salmon in the Columbia basin (Fig. 1). These three stocks are Deschutes River, Snake River (natural origin), and Columbia River mainstem (plus Lyons Ferry Hatchery). Sample collections from hatchery (Priest Rapids Hatchery, Lions Ferry Hatchery) and wild (Hanford Reach) stocks were not significantly different and displayed little genetic differentiation ( $F_{ST} = 0.005$ ). Temporal collections within locations also displayed little genetic differentiation and none were significantly different. Samples from 424 unknown origin upriver bright fall chinook passing Bonneville Dam in 1999 were assigned to one of the three genetic stocks detected in this study. The majority of unknown samples assigned to the Columbia River mainstem stock (68%), 20% assigned to the Deschutes River, and 12% assigned to the Snake River.

| Population     | n   | AR   | Gene Diversity |
|----------------|-----|------|----------------|
| Snake River    | 104 | 21.6 | 0.913          |
| Columbia River | 362 | 22.8 | 0.888          |
| Deschutes      | 228 | 20.4 | 0.891          |

**Fig. 1.** Neighbor Joining Tree constructed with chord distances (Cavalli-Sforza and Edwards 1967) from 1000 iterations. Bootstrap values above 50% are shown at branch nodes. Scale chord distance of 0.01 is shown.



The populations of fall chinook salmon in this study are some of the healthiest stocks in the Columbia River Basin, but intermingled among them is the threatened Snake River fall chinook stock. Genetic analyses may be useful in determining the presence of Snake River stock in unknown catches of fall chinook salmon in mixed stock fisheries in the North Pacific Ocean (Shaklee et al. 1999). Results from this study indicate that data from additional microsatellite loci are necessary to obtain more precise stock structure (specifically for Lyons Ferry Hatchery as per Marshall et al. 2000) and increased assignment fidelity. Seven genetics laboratories in North America (including our lab in Hagerman, Idaho) have begun efforts to standardize microsatellite loci for generating a North American coastwide chinook salmon database. Upon completion of standardized loci between the seven labs, we plan to further analyze this data set with these loci to provide standardized baseline data of Columbia Basin upriver bright fall chinook salmon stocks.

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# Conservation and Genetic Stock Identification: A Study Investigating the Stock-Specific Distribution and Performance of Juvenile Chinook Salmon in the Columbia River Estuary

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Keywords: Chinook salmon, genetic stock identification, individual assignment tests, microsatellites

Research, management and conservation of salmon populations have primarily targeted the freshwater phase of the salmon lifecycle, however recent attention has focused on the importance of the estuarine and ocean phases in the abundance and distribution of Pacific salmon (Brodeur et al. 2000). One potentially critical and interesting lifehistory phase of Pacific salmon is early entry into salt water (Casillas 1999). Coded wire and PIT tag recoveries, scale analysis and length at age data have all provided insight into the use of estuarine and nearshore habitats by juvenile salmonids. Nevertheless, there are significant gaps in our understanding of how and when juveniles use these environments. The research presented here is the genetic component of a multidisciplinary, multiagency collaboration investigating the stock-specific temporal and spatial distribution of juvenile chinook salmon (*Oncorhyncus tshawytscha*) in the Columbia River estuary.

The first goal of our study was to establish a Columbia River microsatellite genetic baseline to evaluate genetic structure and differentiation among populations and evolutionarily significant units (ESUs) of chinook salmon in the Columbia River basin. The second goal of the study was to use the genetic baseline and individual assignment tests to identify putative source populations and ESUs of juvenile chinook salmon mixtures sampled at different spatial and temporal scales in the Columbia River estuary. We collected genotypes at eight microsatellite loci for 3,040 individuals from 65 chinook salmon populations representing all eight ESUs and nearly all major production areas in the Columbia River basin for the genetic baseline. Two hundred and sixty-six juvenile chinook salmon of unknown origin were collected from marsh, forested and shrub habitats in the Columbia River estuary and genotyped at the same eight loci. Fish were assigned to their most likely population, ESU, or larger reporting group (i.e., pooled genetically similar ESUs) of origin using individual assignment tests (implemented in WHICHRUN; Banks and Eichert 2000).

Preliminary genetic analyses indicate that there is significant geographic structuring in the Columbia River basin. The observed genetic relationships between populations were broadly concordant with previous genetic studies (Waples et al. in press and references therein). There was a compelling separation of populations

representing "stream-type" and "ocean-type" lineages (Healy 1991; Myers et al. 1998) based on the  $F_{ST}$  neighbor joining dendogram (Fig. 1). Consistent with expectation, the multilocus individual assignments suggest that most juveniles collected from the estuary originated from populations in the lower Columbia River ESU (Fig. 2). Juveniles from many of these populations are fry or fingerling migrants and are thought to spend significant time rearing in the estuary. We also observed fish in our juvenile mixtures from the Upper Willamette River, Mid-Columbia River, and Upper-Columbia River spring run ESUs. Juveniles originating from these areas were formerly thought to migrate quickly through the estuary

**Fig. 1.** Condensed representation of a mid-point rooted neighbor joining dendogram depicting genetic relationships ( $F_{ST}$ ) among chinook salmon ESUs, genetic reporting groups (I-IV), and lineages . Genetic reporting groups are identified as follows: I = Lower Columbia River (LCR), II = Upper Willamette River (UWR), III = Deschutes and Snake River fall (DR/SR fa), IV = Upper Columbia River summer/fall (UCR su/fa), and V = Interior spring (Int. sp).



**Fig. 2.** Summary of reporting group assignments for chinook salmon juveniles sampled in different habitats in the Columbia River Estuary.



to the ocean (Healy 1991). However, our data suggest that off-channel wetland habitat may also provide rearing habitat for juveniles from at least some spring run populations.

Quantitative evaluation of assignment error is in progress, but preliminary evidence suggests that ESU assignment is relatively certain, whereas assignment to specific population is more tenuous. We are conducting a comprehensive evaluation of statistical power and accuracy of our genetic baseline that includes simulations and analysis of coded-wire-tagged fish. Given our preliminary results, we are expanding our sample coverage in the lower Columbia River and assaying nine more microsatellite markers for the genetic baseline and mixture analysis. We are also analyzing additional juvenile mixtures from the Columbia River estuary to test for stock-specific habitat associations, condition factors and contaminant loads. Our data represent an integral part of a much larger study to

characterize and quantify a broad range of ecological parameters in estuarine and nearshore marine environments. The goal is to develop empirical (and theoretical) associations between habitat attributes (e.g., salinity, depth, channel morphology, vegetation type, prey resources, etc.) and the distribution and performance of juveniles (e.g., abundance, residence time, condition, and growth). This improved understanding will help evaluate specific mitigation and restoration actions and will help predict juvenile salmon response to environmental change.

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# Clues to Chinook Salmon Nearshore Migration in Southeast Alaska from Estimates of Stock Composition in Troll Harvests

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Keywords: Chinook salmon, genetic stock identification, migration

The Southeast Alaska troll fishery harvests stocks of chinook salmon *Oncorhynchus tshawytscha* originating from Alaska, British Columbia, and the Pacific Northwest. Management of the chinook salmon harvest in Southeast Alaska depends, in part, on information from coded-wire tag recoveries, a marker applied only to a subset of populations (mainly hatcheries). Genetic stock identification can provide stock composition information complementary to tag data. This method has been used extensively to estimate the composition of mixed-stock fisheries for chinook salmon in the Pacific Northwest (e.g. Marshall et al. 1991; Miller et al. 1993) and is possible because standardized baseline data for allozyme loci are available from throughout the species range (Teel et al. 1999). Since 1999, the Alaska Department of Fish and Game has used allozymes to provide independent estimates of the stock composition of the harvest throughout the year in Southeast Alaska troll fisheries (Crane et al. 2000). Legal-sized ( $\geq$  71 cm in length) chinook salmon are sampled from landings during the early winter (October to December), late winter (January to April), spring (May to June), and summer (July to September) troll fisheries. Sublegal-sized (<71 cm in length) chinook salmon incidentally caught during the summer fisheries are also sampled.

Estimates of the contribution of major stock groups to the troll harvest reflect the migratory behavior of these stock groups in the nearshore waters of Southeast Alaska. Analysis of the temporal changes in estimates of abundance and relative contribution to the 2000, 2001 and 2002 troll harvests shows the following major trends (Fig. 1). First, chinook salmon from the Oregon and Washington coasts are major contributors to the summer troll fishery, but are absent during the winter and spring. Second, chinook salmon from the Upper Columbia River summer and fall and Snake River fall stocks are major contributors to the fishery all year except during the spring. The abundance of these stocks in the harvest is greatest in the summer. Third, chinook salmon from the Thompson River watershed (Canada) are usually present in large numbers only in July. Fourth, chinook salmon from southern Southeast Alaska are present year round but are mainly harvested during the spring fishery.

**Fig. 1.** Seasonal variation in abundance and relative contribution of selected stock groups to the Southeast Alaska chinook salmon troll fishery, 2000–2002. Solid lines indicate the relative contribution to the harvest and bars indicate the number of chinook salmon harvested. No samples were taken from the spring (May to June) troll fishery in 2000, denoted with an asterisk (\*).



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Differences in the stock composition of samples from legal- and sublegal-sized chinook salmon present in Southeast Alaska during the summer indicate that different stock groups of chinook salmon use the nearshore waters of Southeast Alaska at different lifestages (Fig. 2). chinook salmon from the Oregon and Washington coasts and Thompson River are generally present as larger, more mature individuals. chinook salmon from the Upper Columbia River summer, fall and Snake River fall stock groups are abundant in both size classes. chinook salmon from the Lower Columbia River, Willamette River, Puget Sound, coastal British Columbia and southern Southeast Alaska are generally present as smaller, less mature individuals during the summer.

**Fig. 2.** Relative contributions of legal-sized ( $\geq$  71 cm) and sublegal-sized (< 71 cm) chinook salmon from selected stock groups to samples taken during the summer (July to September) troll fisheries, 1999–2002.



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# Stock Origins of Chinook Salmon in Incidental Catches by Groundfish Fisheries in the Eastern Bering Sea, 1997–1999

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Keywords: Chinook salmon, age composition, stock identification, eastern Bering Sea, groundfish trawl bycatch

The effect on western Alaska chinook salmon (*Oncorhynchus tshawytscha*) of incidental catches by commercial groundfish fisheries has been a major concern since 1977, when the U.S. National Marine Fisheries Service (NMFS) began to monitor and estimate salmon bycatch by groundfish vessels operating in the U.S. Exclusive Economic Zone (EEZ; Berger 2003). Most (> 99%) of the chinook salmon bycatch is taken by the walleye pollock (*Theragra chalcogramma*) trawl fisheries operating in areas with bottom depths of 100 m to 200 m, and high bycatch rates can occur in any location throughout the Bering Sea and Aleutian Islands (BSAI) area (Witherell et al. 2002).

Myers and Rogers (1988) used scale pattern analysis to estimate the age, regional stock composition, and interceptions of western Alaska chinook salmon in incidental catches by foreign and joint-venture groundfish fisheries operating in the BSAI area of the U.S. EEZ in 1979–1982. This was a period (1977–1986) of high abundance of western Alaska chinook salmon, and an estimated 60% of the total chinook salmon bycatch in the Bering Sea groundfish fisheries was western Alaska stocks (Myers and Rogers 1988). During the late 1990s returns of chinook salmon to western Alaska rivers declined to record lows. Because of this decline in abundance, Witherell et al. (2002) hypothesized that the stock composition estimates of Myers and Rogers (1988) may overestimate the contribution of western Alaska chinook salmon to the groundfish bycatch in recent years. When salmon returns to rivers are low, however, even relatively low incidental catches of salmon by non-target marine fisheries may reduce local utilization of chinook salmon resources and impede management and conservation efforts in western Alaska.

We used scale pattern analysis (Myers and Rogers 1988; Patton et al. 1998) to estimate the age and stock composition of chinook salmon in 1997–1999 BSAI groundfish fishery bycatch samples collected by the North Pacific Groundfish Observer Program, NMFS. Scale measurement data (14 variables) from mature chinook salmon returning to major production regions in Asia and North America were used to establish five brood-year specific baselines (BY 1991–1995). Maximum likelihood estimates (MLE) of the proportions of regional (Russia, Western Alaska, Central Alaska, and Southeast Alaska/British Columbia) and western Alaska subregional (Yukon, Kuskokwim, and Bristol Bay) stock groups in the fishery (mixture) bycatch samples were calculated (Millar 1987, 1990). Accuracies of the brood-year models were evaluated by computer simulations and by test mixture samples of baseline scales that were not included in the 4-group regional models.

During the period of our study, the largest bycatch samples were taken during winter (January and February) and late summer–fall (September and October) in the BSAI area east of 170°W. The 1997–1999 bycatch samples were dominated by age 1.2 fish in summer and ages 1.3 and 1.4 fish in winter (Fig. 1). In contrast, Myers and

Rogers (1988) found that younger (age 1.2) fish dominated winter bycatch samples in 1979–1982. This difference may be related to an eastward shift in the fishery area from offshore areas (west of 170°W)

in 1979–1982 to inshore areas (east of 170°W) in

1997–1999. In winter, immature ocean age-.2 chinook salmon may be distributed farther offshore than older age groups of immature and maturing fish.

Our results indicate that western Alaska was the dominant regional stock of chinook salmon in bycatch samples from the U.S. groundfish fishery in the eastern Bering Sea in 1997–1999 (Fig. 2). The estimated regional stock composition of chinook salmon in the five brood-year strata averaged 56% Western Alaska, 31% Central Alaska, 8% Southeast Alaska–British Columbia, and 5% Russia (BY 1991, Fig. 1. Estimated age composition of chinook salmon in eastern Bering Sea groundfish fishery bycatch samples.



**Fig. 2.** Estimated regional stock composition of chinook salmon in eastern Bering Sea groundfish fishery bycatch samples, 1997–99.



n = 373 fish; BY 1992, n = 530 fish; BY 1993, *n* = 1,111 fish; BY 1994, *n* = 762 fish; BY 1995, *n* = 481 fish). In winter age-1.4 western Alaska chinook salmon were largely from the Yukon and Kuskokwim sub-regions, and percentages of Bristol Bay fish were highest in samples of age-1.3 fish (Fig. 3). In summer age-1.2 western Alaska chinook salmon were predominantly from the Kuskokwim and Bristol Bay subregions (Fig. 4). In ongoing analyses, we are calculating estimates of interception (numbers of fish) of Yukon, Kuskokwim and Bristol Bay chinook salmon by the BSAI groundfish fishery and evaluating the effects of the salmon bycatch in 1997-1999 on

western Alaska chinook salmon runs using methods similar to Witherell et al. (2002). Scale samples and associated catch and biological data used in this study were provided by KamchatNIRO, the Alaska Department of Fish and Game, the Pacific Biological Station of the Department of Fisheries and Oceans Canada, and the North Pacific Groundfish Observer Program, NMFS. Funding for this research was provided by the Yukon River Drainage Fisheries Association.

**Fig. 3.** Estimated sub-regional stock composition of western Alaska chinook salmon in eastern Bering Sea groundfish fishery bycatch samples, winter 1997–99.



**Fig. 4** Estimated sub-regional stock composition of western Alaska chinook salmon in eastern Bering Sea groundfish fishery bycatch samples, summer 1997–99.



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# Genetic Stock Identification of Chinook Salmon, Oncorhynchus tshawytscha (Walbaum)

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Keywords: Chinook salmon, Oncorhynchus tshawytscha, genetic diversity, allozyme

The Kamchatka Peninsula is the only area in Asia where chinook salmon, *Oncorhynchus tshawytscha* (*Walbaum*), are abundant. There are two big river drainages (Kamchatka River on the east coast and Bolshaya River on the west coast of the Peninsula) with abundant populations of chinook salmon and many small chinook populations inhabiting smaller Kamchatkan rivers.

Using starch gel electrophoresis, we studied the protein gene frequencies at approximately 70 loci in 14 collections from 7 rivers including the Kamchatka and Bolshaya rivers. Thirty loci were found to be variable in Asian populations. Genetic diversity was highly significant among Kamchatkan populations. The most extensive differences between populations of Kamchatka and Bolshaya rivers were found at *ALAT*\*, *sAH*\*, *sIDHP*-2\*, *mMDH*-2\*, *MPI*\*, *mSOD*-1\*, *TPI*-4\*, *sMEP*-1\*, *sMEP*-2\*, and *GPI*-A\*. We did not find significant genetic differences within the same watershed, and the spring and summer adult runs in Kamchatka River did not differ significantly. The genetic distances were analyzed by cluster and principal component analysis (Fig. 1, 2), which revealed similar patterns. The western and eastern Kamchatka populations formed different clusters and the West Kamchatka populations divided further into three clusters – northern (Utka, Vorovskaya, Hairusova) and southern (Bolshaya, Bistraya) ones.

We made a comparison with allozymes data on American populations selected from preliminary publications and international baseline (Bartly and Gall, 1990; Gall et al., 1992; Teel et al., 1999). It revealed the great differentiation between Asian and North American populations of Chinook salmon. The allelic frequencies at 24 loci were different among regional groups of Asia and North America (*sAAT-1,2\*, mAAT-1\*, mAAT-2\*, ADA-2\* sMDH-B1,2\*, PGM-2\*, PEP-LT\*, PEPD-2\*, sAH\*, sIDHP-1\*, sIDHP-2\*, sAAT-3\* PEPA\*, PEPB-1\*, sSOD-1\*, TPI-4\*, GPI-B1,2\*, IDDH-1\*, sMEP-1\*, sMEP-2\* MPI\*, PGK-2\*, PEPD-2\*, and FDHG\*).* The *GPIB1,2\*, sMEP-2\*, PEPA\** and *sSOD-1\** loci were almost monomorphic in Asia and polymorphic in North America. The *IDDH-1\** locus, on the contrary, was highly polymorphic in Asia while almost monomorphic in North America. There were significant differences between regional population groups in *ALAT\*, ADA-2\** and *TPI-4\** loci. The

Asian populations were the most similar to North American populations from northwestern Alaska and Bristol Bay, though there were some loci significantly different between those regional groups of populations (*sAAT-3\**, *sAH\**, *IDDH-1\**, *sIDHP-1\**, *sMDH-B1,2\**, *MPI\**, *PEP-LT\**, *PEPD-2\**, and *PEPB-1\**).

Thus, genetic differentiation affords an excellent opportunity to discriminate Asian and North American populations of chinook salmon with high precision and accuracy.

In 1998 the mixed-stock fisheries collections from catches in Russian EZ were analyzed using allozyme genetic data (Fig.3, 4) from international baseline (Teel et al., 1999). The predominant stocks were from Kamchatka and Bolshaya River drainages, and also from northwest of Kamchatka peninsula (Fig.3). Among American stocks only Kodiak populations contributed more then 1% in the catches, but still the percentages were low (Fig.3). We estimated stock compositions in different seasons and in mature fish. The Alaskan fish were found in catches in May and mostly in immature fish when their percentage reached as much as 25% (Fig. 4). Fig. 1. UPGMA –dendrogram calculated by Nei's standard genetic distances on 43 allozyme loci in 10 populations of chinook salmon from Kamchatka Peninsula (sAAT-1,2\*; sAAT-3\*; mAAT-1\*; mAAT-2\*; ADA-1\*; ALAT\*; sAH\*; mMDH-2\*; CK-A1\*; CK-A2\*; CK-C2\*; CK-B\*; GPI-A\*; GR\*; IDDH-1\*; IDDH-2\*; mIDHP-2\*; sIDHP-1\*; sIDHP-2\*; LDH-B1\*; LDH-B2\*; LDH-C\*; sMEP-1\*; sMEP-2\*; mMEP-2\*; sMDH-B1,2\*; mMDH-2\*; MPI+\*; PGDH\*; PGK-1\*; PGK-2\*; PK-1\*; PEPA\*; PEPB-1\*; PEPD-1\*; PEPD-2\*; PGM-2\*; mSOD-1\*; sSOD-1\*; TPI-1\*; TPI-2\*; TPI-3\*; and TPI-4\*).



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Fig. 2. Principal component analysis of genetic distances on 43 allozyme loci in 10 populations of chinook salmon from Kamchatka peninsula (the same set of populations and loci as on Figure 1).



Fig. 3. Stock composition (%) of collections from chinook salmon mixed-fisheries in the Russian Economic Zone in May-July of 1998.



5 - Northwestern Kamchatka, 6 - Western Kamchatka, 7 - Southeastern Kamchatka,
 8 - Kamchatka River Drainage.

Fig. 4. Stock composition (Oregon, Alaska, Kamchatka) in May, June and July (to the left), and in immature and mature (to the right) chinook salmon collections from mixed-fisheries in the Russian Economic Zone in 1998.



### Scale Criteria Identify Chum Salmon, *Oncorhynchus keta* (Walbaum) Stocks in Gillnet Catches within Economic Zone of Russia

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Keywords: Stock identification, chum salmon, scale pattern analysis

In our study, stock identification of chum salmon was accomplished based on the scale structure criteria in the first year of growth according to techniques described by Davis et al. (1990) and Patton (1998). Fifteen variables were measured including: the number of sclerits for the first year of growth, the total radius of the first year growth zone, the total radius of the first year growth zone divided by two, the triplets of intersclerital distances from the first sclerit (six measures), and the retrospective triplets of intersclerital distances from the last winter sclerit of the first oceanic period of the growth (six measures).

The data baselines were created for the fish of 0.3 and 0.4 ages from the rivers of East and West Kamchatka, Sakhalin, Japan, and North America for the period of 1997–2000 (see Fig. 1 for location of sample collections). Scale samples were also collected from marine catches of chum salmon fisheries with the economic zone of Russia (Fig. 2).

**Fig. 1.** Sampling area of the chum salmon scale standards, used for the basis lines in 1997-2000: 1 – Kukhtuy River; 2 – Tauy R.; 3 – Nayakhan R.; 4 – Krutogorova R.; 5 – Kikhchik R.; 6 – Utka R.; 7 – Bolshaya R.; 8 – Tym R.; 9 – Kalininka R.; 10 – Tokushibetsu R.; 11 – Yurappu R.; 12 – Gakko R.; 13 – Tokachi R.; 14 – Abashiri R.; 15 – Avacha R.; 16 – Zhupanova R.; 17 – Kamchatka R.; 18 – Khaylula R.; 19 – Apuka R.; 20 – Yukon R.; 21 – Big Eddy R.; 22 – Kuskokwim R.; 23 – Nushagak R.



Fig. 2. The scheme of Russian (A) and Japan (B) gill-net fishery areas within Russian Federation economic zone.



Cluster analysis revealed the substantial differences among regional groups of stocks such as West and East Kamchatka, Sakhalin, Magadan, Japan and Alaska. Russian stocks, mostly from Kamchatka and North Okhotsk Sea, dominated the catches in all years. North Okhotsk Sea chum salmon dominated in May. Later their percentage decreased while chum salmon from Kamchatka Peninsula dominated. The frequency of Sakhalin chum salmon was insignificant. Chum salmon originating from Japan were found in catches over the whole period of the fisheries, their frequency being largest in August. American chum salmon were found in the catches in small numbers.

Based on the frequencies of principal local chum salmon stocks in these gillnet catches, it is reasonable to conclude that stocks of North Okhotsk Sea and Kamchatka have the earliest run timing in the fishery. In May and June the contribution of these stocks was in range of 70–90% (Fig. 3, 4). Relative abundance of Japanese chum salmon for the same period was in range of 10–20% and increased during August. The distribution of stocks revealed by these scale criteria was similar to that described by genetic analysis (Varnavskaya, 2001).

**Fig. 3.** Frequency of the principal chum salmon local stocks in gill-net catches in Karaginskaya subzone - 61.02.1 (district N 1) (I), Petropavlovsk-Commanders subzone - 61.02.2 (II), Pacific subzone - 61.03.1 (district N 3) (III) in 1999.

**Fig. 4.** Frequency of the principal chum salmon local stocks in gill-net catches in Karaginskaya subzone - 61.02.1 (district N 1) (I), Petropavlovsk-Commanders subzone - 61.02.2 (II), Pacific subzone - 61.03.1 (district N 3) (III) in 2000.



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# Stock Identification of Chum Salmon by Mitochondrial DNA Sequence Analysis

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Keywords: Chum salmon, mitochondrial DNA, haplotypes, genetic stock identification

Polymorphic nucleotide sites were found in about 500 bp sequence from the 5' end of the control region of the mitochondrial (mt) DNA of chum salmon (*Oncorhynchus keta*) (Sato et al. 2001). The observed nucleotide sequence variation has so far defined 30 mtDNA haplotypes of three genealogical groups (clade A, B and C) in more than 2,100 individuals representing 48 populations from Japan, Korea, Russia, and North America including Alaska, British Columbia and Washington (Sato et al. in press). The observed haplotypes were mostly associated

with geographic regions, in that clade A and C haplotypes characterized Asian populations and clade B haplotypes distinguished North American populations (Fig. 1).

The haplotype diversity was highest in the Japanese populations (0.64), followed by Russian (0.43) and North American populations (0.34), suggesting a greater genetic variation in the populations of Japan than those of the other two regions. The AMOVA, contingency chi-square test and pairwise population  $F_{ST}$  estimation showed a distinct genetic differentiation among Japanese, Russian and North American populations (Sato et al. in press). In addition, a moderate but significant differentiation was suggested in the populations within Japan and among northwestern, central and southeastern Alaska, British Columbia and Washington in North America (Fig. 2). The populations from Northwest Alaska were found to be genetically distant from other North American populations but closer to Russian populations (Sato et al. in press).

Using the above mtDNA data as a baseline, SPAM estimation was performed for regional stock contribution in the mixed ocean chum samples. In summer, Japanese and Russian stocks were nearly 100% in the central Bering Sea (Fig. 3A), whereas North American stocks were predominant in the Gulf of Alaska, particularly in the north (about 94%) (Fig. 3B). In the central North Pacific Ocean, Russian stocks were more abundant than Japanese and North American stocks (Fig. 3C). **Fig. 1.** A. Geographical distribution of the three mtDNA haplotype lineages for three regions of chum salmon in the Pacific Rim. Dots indicate the sampling locations in the Pacific Rim. B. Parsimony network for genealogy of the 30 mtDNA control region haplotypes (481 bp) of chum salmon with geographical association. J: Japan, R: Russia, and A: North America.





The present results suggest that mtDNA sequence analysis will become a useful means for genetic stock identification of chum salmon (Abe et al. 2002) with improvement of the baseline data by incorporation of more populations from Russia and North America.

**Fig. 2.** Genetic differentiation of 48 chum salmon populations among or within regions inferred from AMOVA (analysis of molecular variance, Sato et al., in press). Dots indicate the sampling locations in the Pacific Rim. Degree of differentiation is shown by the percent of variation with indicated statistical support.



**Fig. 3.** Stock composition of chum salmon estimated by the SPAM program on mtDNA haplotype data. A. Chum salmon collected in the Bering Sea (178°W 56°N) July 2000 (50 fish) and 2001 (64 fish). B. Chum salmon collected in the North (145°W 56°N, 45 fish) and South station (145°W 50°N, 79 fish) of the Gulf of Alaska July 2000. C. Chum salmon collected in the central North Pacific Ocean (180° 41°N–46°N, 60 fish) July 2000.







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# Identification of Two Ecological Forms of Chum Salmon by Analyzing Microstructure of Otoliths

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Keywords: Chum salmon, otoliths, run timing

We analyzed otoliths (sagitta) of chum salmon caught in 2002 in the Taui River, the largest river of the Taui Gulf on the continental coast of the Sea of Okhotsk. While conducting biological analyses, we sampled otoliths from 818 individuals of chum salmon caught with a drag seine during controlled test fishing. Otoliths were removed during biological analysis and stored dried until processing. Some otoliths were rejected due to indistinct micro-increments (aberrant otoliths). Thermoplastic cement (Buehler, USA) was used to prepare sections. Sections were polished with "Mark-52" polisher (USA) using aluminum oxide abrasive paper disks with different particle sizes (Struers, Copenhagen/Denmark). Otoliths were sectioned along the center of growth, polished, and examined under a microscope. Sections were turned over when necessary and polished from the other side. Sections were examined with a Galen-3 microscope in transmitted light with magnifications 10x10, 10x20, 10x40, 10x100.

Analysis of otolith patterns of chum during anadromous migration permits not only division of the entire population into hatchery released and wild fishes, but also identification of local groups within wild populations.

For this purpose the most descriptive information is the analysis of otolith patterns formed during fresh-water ontogenesis up until the switch to exogenous feeding.

Based on analysis of otolith sections (sagitta) made along the sagittal plane through the core, we divided chum population of Taui River into two groups – group A and group B. Group A is characterized by a large number of similarly grouped increments (Fig. 1) with high optical brightness in transmitted light. Group B consists of individuals with otolith zone, corresponding to embryonic period, characterized by different combinations of hyaline and opaque rings. Groups of increments in their otoliths are characterized by diffuseness and lower optical brightness. Only eye pigmentation and hatching rings can be distinctly identified (Fig. 2).

Statistical analysis of biological characteristics of individuals of both groups showed distinct differences in weight and linear parameters (Figs. 3 and 4)..

Analysis of age and sex composition of both groups of chum salmon showed a significant difference between them. Males prevailed in group A (53.4%), females – in group B (51.6%) (Fig. 5).

As you can see from Fig. 6, both groups are represented by fish of three ages, however the relative number of fishes in each age group is different.

Group A is characterized by an almost even distribution of fishes by ages with a slightly larger quantity of the oldest fish. At the same time, group B is characterized by significant differentiation with a peak number of individuals at the age of 3 (48.96%) and a minimum number of fish at the age of 5 (11.34).

**Fig. 1.** Otolith pattern No. 1 - otolith of individual belonging to group A.



**Fig. 2.** Otolith pattern No. 2 – otolith of individual belonging to group B.



**Fig. 3.** Fork length of chum in groups A and B caught in Taui River.



Fig. 5. Sex composition in groups A and B.



**Fig. 4.** Weight of chum salmon in groups A and B caught in Taui River.







Differences in groups A and B were apparent not only for morphological parameters but also for spawning dynamics. Fish of group A prevailed in the beginning of spawning (69.57%). Then the proportion of fish in both groups became equal. By the middle of spawning, the proportion of fish in group A declined to a minimum and remained at that level until the end of anadromous migration (Fig. 7).

The preceding data support the assumption that two groups of chum salmon, differentiated by otolith microstructure, belong to different ecological forms. Based on previous studies of chum salmon of Taui River (Volobuev 1983; Mednikov 1988; Volobuev 1990, 2001), these groups of chum salmon can be classified as early (group A) and late (group B) forms.

Differences in visualization of increments are caused by the fact that these ecological forms reproduce at different types of spawning grounds: early chum salmon are spawning in river channels, and the late form of chum salmon reproduces in spring-fed grounds (Volobuev 1990). Hydrological conditions of spawning grounds determine differences in length of a time period required for embryonic and larval development. High water

temperature of the spawning grounds of the early form of chum salmon in summertime  $(9-13^{\circ}C)$  was the reason for higher rate of development in the early stages of embryogenesis. This is very different from the late form of chum salmon which reproduces in spawning grounds with water temperature ranging from 7 to 8°C.

Analysis of microstructure of otoliths divides chum salmon populations into ecological forms. Large numbers of similarly grouped increments with high optical brightness on otolith section of individuals indicate that they belong to the early form of chum salmon. Chum salmon individuals belonging to a certain ecological form can be identified by applying the above method at any stage of ontogenesis. Fig. 7. Spawning dynamics in both groups of chum salmon in Taui River.



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## Juvenile Chum Salmon in the Okhotsk Sea: Their Origins Estimated by Genetic and Otolith Marks

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Keywords: Juvenile chum salmon, Okhotsk Sea, genetic stock identification, otolith mark

Juvenile chum salmon (*Oncorhynchus keta*) are abundant in the Okhotsk Sea during the summer and fall (Ueno 1997; Melnikov et al. 1999a, 1999b; Lapko and Glebov 2001; Volvenko 2003). A genetic stock identification (GSI) study suggested that Japanese stocks dominated juvenile chum salmon catches in the southern Okhotsk Sea in the fall of 1993 (Urawa et al. 1998, 2001), while Russian stocks dominated in the southwestern water during the fall of 2000 (Urawa et al. 2003).

Thermal and dry markings of salmonid otoliths have been well developed as a reliable tool to determine the hatchery origin of salmon. In the spring of 2002, approximately 44 million thermally-marked chum salmon fry were released from 5 hatcheries in Japan (Kawana et al. 2002), and 18 million chum fry with dry or thermal marks were released from 5 Russian hatcheries along the Okhotsk Sea coast (Akinicheva and Rogatnykh 2002).

In the present study, the origins of juvenile chum salmon caught in the Okhotsk Sea in 2002 were determined by using genetic and otolith marks. Fish were caught at 27 stations (45–55°N and 146–152°E) by a surface trawl net (1 hour) of R/V *Torishima* in October 2002. The fork length, body weight and gonad weight of each fish were recorded, and scales were removed for the age determination. The sagittal otoliths, muscle, heart, and liver were collected from each fish. The sagittal otoliths were dried and kept in cell well plates until the detection of otolith makers. The other tissues (muscle, heart, and liver) were immediately frozen in -80°C freezer for genetic analysis.

Samples were examined for protein electrophoretic variation on horizontal starch gels using standard procedures described by Aebersold et al. (1987). Alleles were compared and standardized for 20 polymorphic loci. We used Asian baseline data set (43 stocks/20 loci) collected by Winans et al. (1994), Wilmot et al. (1998) and Urawa et al. (2003). Estimates of stock contributions were made with a conditional maximum likelihood algorithm (Pella and Milner 1987) using the Statistics Program for Analyzing Mixtures (SPAM version 3.5, Debevec et al. 2000). Standard deviations and 90% confidence intervals were estimated by 1000 bootstrap resamplings of the baseline and mixture samples. Estimates were made to individual stocks and then pooled to regional stock groups: Japan, Sakhalin, Premorye, Amur River, and northern Russia (Magadan/Kamchatka/Anadyre).

The left sagittal otoliths were mounted on slide glasses using thermoplastic cement, and then ground to expose the primordia. If the left sagittal otoliths were not available, the right sagittal otoliths were used. Otolith microstructures were observed under a light microscope, and the microstructure patterns were compared to the thermal mark patterns of voucher specimens collected from hatcheries before releases.

A total of 2,776 juvenile chum salmon were caught by 27 trawls. Fish were relatively abundant in waters between 50°N and 53°N, where the sea surface temperature (SST) ranged between 7°C and 9°C. The regional stock composition estimates of juvenile chum salmon was 37.6% Japan, 6.6% Sakhalin, 0.6% Premorye, 4.2% Amur River, and 49.7% northern Russian stocks. The estimated stock composition was apparently different among the catching locations. The percentage of Japanese stocks was high in southern water, but low in northern water. The northern Russian stocks showed the opposite trends in their distribution. Sakhalin and Amur River stocks appeared in the western water.

Nineteen otolith marked fish released from 3 Japanese (Chitose, Shizunai and Ichani in Hokkaido) and 3 Russian (Bereznykovsky and Sokolovsky in Sakhalin, and Ozerki in western Kamchatka) hatcheries were found from juvenile chum salmon caught in the Okhotsk Sea. Japanese marked fish (n=14) were widely distributed in the

waters south of 53°N. We are the first to document that Japanese chum salmon juveniles migrate even from the Pacific coast (Shizunai Hatchery) to the Okhotsk Sea. Four otolith marked fish released from two hatcheries in southern Sakhalin were caught in the western water near the island.

The Okhotsk Sea is indispensable for Asian chum salmon as a feeding ground during the early ocean life. It is important to continue the monitoring program for juvenile salmon in the Okhotsk Sea using stock identification and abundance estimate techniques in order to understand the population dynamics of Asian chum salmon.

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# Hatchery and Wild Stock Interactions of Juvenile Chum Salmon in Marine Waters of Southeastern Alaska: A Bioenergetics Approach

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Keywords: Bioenergetics, juvenile chum salmon, hatchery interactions, southeastern Alaska

As part of the Southeastern Alaska Coastal Monitoring project, the interactions of hatchery and wild stocks of juvenile chum salmon (*Oncorhynchus keta*) migrating seaward were studied in littoral (nearshore) and neritic (epipelagic offshore) marine habitats in southeastern Alaska. Bioenergetics modeling was used to estimate prey consumption by different salmon stock groups during their first five months at sea. Model runs were completed using biophysical data collected in Icy Strait, a regional salmon migration corridor, in May, June, July, August, and September of 2001 (Fig. 1). These data included: temperature (1-m surface versus surface to 20-m average), zooplankton standing crop (surface to 20-m depth versus entire water column), salmon diet (percent weight of prey type consumed), and energy densities, weight, and growth of juvenile chum salmon. Literature values were used for energy densities of salmon prey items. Known numbers of hatchery releases were used in a cohort reconstruction model to estimate total abundance of hatchery and wild chum salmon in the northern region of southeastern Alaska assuming average survival to adults, and for two different (low and high) early marine littoral mortality assumptions.

Total prey consumption was relatively insensitive to temperature differences associated with the depths potentially utilized by juvenile chum salmon. However, the magnitude and temporal pattern of total prey consumed differed dramatically between the low and high mortality assumptions (Fig. 2). Daily consumption rates from the bioenergetics model and juvenile salmon densities from Icy Strait were used to estimate amount and percentage of zooplankton standing crop consumed by hatchery and wild chum salmon (Table 1). We estimated that only a small percentage of the available zooplankton was consumed by juvenile chum salmon, even during peak abundances of hatchery and wild fish in July (Fig. 3). Under the modeling assumptions, these results indicate that current levels of hatchery production in southeastern Alaska do not represent a significant impact on the prey resource available to wild chum salmon stocks in neritic marine habitats represented by the Icy Strait migration corridor. As with any modeling exercise, model outputs can be misleading if input parameters and underlying assumptions are not valid; therefore, additional studies are warranted, especially to refine physiological input parameters specific to juvenile chum salmon.

Fig. 1. Habitats sampled within the Icy Strait study area (northern region of southeastern Alaska), from May to September 2001. Three primary chum salmon hatcheries in the region are identified (Macaulay, Hidden Falls, and Gunnuk Creek). Principal migration routes to the Gulf of Alaska are indicated with dashed lines.



**Fig. 2.** Total zooplankton consumption estimated for wild and hatchery stocks of chum salmon, based on bioenergetics model runs of two simulated early littoral mortality rates and two neritic thermal conditions in the northern region of southeastern Alaska, May to September, 2001.



**Fig. 3.** Estimates of zooplankton standing crop and consumption by juvenile chum salmon in the neritic habitat of Icy Strait, Alaska, for June, July, August, and September, 2001. Panel a) shows two estimates of zooplankton standing crop at two different sampling depths, overlaid by juvenile chum salmon density estimates. Panel b) shows the percentage of available zooplankton consumed by unmarked and hatchery stocks of chum salmon for the two estimates of zooplankton standing crop. Detailed stock-specific consumption rates are shown in **Table 1**.



**Table 1.** Stock-specific estimates of zooplankton consumption by juvenile chum salmon in the neritic habitat of Icy Strait in June to September, 2001. Stock-specific consumption rates are from model runs. Total weight of juvenile chum salmon stock groups is based on stock compositions, date-specific weights, and salmon density estimates. Total consumption of zooplankton by juvenile stock groups is based on the stock-specific consumption of the total weight of the stock groups. The abbreviations for chum salmon stocks are: unmarked (UM), Macaulay hatchery (MC), and Hidden Falls hatchery (HF).

| Neritic period | Stock-s<br>(g pr | specific const<br>ey/g predato | umption<br>r · d <sup>-1</sup> ) | Total wt of<br>stock | juvenile chur<br>k groups (g/kr | n salmon<br>m²) | Total consumption of<br>zooplankton by juvenile stock<br>groups (g/km <sup>2 ·</sup> d <sup>-1</sup> ) |       |       |  |
|----------------|------------------|--------------------------------|----------------------------------|----------------------|---------------------------------|-----------------|--------------------------------------------------------------------------------------------------------|-------|-------|--|
|                | UM               | MC                             | HF                               | UM                   | MC                              | HF              | UM                                                                                                     | MC    | HF    |  |
| Late June      | 0.0746           | 0.0909                         | 0.0652                           | 2,843                | 4,322                           | 0               | 211.5                                                                                                  | 393.1 | 0.0   |  |
| Late July      | 0.0843           | 0.0867                         | 0.1048                           | 13,240               | 1,112                           | 5,253           | 1,116.2                                                                                                | 96.3  | 550.3 |  |
| Late August    | 0.0964           | 0.0837                         | 0.0963                           | 7,391                | 503                             | 678             | 712.8                                                                                                  | 42.1  | 65.3  |  |
| Late Sept.     | 0.0701           | 0.0628                         | 0.0702                           | 4,514                | 0                               | 190             | 316.5                                                                                                  | 0.0   | 13.3  |  |
| Total          |                  |                                |                                  |                      |                                 |                 | 2,356.9                                                                                                | 531.5 | 628.9 |  |

# Emerging Baselines to Estimate the Migration Patterns of Dolly Varden Charr Nearshore and on the High-seas

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Keywords: Dolly Varden, microsatellites, mitochondrial DNA, mixed-stock analysis, Salvelinus malma

Dolly Varden (*Salvelinus malma*) is a dominant freshwater species in arctic and subarctic eastern Russia, Alaska, and western Canada and an important food source for indigenous people in these regions. Anadromous Dolly Varden are distributed throughout Alaska and occupy a wide variety of habitats, from coastal streams in the southeast panhandle to rivers draining the North Slope. Anadromous Dolly Varden show highly complex migration patterns. They typically feed in marine waters in the summer, home to spawn in their natal streams, and overwinter in natal and non-natal freshwater lakes and rivers in mixed aggregations.

Two subspecies have been described in North America. The northern form (*S. m. malma*) is distributed from the Mackenzie River to the north side of the Alaska Peninsula, while the southern form (*S. m. lordi*) is distributed from the south side of the Alaska Peninsula to Puget Sound. The forms appear to differ greatly in their marine migrations in Alaska. The median migration distance reported in studies of southern form Dolly Varden is less than 60 km with the longest documented migration of 250 km (Bernard et al. 1995). Longer migration distances have been reported for northern form Dolly Varden. Dolly Varden spawning and overwintering in Beaufort Sea drainages routinely travel distances of 100 to 350 km during summer feeding migrations (Krueger et al. 1999 and references therein). Dolly Varden tagged in the Wulik River north of Kotzebue Sound have been recovered not only in other rivers of Kotzebue Sound, but also in Norton Sound, St. Lawrence Island, and several locations in the Russian Far East, migrations ranging from 150 km to 1,690 km (DeCicco 1992, 1997). Differences in migration patterns between the forms may be due to frequency of spawning, reliance on lakes for overwintering, and other life history differences.

Migration patterns have typically been evaluated using tagging and radio telemetry studies. Our objectives are to develop datasets based on microsatellites and mitochondrial DNA markers to estimate population structure in Alaska and use mixed stock analysis (MSA) to identify the origin of Dolly Varden sampled from overwintering areas, coastal catches, and offshore locations to evaluate population specific migration patterns.

Dolly Varden have been collected from 37 locations in Alaska (Fig. 1). These collections are being assayed for genetic variation at seven microsatellite loci (*Sma-3*, -5, -10, -17, -21, -22, and -24). A subset of 20 populations (N = 10) is being assayed for genetic variation in three segments of the mitochondrial DNA mtDNA) genome (cytochrome b, ND1/2, and ND5/6) using 14 enzymes.

Fig. 1. Locations of Dolly Varden populations sampled for genetic analysis in Alaska.



Preliminary results are presented for eight populations analyzed for both marker types: Kongakut River, Saviukviak River, Kivalina River, Kelly River, Cobblestone River, Sinuk River, Solomon River, and Kashaiak River (Fig. 1, closed circles). Microsatellite loci were highly polymorphic; average observed heterozygosity was 0.684 and the average number of alleles per locus was 20. Fourteen mtDNA haplotypes were observed. Haplotype diversity was 0.532 and nucleotide diversity was 0.002. In a multidimensional scaling of genetic distances calculated from microsatellite data, four population clusters were apparent: the North Slope (Kongakut and Saviukviak Rivers), Kotzebue Sound (Kivalina and Kelly Rivers), Norton Sound and Imuruk Basin (Cobblestone, Sinuk, and Snake rivers), and Bristol Bay (Kashaiak River) (Fig. 2), indicating that genetic relationships follow geographic proximity as well as life history variation. Dolly Varden in Norton Sound drainages south to the Alaska Peninsula are smaller than those found in Kotzebue Sound and Beaufort Sea drainages, and their movement patterns for feeding follow the movements of Pacific salmon (DeCicco and Reist 1999). Few differences in haplotype frequencies were detected among the northern form populations though large differences between southern and northern form populations were observed. MtDNA data collected in this study can be merged with mtDNA survey for Asian Dolly Varden (Oleynik et al. in press).

A simulation analysis was conducted in SPAM version 3.7 (Debevec et al. 2000) to test whether allele frequency differences among collections were large enough for MSA. Rare alleles were pooled and a Bavesian estimator of baseline allele frequencies was used to account for sampling error. Conditional maximum likelihood estimates of populations contributions were made for 1,000 artificial mixes (n = 400) from a single population so that mean contribution estimates should equal 100%. Mean contribution estimates approximated 90% for all populations (Fig. 3). Individual assignment tests were conducted using the direct classification method in Geneclass (Cornuet et al. 1999) because incidence of Dolly Varden in coastal waters is likely to be low. The percent of individuals classified to the correct region was approximately 80% for individuals from the North Slope, Kotzebue Sound, and Norton Sound and 70% for the Kashaiak River.

Significant global warming is predicted in this century; the greatest warming is expected at high latitudes. Warming may cause profound changes in Dolly Varden life history phases including changes in movement patterns in marine waters as ocean productivity and fish metabolism alter and location and stock composition in overwintering areas as stream discharge and freeze cycles alter. Genetic methods will provide stock-specific information to aid in assessing predicted changes in marine and freshwater habitat use. **Fig. 2.** Multidimensional scaling analysis of genetic distances calculated from allele frequencies for seven microsatellite loci for eight populations of Dolly Varden in Alaska.



**Fig. 3.** Mean contribution estimates for 1000 artificial mixtures created from individual populations of Dolly Varden.



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# The Quality of Marks Obtained under Different Regimes of Salmon Otolith Marking

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The method of creating otolith marks using specific regimes of rearing water temperature, feeding and photoperiod has been described in many works (including Brothers 1984, 1985; Volk et al. 1987; Brothers 1990; Akinicheva and Rogatnykh 1997). This method allows the creation of marks during the period of juvenile growth in the hatchery, and has been used for the purpose of identification of large managed stocks of fish produced by artificial reproduction.

Thermal marks have been created through rearing the embryos and larvae under the temperature that are periodically different from the background, i.e. from water temperature supplied before the time of marking. Decreased water temperature results in slowing down calcium metabolism in fish creating a clear dark stripe. Returning to the background temperature restores the otolith calcium sedimentation rate where every new stripe becomes quite wide to frame out the next doubled dark stripe obtained via the next water temperature decrease (Akinicheva and Rogatnykh 1997). The perfect case for marking is when two water supply systems occur in a hatchery - «cold» and «warm» with a difference no less than 3°C. Total (100%) marking is possible if warming occurs in the hatchery.

Malkinsky Salmon Hatchery (Bolshaya River basin, West Kamchatka) uses thermal water from Malkinsky geothermal outfall for the purpose of warming the cold water from the river. Hatchery incubation and rearing the fry and juveniles usually takes place under the higher water temperature that are never observed in nature (+7-10°C).

Thermal otolith marking has been used in Malkinsky Hatchery since 1995 (Vasylkov 1995, 1996). The schemes of marking suggested in Figs. 1, 2 were used on sockeye and chinook salmon in order to create a mark resulting from an 8-hour period water temperature decrease. The image of the mark for 1995 looks like III I II, and for 1996 - II I III.

Since the schemes were worked out without taking into consideration a circadian rhythm, the mark observed in adult sockeye that returned to the hatchery was of poor-quality (Fig. 3).

Fig. 1. The scheme of chinook and sockeye salmon marking in Malkinsky Salmon Hatchery in 1995.

| 10ºC | 1  | 40h |    | 40h |    | 88h |    | 88h |    | 40h |    | Background temperatures |
|------|----|-----|----|-----|----|-----|----|-----|----|-----|----|-------------------------|
| 5°C  | 8h |                         |

Fig. 2. The scheme of chinook and sockeye salmon marking in Malkinsky Salmon Hatchery in 1996.

| 10ºC | 1  | 40h |    | 88h |    | 88h |    | 40h |    | 40h | Background temperatures |
|------|----|-----|----|-----|----|-----|----|-----|----|-----|-------------------------|
| 5°C  | 8h |     |                         |

**Fig. 3.** The photos of otolith mark from sockeye salmon in the return to Malkinsky Hatchery (a - marked area - ocular 10x/22, objective 10x/0,25; b - the mark of 1995, ocular 10x/22, objective 20x/0,40; c - the mark of 1996, ocular 10x/22, objective 40x/0,65).



Since 1997 the scheme of marking has been changed (Fig. 4). Periodic temperature decreases were carried out for 48 hours. As a result a mark of a high quality in the otoliths of sockeye and chinook salmon has been formed, and consisted of two blocks: first block-2 stripes, second block-3 stripes. The image of the mark in 1997 was II III. The photos of otoliths are shown in the Fig. 5.

High-quality marks can be seen in the standards of juvenile sockeye salmon otoliths sampled at release in 1998, and in adult fish in the returns of 2000 and 2001. The scheme of marking is shown in Fig. 6. The water temperature was decreased periodically during a 24 hour period. The marks of 1998 looks like III II.

The photos of the juvenile sockeye salmon standard otolith mark, and of the mark recovered in adult fish in the return are shown in Fig. 7. It is clear from the figure that the regime of a periodic 24-hours temperature decrease for sockeye salmon provides a high-quality (reliable) mark. There were no marks from 1998 found in adult chinook salmon otoliths, although the mark can be recognized in juvenile otolith at significant magnification (Fig. 7).

In 1999, juvenile chinook and sockeye salmon were marked according to the scheme shown in Fig. 8. The mark from 1999 looked like I I III. Clear marks were observed in neither standard juvenile or in adult fish.

Fig. 4. The scheme of chinook and sockeye salmon marking in Malkinsky Salmon Hatchery in 1997.

| 11⁰C |     | 48h |     | 96h |     | 48h |     | 48h |     | Background temperatures |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------------------------|
| 5°C  | 48h |                         |

Fig. 5. Photos of otolith marks from sockeye salmon in the return to Malkinsky Hatchery (a - the mark of 1997 in sockeye salmon otolith - ocular 10x/22, objective 40x/0,65; b - the mark of 1997 in chinook salmon otolith - ocular 10x/22, objective 20x/0,40; c - the mark of 1997 in chinook salmon otolith - ocular 10x/22, objective 40x/0,65).



Fig. 6. The scheme of chinook and sockeye salmon otolith marking in Malkinsky Hatchery in 1998.

| 8°C | 1   | 48h | 48h | 96h | 96h | Background temperatures |
|-----|-----|-----|-----|-----|-----|-------------------------|
| 5°C | 24h | 24h | 24h | 24h | 24h |                         |

Fig. 7. Photos of Malkinsky Hatchery chinook and sockeye salmon otolith mark, ocular 10x/22, objective 40x/0,65 (a - standard chinook salmon otolith, b - standard sockeye salmon otolith, c - the otolith of adult sockeye salmon).



Fig. 8. The scheme of chinook and sockeye salmon marking in Malkinsky Salmon Hatchery in 1999.

| 10°C | 1   | 96h | 96h |     | 48h |     | 48h |     | Background temperatures |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-------------------------|
| 5°C  | 24h | 24h |     | 24h |     | 24h |     | 24h |                         |

Until 2000 the marking of Malkinsky Hatchery salmon was carried out in late March and April when juvenile sockeye and chinook salmon had begun feeding. According to Akinicheva and Rogatnykh (1997) the results of several experiments carried out on juvenile, larval and embryonic salmon show that the best period in ontogenesis for creation of thermal marks in the otolith structure is the period when a clear nucleus area has been formed. Thermal marks in this area are clearly seen. A stable working calcium metabolism rhythm is established in the otolith of actively swimming juvenile fish, and they are not effected by environmental factors. Therefore, the mark cannot be recognized among the number of bright and contrast rings. However, the marks of all previous years have been observed in the otoliths of adult sockeye salmon returning to Malkinsky Hatchery for 2000–2001, with the exception of the mark from 1999. In the otoliths of adult chinook salmon the mark from 1997 is easily recognized.

In 2000 the work on thermal otolith marking of Malkinsky sockeye and chinook salmon was continued. Sockeye salmon marking was carried out at the eyed stage according to the scheme of background temperature increases with a 24-hours periodicity (Fig. 9), and there were  $770 \times 10^3$  sockeye salmon individuals marked. The mark of 2000 was III III. There were  $485 \times 10^3$  chinook salmon individuals marked as before. The marking was carried out at the passive embryo stage via background temperature decrease to  $4.6^{\circ}$ C according the scheme shown in Fig. 10. Water exchange in the basins and in the incubators took one hour. The mark formed in the otolith in 2000 was III IIII. Photos of standard chinook and sockeye salmon juvenile otoliths are represented in the Fig. 11. In conclusion, the thermal marks in otoliths of chinook and sockeye salmon show the best contrast in the clear area, i.e. within the eyed stage and the passive embryo stage. However, the variations of the period of temperature decreases (up to 48 hours) can provide high quality marks at later stages of juvenile salmon development.

Fig. 9. The scheme of Malkinsky Hatchery sockeye salmon otolith marking in 2000.

| 4.6°C |     | 24h | 2   | 24h | 48h |     | 24h |     | 24h | Background   |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------------|
| 7.5°C | 24h |     | 24h | 24h |     | 24h |     | 24h | 24h | temperatures |

Fig. 10. The scheme of Malkinsky Hatchery chinook salmon otolith marking in 2000.

| 7.5 °C |     | 24h | 24  | 4h  | 48h | 2   | 24h | 24h |     | 24h |     | 24h |     | Background   |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------------|
| 4.6 °C | 24h |     | 24h | 24h |     | 24h | 24h |     | 24h |     | 24h |     | 24h | temperatures |

**Fig. 11.** Photos of the mark of 2000 in Malkinsky Hatchery chinook and sockeye salmon otoliths, ocular 10x/22, objective 40x/0,65 (a - standard chinook salmon otolith, b - standard sockeye salmon otolith).



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# Strontium Chloride (SrCl – 6H<sub>2</sub>O) as a Mass-Marker for Salmonid Otoliths in Alaska

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Keywords: Otolith, strontium, mass-marking

As the number of thermally marked salmonids released from hatcheries increase, fewer mark patterns become available, and the risk of repetitive mark releases increases. Consequently, there is a need to develop alternative, reliable, and cost-effective methods of mass-marking hatchery-raised salmon. We evaluated the usefulness of strontium chloride hexahydrate (SrCl - 6H<sub>2</sub>O) to mass-mark salmonid otoliths in Alaska. In 2000, emergent sockeye fry raised at the Gulkana Hatchery, Prince William Sound, Alaska, were exposed to dilute concentrations of SrCl (500, 1,000 and 3,000 ppm) for a variety of durations (2, 4, 8, 12, and 24 hours) to determine which protocol resulted in a clear, unambiguous mark. Voucher specimens were collected spring 2001 from each mark group and held for two weeks in collection boxes to allow additional growth to occur on the otolith beyond the strontium mark to eliminate the potential for edge effects that may occur while viewing the mark.

Approximately 20 fry were sampled from each mark group, preserved in 90% ethanol, and sent to Alaska Department of Fish and Game's Mark, Tag, and Age Laboratory in Juneau, Alaska for dissection and otolith preparation. Approximately 1 million fry exposed to strontium chloride for 24 hours at 3,000 ppm were released into Paxson, Crosswind and Summit Lakes. All remaining fry were destroyed. Adults are expected to be recovered as part of the Copper River salmon fishery beginning in the summer of 2003. Otoliths were removed from fry and mounted directly onto petrographic glass slides with clear thermoplastic cement. Only left sagittal otoliths were mounted. Right sagittae were stored dry to determine if the mark could be detected without any kind of sample preparation (e.g. grinding and polishing). All mounted otoliths were ground down to the primordia using 9-micron aluminum oxide lapping film, then finished with three and one micron polishing film to eliminate scratches. During this preparation process, the smoothness of the otolith's surface was evaluated using a Leica reflected light microscope. Once polished, specimens were examined for mark presence using an FEI Quanta 600 environmental scanning electron microscope (ESEM) capable of operating at high pressure (low vacuum) that employed backscatter electron detectors (BSE) as the primary imaging source.

Fry exposed to the highest concentration (3,000 ppm) for the longest duration (24 hours) experienced minimal stress and average survival rates. Mortality did not exceed 0.8%, which was considered to be an average rate of mortality at the Gulkana Hatchery. During growth, strontium displaced calcium in structures made of calcium carbonate (CaCO<sub>3</sub>) to form strontium carbonate (SrCO<sub>3</sub>). Sequential exposure produced a series of strontium carbonate bands in otoliths that were similar to those patterns produced using thermal marking procedures. The higher molecular weight strontium layer scattered a greater number of electrons than the surrounding matrix and was consequently displayed as a bright band against the darker lower molecular weight calcium of the otolith (Fig. 1). Strontium rings were only visible in polished specimens. The surface topography of whole, unprepared otoliths scattered the electron beam to such a great degree that the strontium mark was not detectable. Mark recovery procedures indicated exposure duration was more important than concentration and that modern, low vacuum ESEMs with BSE had high enough sample processing rates to support mark recovery efforts while a fishery was in progress. From a purely subjective standpoint, otoliths exposed to at least 1,000 ppm of SrCl for 24 hours consistently exhibited the clearest, most unambiguous marks (Fig. 1). Advantages of strontium for mass-marking include 100% effectiveness, relatively short exposure times, generation of marks that can be detected in the otolith any time after hatch, and creation of marks at a reasonable cost, especially under circumstances where thermal marking is impractical. Because strontium marks cannot be detected with a light microscope, the primary disadvantage is the high cost of the ESEM needed for mark recovery. Traditional SEMs operate only at high

vacuum, which cannot be used to examine non-conductive materials without preparation (e.g. conductive coating), both of which increase sample handling times. ESEMs operate at low vacuum and require no sample preparation, which significantly increases sample processing rates. Because strontium occurs naturally in the environment in dilute concentrations, there should be little environmental concern over its handling or disposal.

Fig. 1. Otoliths from sockeye salmon fry exposed to dilute concentrations of strontium chloride for a variety of durations. The bright ring occurring towards the edge of each otolith is the strontium mark.









## Post-Cephalic White Spot Syndrome in Salmonids

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Keywords: Post-cephalic, white spots, melanocytes, anomaly, teratogeneic

This report documents a phenomenon in salmonids involving one or more white spots generally located in the mid-dorsal nape or occipital region immediately posterior of the fishes' head. The syndrome, post-cephalic white spot (PCWS), varies widely among populations. When present it often is found in less than 0.1% of individuals in a population although in some instances occurrence rates can be considerably higher. This anomaly is easily overlooked and often goes unnoticed, especially when viewing a large number of fish. The white pigment spots, only a few millimeters in diameter in juveniles, grow allometrically and are larger in older fish.

I first observed PCWS in juvenile sockeye salmon in 1975 (Fig. 1). These fish were progeny of adults from Nakvassin Creek at the west end of Port Herbert, a 4.8 km fiord on Baranof Island in Southeast Alaska. Adult salmon were collected and taken to the nearby National Marine Fisheries Service Field Station at Little Port Walter where they were spawned as part of a study to evaluate rearing of smolts in estuarine net pens (Wertheimer et al. 1983).

Since first observing PCWS the anomaly has been found to occur in all five species of North American salmon, rainbow trout, Dolly Varden char, and Atlantic salmon. Documentation of these findings was accomplished through careful observations by myself and by the solicited assistance of many other individuals. Much of this information documents the presence of PCWS in 37 different populations of salmonids from Alaska, Washington, Oregon, British Columbia, New Hampshire, Australia, and Hokkaido, Japan. In some instances sufficient data were collected in certain stocks of wild-and hatchery-origin sockeye and coho salmon to provide preliminary estimates on PCWS occurrence rates in the populations (Fig. 2). Occurrence rate data were also collected on eight populations of hatchery-origin chinook salmon smolts from Alaska, British Columbia, and Oregon that ranged from 0.01 to 0.86 percent.

While PCWS is found in all life stages including adults (Fig. 3) it is more commonly seen in juveniles suggesting, when present, the syndrome carries a distinct survival liability. Although it has been observed in both wild- and hatchery-origin salmonids PCWS is more commonly seen in hatchery populations (Fig. 4), perhaps because large number of hatchery-origin juveniles can often be more readily observed than large numbers of wild juveniles. However, in some instances, for example during monitoring of wild sockeye salmon smolts outmigrating from the Hugh Smith Lake system in southern Southeast Alaska, considerable numbers of PCWS juveniles are found (Fig. 5).

Initial histological comparisons of juvenile chinook and sockeye salmon with and without PCWS show subcutaneous differences including a lack of melanocytes in epidermal tissues and the likely presence of purine

crystals in vacuolated area causing the white pigments in dermal tissues (Fig. 6). Pathological examination of sibling sockeye salmon with and without white spots revealed no significant differences although one spacimen without PCWS did have a protozoal gill infection.

A different but perhaps related white pigmented anomaly has also been observed in juvenile chum and coho salmon. In these cases a white slash from the post-cephalic dorsal region traverses downward ventrally toward the pectoral fin. White slashes occur either on the right or left side and are associated with deformation of the pectoral girdle, fin, or operculum (Fig. 7). I have only seen white slash anomalies in hatchery-origin fish. Fig. 1. Hatchery-origin sockeye salmon with post-cephalic white spots from Nakavassin Creek parents.



**Fig. 2.** Occurrence rates of post-cephalic white spots in sockeye salmon above, and coho salmon below.



**Fig. 4.** Tahani River chinook salmon smolts at DIPAC Hatchery near Juneau have at least one post-cephalic white fish in the group. Can you find it?



**Fig. 6.** Cross sections of juvenile chinook salmon: (A) pre-PCWS region showing prominent chromatophores beneath epidermal cell layer (1); and (B) PCWS region showing lack of chromatophores between dermal and epidermal tissues and vacuolated areas in dermal tissue that may contain pruine crystals causing white pigment (2).

Fig. 3. Adult salmon with post-cephalic white spots: left chinook; middle pink; right chum.



Fig. 5. Wild Hugh Smith Creek sockeye salmon smolts with post-cephalic white spots May 19, 1988.



Fig. 7. Perhaps related to post-cephalic white spots, a coho salmon parr with a "white slash" along pectoral girdle. Note deformed operculum.





Causes of either PCWS or white slashes is unknown although some possibilities could include teratogenic malformed anomalies due to exogenous environmental factors causing chromosomal aberrations in one or the other parent, inappropriate mate selection, or interactions of genetic and environmental factors during embryogenesis. Preliminary DNA comparisons of sibling hatchery-origin chinook salmon with and without white spots suggest a genetic linkage; in one instance 7 of 27 fish with white spots from a population of over 218,000 juveniles came from the same parents.

Various literature searches (Dawson 1964) failed to find previous accounts of these phenomena although I am certain others have noted post-cephalic white spots and perhaps the white slashes in salmonids. A popular internet search engine was queried for "white spots in fishes" that yielded thousands of hits. Many hits were associated with the common white spot disease, or ich, caused by a ciliated protozoan. Other references have identified "white spot condition" with abraded skin on salmonids caused from sea lice parasitism (White 1940).

Dorothy Leonard prepared the histological tissue sections, these individuals provided information or observations on PCWS; Jim Cochran, Kent Crabtree, Ted Meyers, Monte Miller, Jerry Koener, Trish Mc Hugh, Al Hemmingsen, Kenneth Johnson, Joe Verret, Diana Tersteeg, Sam Rabung, Dick Crone, Lou Barr, Bill Farris, Lauran Donaldson, D. Asuburner, Hiroshi Kawamura, Adrian Celewycz, Andy Gray, Jeff Hard, Jim Miles, Jerry Taylor, Frank Thrower. I would appreciate hearing from others who may have observations or comments on these phenomena.

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## Identification of Source Populations of Mixture Individuals from their Genotypes

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The source populations of individuals of unknown origin can be surmised from their genotypes. In many applications, the sources for more than a single individual are desired and a list of the c (say) potential source populations is available. When such is the case, misidentifications are minimized by assigning each individual with the maximum *a posteriori* probability (MAP) rule. The MAP rule assigns an individual with genotype X to the population for which the posterior source probability,

(1) 
$$p(s \mid X) = \frac{p_s g_s(X)}{\sum_{i=1}^{c} p_i g_i(X)}$$
  $s = 1, ..., c,$ 

is greatest. Here  $p_i$  is the prior (before seeing its genotype) probability that the individual comes from population *i*, and  $g_i(X)$  is the relative frequency of the genotype *X* in population *i*. Intuitively, p(s | X) is the fraction of individuals having genotype *X* that is contributed by population *s* to a mixture composed of *c* populations with proportions,  $p = (p_1, \dots, p_c)$ .

If only a single individual is to be identified to source, the equi-probable prior is an obvious choice, i.e.,  $p_1 = ... = p_c = 1/c$ . The MAP rule with equi-probable prior produces the same assignments as WHICHRUN<sup>1</sup> (Banks and Eichert 2000), which assigns individuals to the population *k* in which the genotype is most frequent (MFG rule), i.e.,  $g_k(X) = \max_i \{g_i(X), i = 1,...,c\}$ . However, if sources of several individuals are to be identified, they are better viewed as a sample from a mixture whose unknown source proportions are the prior probabilities in eq. 1. These prior probabilities can be estimated by conditional maximum likelihood with program SPAM<sup>2</sup> (Debevec et al. 2000; Alaska Department of Fish and Game 2003), or by Bayesian methods with program BAYES<sup>3</sup> (Pella and Masuda 2000). Currently, SPAM simply evaluates eq. 1 once using the conditional maximum likelihood point estimates, but BAYES evaluates eq. 1 from draws of all unknowns at each of many cycles.

Paetkau et al. (1995) obtained data for eight microsatellite loci from four Canadian polar bear populations: northern Beaufort Sea, southern Beaufort Sea, western Hudson Bay, and Davis Strait-Labrador Sea (Table 1). Average heterozygosity was near 60% for each population and allele frequency distributions were significantly different between all pairs of populations (Paetkau et al. 1995). Paetkau et al. (1999) in a more extensive genetic study of circumpolar populations of polar bears expanded the number of loci to 16 and the number of populations to 16 (Table 1). A simulation experiment was performed to compare methods of individual assignments using the polar bear data<sup>4</sup>. Only the original four populations were included in our study. The variables controlled in the experiment were the number of loci (either the initial eight loci, or the later 16 loci), the proportions of contribution

|                          |                   | Sample size<br>Population |                          |                       |                              |  |  |
|--------------------------|-------------------|---------------------------|--------------------------|-----------------------|------------------------------|--|--|
| Study                    | Number<br>of loci | Southern<br>Beaufort Sea  | Northern<br>Beaufort Sea | Western<br>Hudson Bay | Davis<br>Strait-Labrador Sea |  |  |
| Paetkau et al.<br>(1995) | 8                 | 22                        | 30                       | 30                    | 26                           |  |  |
| Paetkau et al.<br>(1999) | 16                | 30                        | 30                       | 33                    | 30                           |  |  |

<sup>&</sup>lt;sup>1</sup> WHICHRUN can be obtained from http://www.bml.ucdavis.edu/whichrun.htm.

<sup>&</sup>lt;sup>2</sup> SPAM can be obtained from http://www.cf.adfg.state.ak.us/geninfo/research/genetics/Software/SpamPage.htm.

<sup>&</sup>lt;sup>3</sup> BAYES can be obtained from ftp://ftp.afsc.noaa.gov/sida/mixture-analysis/bayes/.

<sup>&</sup>lt;sup>4</sup> Dr. David Paetkau generously made the data available for this study.

from the four populations (either equal proportions of 1/4, or an uneven set with western Hudson Bay comprising 75–88.5% of the population mixture and the other stocks comprising equal thirds of the remaining mixture), and mixture sample size (12, 52, or 100 bears). The original baseline samples were independently resampled for each of the 12 cells of the design to provide simulated sets of baseline and population-mixture genotype samples of polar bears, and each cell was independently replicated three times. For each simulated mixture sample, source populations of mixture individuals were identified with the MAP rule applied to the posterior source probabilities using SPAM, the MAP rule applied to average posterior source probabilities using BAYES, and the MFG rule using WHICHRUN. SPAM was run with a Bayesian model of baseline allele frequency distributions, specifically, the mean of the Rannala and Mountain (1997) baseline posterior. BAYES generated a single fixed sequence of 5,000 samples of the unknowns (first 2,500 was discarded as burn-in) for each cell and replicate. The experiment was summarized with the percentage of mixture individuals correctly assigned to their source population.

Although the average correct (%) is imprecisely determined with only three replications per experimental cell, the following generalizations are discernible (Fig. 1). First, with fewer loci and unequal mixture proportions (Fig. 1b), the method used becomes increasingly important with increase in number of individuals to be identified. SPAM and BAYES perform better with increase in mixture sample size, whereas WHICHRUN performance falls progressively below that of the others. The same is true with more loci and unequal mixture proportions (Fig. 1d), but performance differences are smaller. Second, under the contrived equal-proportions mixture for which WHICHRUN is expected to perform best because it is effectively given the correct prior, it performed on average only comparably to SPAM and BAYES for fewer loci (Fig. 1a) and apparently slightly worse for more loci (Fig. 1c).

As the number of individuals to be identified to their source populations increases, the maximum *a posteriori* (MAP) rule with posterior source probabilities computed by likelihood or Bayesian methods performs better than the most frequent genotype (MFG) rule, especially if genetic information is limited. At small sample sizes, little, if any, loss in performance occurs by use of the MAP rule with estimated prior probabilities as compared to the MFG rule. At present, the two programs—SPAM and BAYES—that compute and output the posterior source probabilities of individuals are not designed to perform the assignments by the MAP rule, and the researcher must make the assignments manually (hence the limited number of replications in this study).

**Fig 1.** Average (n = 3) percentage of mixture individuals (white break in vertical bar) correctly assigned to the population for varying mixture sample sizes, 8-locus (a and b) or 16-locus data set (c and d), equal (a and c) or unequal mixture proportions (b and d), and assignment method: BAYES (a), SPAM (a), and WHICHRUN (b). Extreme ends of bars indicate ±1 standard deviation.



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## Reducing Bias in Mixture Estimates: a Computer Program to Bin Alleles

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Keywords: DNA, microsatellites, data reduction, homogeneity, exact test, mixture analysis

Mixed-stock analysis (MSA) using genetic characters is an integral part of research programs estimating stock composition of catches of anadromous salmon and describing migration patterns of anadromous salmon in the highseas. Widespread adoption of DNA techniques has lead to increased use of highly polymorphic loci in MSA. Greater polymorphism can enhance the power of MSA, but is not always beneficial. Researchers often bin alleles to reduce the effect of sampling error in baseline allele frequencies in studies using conditional maximum likelihood. Alleles are typically binned based on allele size or frequency, but these methods may result in a loss of information. We present a program for binning alleles to reduce the number of dimensions in a baseline while simultaneously maintaining the ability of the data to differentiate populations.

Exact tests of homogeneity can be used to test if alleles are similarly distributed across populations, with Monte Carlo simulation to estimate significance, to determine binning strategy. For any two alleles, the hypothesis of homogeneity is tested using either a likelihood ratio or Pearson test statistic. The P (number of populations) by A (number of alleles) matrix of allele frequencies is permuted such that the marginal allele and population frequencies of the entire P by A matrix remain fixed. For each permutation of the matrix, the test statistic is computed for all allele pairs and its value ( $\Psi_p$ ) is compared to the test statistic from the full model ( $\Psi_o$ ) providing an estimate of the probability of the distribution of the test statistic. The number of alleles having the larges value of k is identified. The ratio p = k/K is an estimate of the significance of an exact test of the hypothesis that the allele proportions are equal across all populations, and large values of p indicate the allele proportions are not statistically different among the populations. If p exceeds a specified threshold  $p_{max}$ , the two alleles are binned to form a new allele, A is reduced by 1, and the process is repeated with the new P by A data matrix, otherwise the process terminates.

The program OptiBin uses baseline files (\*.bse) for SPAM (Debevec et al. 2000) as input. The program options include choice of test statistic, threshold p value, number of permutations for Monte Carlo tests of significance, random seed, and whether to test all possible pairs of alleles or only alleles adjacent in size. The program outputs a \*.bse file readable by SPAM and a log file of which alleles were binned and p-value for homogeneity test. The log file can be used by OptiBin to bin aleleles of mixture files for estimation. The program will be available at <a href="http://www.r7.fws.gov/fish/genelab/home.html">http://www.r7.fws.gov/fish/genelab/home.html</a>.

The binning algorithm was tested on two data sets, allele frequencies for six microsatellite loci for five populations of Dolly Varden in western Alaska and allele frequencies for 11 microsatellite loci for ten populations of chum salmon from Yukon River. The binning program reduced the average number of alleles per locus by 50% and greatly reduced the number of sampling zeros. Bias was reduced in conditional maximum likelihood estimates of simulated mixtures. Further, a slight reduction in bias was also apparent when a Bayesian estimator of baseline allele frequency distributions was employed.

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## Diel Feeding and Gastric Evacuation of Juvenile Pink and Chum Salmon in Icy Strait, Southeastern Alaska, May–September 2001

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Keywords: Trophic ecology, bioenergetics, daily ration, juvenile salmon, Oncorhynchus spp., Alaska

We studied seasonal diel feeding and gastric evacuation rates of juvenile pink (Oncorhynchus gorbuscha; n = 458) salmon and chum (O. keta; n = 464) salmon in the marine waters of the northern region of southeastern Alaska, from May through September of 2001. These process studies were conducted as part of the Southeast Coastal Monitoring (SECM) Project of the Auke Bay Laboratory, National Marine Fisheries Service. For each of the past seven years (1997-2003), SECM scientists have monitored the abundance, distribution, stock composition, and energy content of juvenile salmon as the fish transit through the principal migration corridor in the region, and also have monitored habitat biophysical parameters and carrying capacity (Orsi et al. 2002). For this project, we sampled monthly in Icy Strait by beach seining two sites near shore in May and by surface trawling at a station located 6.4 km offshore in June through September (Sturdevant et al. 2002). Surface (2-m) temperature was taken, and samples of fish (size and stomach analysis) and zooplankton (surface to 20-m oblique hauls, 333 µm mesh, 60 cm diameter bongo net) were examined from seven intervals within a 24-hr day. The objectives of the diel study were to monitor juvenile salmon feeding rhythms (stomach % fullness, numbers of prey, and prey percent body weight (%BW)) and prey composition (% number and % weight by taxon), as well as zooplankton displacement volumes (DV, ml·m<sup>-3</sup>), densities (number  $\cdot$  m<sup>-3</sup>), and composition (% number  $\cdot$  m<sup>-3</sup>). The objectives of the gastric evacuation study were to monitor the passage of food out of the stomachs (decline in prey number and biomass) of juvenile salmon that were caught in single hauls at different times of day in May and July, and to compute exponential evacuation rate, ER. Results of these studies were used to compute daily ration (DR) for each month, where DR = 24 mean diel prey %BW·ER (Williams et al. 2001).

Biophysical habitat parameters exhibited strong seasonal and diel patterns. Seasonal surface temperatures rose from approximately 7°C in May to a peak greater than 13°C in June, declining to approximately 9°C in September; we observed little diel change except in June. Zooplankton DV and densities were also highest in June, and peaked late in the day each month. Prey composition changed seasonally with zooplankton composition (Fig. 1). In May and June, small calanoids were prominent in the varied diets, the only time this taxon did not dominate zooplankton composition. In June, large calanoids, euphausiid larvae and juveniles, and larvaceans were also prominent prey, while in later months, hyperiid juveniles and larvaceans were prominent; however, no consistent patterns in diel prey composition were observed. Prey selection for hyperiids, euphausiids, and larvaceans was indicated by high percentages of these taxa in the diets compared to their low abundance in the 333  $\mu$ m mesh zooplankton (Fig. 1); juvenile salmon avoided the prominent, small prey taxa.

Juvenile salmon fed continuously throughout the day, with average stomach fullness indices of 60–95% per diel period in all five months. However, numbers of prey in stomachs were generally highest early and late in the day (Fig. 1), coincident with peak zooplankton density and DV late in the day. Fullness index and DR peaked in June, coincident with the seasonal peak in zooplankton density (Fig. 1). ER approximately doubled from May to July (Fig. 2), when 1) time to completely empty the guts increased from approximately 8 hrs to 12 hrs, 2) temperature increased from 7.1°C to 12.7°C, 3) prey composition changed from principally hard-bodied (crustaceans) to principally soft-bodied (larvaceans) taxa, 4) starting numbers of prey at time of capture for evacuation experiments were at least five times greater, and 5) fish length tripled (approximately 40 mm to 120 mm fork length) and weight increased by two orders of magnitude. The lower DR at summer's end coincided with the decline in zooplankton density.

Further analysis of these preliminary results will be supplemented with data from zooplankton samples collected in different mesh nets and fish diet composition by weight. Our study provides input parameters for bioenergetic modeling of juvenile salmon (Orsi et al. 2003a), a tool useful for assessing the demands of increased hatchery production on the carrying capacity of the marine ecosystem in a region of economically important commercial fisheries. It also provides the background necessary for investigations on trophic ecology and interactions between wild and hatchery chum salmon (Orsi et al. 2002, 2003b; Sturdevant et al. 2002).

**Fig. 1.** Monthly diel prey composition (mean % number) and mean total number of prey of juvenile pink and chum salmon, with 20m zooplankton (double oblique bongo, 333-µm mesh) composition (mean % number) and total density (thousands per m<sup>3</sup>); number of fish (n) and daily rations (DR, wet % body weight) shown.



**Fig. 2.** Exponential Evacuation Rate: decline in number of prey over time, with prey composition (mean % number of hard- or softbodied taxa, bar graphs) from time of capture (time 0), in May and July, for juvenile pink and chum salmon; number of fish (n) and  $R^2$  shown.



Number of hours post-capture

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## Even-Year Pink Salmon Pacific Rim Allozyme Baseline and Origin of Juveniles from Gulf of Alaska Coastal Waters, 2003

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Keywords: Even-year pink salmon, allozymes, baseline, mixture

Over the past two decades, even-year pink salmon have been collected and analyzed for allozyme variation from more than 150 river systems around the Pacific Rim. These studies have been accomplished by many individuals and laboratories. Most have been published as independent studies. This study combines data from those studies and unpublished data from North America. Where allele standardization was unavailable, alleles were pooled. The baseline used for simulations in this study attempted to maximize numbers of populations, balanced with a useful number of loci. The number of loci available across all populations was between 6 and 54. A series of analyses such as G-test, genetic distance, and multidimensional scaling was examined on baselines of varying numbers of populations and loci.

Simulations to estimate accuracy of stock allocations in a mixture were run using conditional maximum likelihood analysis. Using a baseline of 31 loci and 77 populations, the proportion (90% confidence interval) of regional simulated mixtures correctly assigned, when the expected is 100%, is as follows:

| Magadan/Kamchatka, Russia        | 0.91 (0.85-0.95) |
|----------------------------------|------------------|
| Japan/Sakhalin, Russia           | 0.95 (0.93-0.98) |
| Alaska/northern British Columbia | 0.95 (0.94-0.97) |
| Snohomish River, Washington      | 0.93 (0.89–0.98) |

Finer regional scale simulations of North American fish were less accurate:

| 0.70 (0.58–0.85) |
|------------------|
| 0.85 (0.73-0.97) |
| 0.90 (0.80-0.97) |
| 0.64 (0.49–0.79) |
| 0.92 (0.87-0.99) |
|                  |

Three groups of juvenile pink salmon were collected (n = 781) from the northern Gulf of Alaska, between Kodiak and Prince William Sound, by the Auke Bay Laboratory's Ocean Carrying Capacity program in July of 2003. Juveniles in the nearshore, mid-shelf, and offshore collections averaged 130, 142, 153 mm in length (p > 0.001). Conditional maximum likelihood analysis using North American populations of the coastwide baseline allocated the regions of origin (with Standard Error) as follows (allocation to Asian populations was 0):

|           | <u>n</u> | <u>Susitna</u> | <u>P.W.S.</u>    | <u>S.E. AK</u>   | <u>Snoho</u> | <u>N. BC</u> |
|-----------|----------|----------------|------------------|------------------|--------------|--------------|
| Nearshore | 300      | 0              | 0.96 (0.89-1.0)  | 0.05 (0-0.11)    | 0            | 0            |
| Mid-Shelf | 241      | 0              | 0.43 (0.24-0.78) | 0.54 (0.21-0.74) | 0            | 0            |
| Offshore  | 80       | 0.12 (025)     | 0.58 (0.30-1.0)  | 0.27 (0-0.47)    | 0            | 0            |

## Stock Abundance Dynamics of Azabachye Lake and Dvukhyurtochnoye Lake Sockeye Salmon (Oncorhynchus nerka) from the Results of Sockeye Salmon Origin Identification in the Coastal and River Catches of Kamchatka River Basin

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Keywords: Sockeye salmon, abundance, sub-stocks, scale identification, Kamchatka River

The Azabachye Lake sockeye salmon "A" stock inhabiting the lower reaches of the Kamchatka River, is very abundant and contributes up to 50–60% of the total Kamchatka River catch in some years. The sockeye salmon "D" stock reproducing in Dvukhyurtochnoye Lake basin is the next most abundant stock and contributes from 3 to 10% (maximum -15-20%) of the Kamchatka River total sockeye salmon stock.

From the results of sockeye salmon complex identification; including the analysis of fresh-water scale zone (Bugayev 1983, 1986), the frequency of *Diphyllobothrium sp.* infection, and the time of harvest; the contribution of "A" and "D" stocks in total Kamchatka River sockeye salmon coastal and in-river escapement has been estimated for the period from 1978 to 2002. The results provided accurate estimation of "A" and "D" stock contributions to the fishery for the period 1957–1977.

It is estimated that 70-80% of the abundance of the "A" and "D" stocks are composed of age 2.3 fish. Therefore, estimation of the sockeye salmon abundance has been made on the 2.3 age group only. The stock abundance of the "A" stock and "B" stock is equal to the stock abundance of mature fish in the sea before the drift gill net fishery started (Fig. 1–2).

From the analysis of sockeye salmon "A" stock abundance, the level of reproduction (RL) has been subdivided: 1 (high RL) – 1957–1988 after the effect of Azabachye Lake fertilization from volcanic ash (1960–1962, 1977–1979) and years with outstandingly favorable conditions of juvenile feeding (1986–1988) (Fig. 1b); 2 (low RL) – 1957–1988 where the fresh-water period of feeding took place in years without the effects of fertilization, and juvenile feeding conditions were below average (1957–1959, 1963–1976, 1980–1985) (Fig. 1c); 3 – 1989–1996 where the RL was enhanced as a result of favorable feeding conditions during their marine period of life (Fig. 1d–f). Analysis indicates similar conditions of stock abundance dynamics in both the early and late runs of "A" stock sockeye salmon.

The differences between Fig. 1d and Fig. 1f consist only in the assessment of the abundance: a standard year in Fig. 1d and an atypical year in Fig. 1f (for example 1995 and 1996 when adult escapement was 690,000 and 268,000 respectively). The high escapements in 1995 and 1996 resulted in an unusually high percentage (up to 40%) of four ocean age fish in 2002 of the "A" stock (SMP) sockeye salmon for the first time since 1946–1947. We estimate that the abundance of the "A" stock for the years 1957-1994 was equal to the abundance of the SMP of the "A" stock in the ocean before the drift gill net fishery started (Bugayev 1983). In 2003 the "A" stock was composed of about 80% age 2.3 fish.

Current escapement to the spawning grounds in Azabachye Lake should be 100,000 fish for maximum effective reproduction. For the period 1957–1988 the escapement should be 100,000 fish in the case of a high RL, and 50,000 in the case of a low RL.

Prior to 1987 (1957-1986) analysis of the "escapement-progeny" relation in the "D" stock for 1957–1996 (Fig. 2) indicates higher progeny abundance in odd years compared to that in even years since parental escapements in 1987 and 1988. Progeny generations of odd and even years from the parental generations of 1957–1986 do not demonstrate any differences. For the years 1957–1996, we estimated the abundance of the "D" stock to be equal to the abundance of mature fish (SMP) in the ocean before the drift gill net fishery started (Bugayev 1983).

The optimum escapement of sockeye salmon for the Dvukhyurtochnoye Lake basin since 1987–1988 should be about 100,000 fish in odd years (since 1987) (Fig. 2e) and 30,000 fish in even years (since 1988) (Fig. 2f). For 1957–1986 the optimum escapement of the "D" stock has been estimated to be 20,000–30,000 fish (Fig. 2b–d).

Figures 3–4 show the abundance of the "A" and "D" stocks in 1957–2002.

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**Fig. 1.** "A" stock brood year abundance and abundance of the mature portion of the "A" stock (SMP) sockeye salmon in the ocean that is dependent on the parental escapement in Azabachye Lake for 1957–1996 (combined data on early and late run seasonal "A" stock), in thousands.

- a combined data for 1957-1996 (returns of 1963-2002);
- b brood years of 1957–1988 were influenced by volcanic ash fertilization of Azabachye Lake (1960–1962, 1977–1979) resulting in excellent rearing conditions (1986–1988);
- c brood years of 1957–1988 resulting from below average rearing conditions after the years of volcanic ash fertilization (1957-1959, (1963–1976, 1980–1985);
- d brood years of 1989–1996 when reproduction levels were principally influenced by ocean feeding conditions;
- e brood years of 1989–1996 (with the exception of 1995 when 690,000 fish spawned);
- f brood years of 1989–1996 (with specified abundance of brood years of 1995–1996).



Fig. 2. "D" stock brood year abundance and abundance of the mature portion of the "D" stock (SMP) sockeye salmon in the ocean dependent on parental escapement in Azabachye Lake for 1957–1996 (combined data for early and late run "D" stock), in thousands.

- a combined data on brood years of 1957-1996 (returns of 1963-2002);
- b brood years of 1957-1986 (all years);
- c brood years of 1957-1985 (odd years);
- d brood years of 1958-1986 (even years);
- e brood years of 1987–1995 (odd years);
- f brood years of 1988-1996 (even years).









**Fig. 4.** Sockeye salmon abundance of the "D" stock in 1957–2002: the mature portion in the sea before drift net fishery (1), run (coastal catch + escapement) (2), escapement in Dvukhyurtochnoye Lake (3), in thousands.



## Results of Identification of Sockeye Salmon (Oncorhynchus nerka) Secondary Local Stocks and Secondary Groups of Local Stocks in the Coastal and River Catches of Kamchatka River for 1978–2001

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Keywords: Sockeye salmon, abundance, sub-stocks, scale identification, Kamchatka River

Sockeye salmon from the early run, spawning in the upper and middle tributaries of the Kamchatka River (Fig. 1), almost totally migrate to the sea in the year of their emergence from the gravel as underyearlings of 35–45 mm in length ("C" group). Juvenile sockeye salmon of the late run inhabiting this region feed in the vicinity of spawning grounds and migrate to the sea at age 1+ ("B" group). General sockeye salmon stock, spawning in the tributaries of the middle and low reaches of the Kamchatka River, migrates (as underyearlings) to the Azabachye Lake for feeding, situated in the low reaches of Kamchatka River ("E" group); a small number of underyearlings from this area migrates to Nerpichye Lake ("N" group) for feeding. At the same time, native sockeye salmon stock reproduces in the basin of Azabachye Lake, i.e. native juveniles spend two winters in the lake and migrate to the sea at age 2+ ("A" stock). "E" group smolts are of 1+ age. Aside from these stocks mentioned above, two native

sockeye salmon stocks - Dvukhyurtochnoye Lake stock ("D" stock) and Nerpichye Lake stock ("N stock) are in the basin of Kamchatka River. "D" stock smolts are of 2+ age, "N" stock and group are represented by the age 1+. In general, Kamchatka River sockeye salmon mature in three marine years.

The complex method of identification of sockeye salmon secondary stocks and secondary groups from the structure of scale fresh-water zone, the frequency of Diphyllobothrium sp. infection and the time of catching was worked out in 1980's (Bugayev 1983, 1986). It has been shown that the fish from different stocks and groups enter the river at different times which results in their different contribution in the fishery catches. Total abundance of fishes (escapement and commercial catch) improved on the basis of the identification methods (Table 1) and provides the possibility of using the results in studying stock abundance dynamics of secondary stocks, secondary groups, and total Kamchatka River stock.

If data from Tables 1 and 2 are compared, one can see that the age and population composition of the catches and the relationship to the spawning stock is similar to that noted for Kamchatka River sockeye salmon long ago (Bugaev 1983).

Figure 2 shows sockeye salmon abundance of Kamchatka River total basin in 1957–2003.

**Fig. 1.** The Kamchatka River basin (diagrammatic) showing samplings sites for sockeye spawners: 1 – Puschino village, 2 – Kashkan River, 3 – Sharoma village, 4 – Kavycha River, 5 – Andrianovka River, 6 – Zhupanka River, 7 – Vakhvina River, 8 – Kirganik River, 9 – Kimitina River, 10 – Kitilgina River, 11-Shchapina River, 12 – Nikolka River, 13 – Tolbachik River, 14 – Bystraya-Kozyrevka River, 15 – Shekhlun River, 16 – Kreruk River, 17 – Ushkovskoye Lake springs, 18 – Kruki River, 19 – Polovinnaya River, 20 – Belaya River, 21 – Yelovka River, 22 – Dvukhyrtochnoye Lake, 23 – Bolshaya Khapitsa River, 24 – Malaya Khapitsa River, 25 – Raduga River, 26 – Azabachye Lake, 27 – Nizovtsevo Lake (Raduga River drainage), 28 – Kursin Lake, 29 – Soldatskaya River (Nerpichye Lake drainage). See text for description of groups A, B, C, D, E, and N.



| Vooro     | 0.3  |      | 1.3       |        | 2    | 2.3  | Sample |
|-----------|------|------|-----------|--------|------|------|--------|
| reals     | С    | В    | E         | N*     | А    | D    | size   |
|           |      |      | Early run | (June) |      |      |        |
| 1985      | 4.1  | 10.3 | 39.2      | 2.6    | 37.1 | 6.7  | 194    |
| 1986      | 12.0 | 10.9 | 26.1      | 3.6    | 26.6 | 20.8 | 192    |
| 1987      | 17.7 | 4.0  | 40.2      | 3.6    | 21.3 | 13.2 | 249    |
| 1988      | 22.1 | 3.1  | 29.5      | 6.3    | 23.2 | 15.8 | 95     |
| 1989      | 20.8 | 0.0  | 33.6      | 4.0    | 20.8 | 20.8 | 101    |
| 1990      | -    | -    | -         | -      | -    | -    | -      |
| 1991      | -    | -    | -         | -      | -    | -    | -      |
| 1992      | 12.1 | 1.6  | 26.1      | 2.8    | 51.0 | 6.4  | 249    |
| 1993      | 17.2 | 1.4  | 31.9      | 2.4    | 43.0 | 4.1  | 291    |
| 1994      | 16.7 | 4.2  | 33.3      | 2.1    | 42.7 | 1.0  | 96     |
| 1995      | 4.2  | 0.0  | 23.1      | 0.3    | 56.2 | 16.2 | 290    |
| 1996      | 5.8  | 1.6  | 31.3      | 0.8    | 52.3 | 8.2  | 243    |
| 1997      | 16.8 | 4.1  | 33.2      | 0.0    | 44.4 | 1.5  | 196    |
| 1998      | 9.5  | 5.4  | 29.9      | 3.2    | 47.9 | 4.1  | 465    |
| 1999      | 24.0 | 1.4  | 29.9      | 1.5    | 33.2 | 10.0 | 521    |
| 2000      | 24.2 | 6.1  | 31.2      | 2.1    | 32.6 | 3.8  | 475    |
| 2001      | 12.4 | 0.8  | 34.7      | 1.9    | 35.7 | 14.5 | 614    |
| Mean      |      |      |           |        |      |      |        |
| 1978–1984 | 9.9  | 6.2  | 51.8      | 2.4    | 25.7 | 4.0  |        |
| 1985–2001 | 14.6 | 3.7  | 31.5      | 2.5    | 37.9 | 9.8  |        |
|           |      |      | Late run  | (July) |      |      |        |
| 1985      | 2.5  | 27.4 | 17.3      | 4.6    | 41.1 | 7.1  | 197    |
| 1986      | 4.3  | 16.1 | 34.4      | 4.9    | 32.8 | 7.5  | 186    |
| 1987      | 9.8  | 18.3 | 24.2      | 1.7    | 32.8 | 13.2 | 235    |
| 1988      | 10.2 | 24.5 | 23.1      | 4.8    | 33.3 | 4.1  | 147    |
| 1989      | 12.1 | 26.6 | 19.6      | 7.0    | 25.6 | 9.1  | 199    |
| 1990      | -    | -    | -         | -      | -    | -    | -      |
| 1991      | -    | -    | -         | -      | -    | -    | -      |
| 1992      | 6.1  | 6.1  | 18.4      | 1.0    | 68.4 | 0.0  | 98     |
| 1993      | 5.4  | 8.1  | 15.6      | 2.0    | 58.1 | 10.8 | 148    |
| 1994      | 8.3  | 9.7  | 25.5      | 3.4    | 49.7 | 3.4  | 145    |
| 1995      | 2.9  | 10.7 | 11.4      | 4.8    | 63.9 | 6.3  | 440    |
| 1996      | 3.2  | 6.4  | 23.3      | 0.5    | 60.2 | 6.4  | 533    |
| 1997      | 4.6  | 4.9  | 22.0      | 1.4    | 64.5 | 2.6  | 346    |
| 1998      | 7.3  | 10.7 | 19.6      | 5.7    | 53.7 | 3.0  | 439    |
| 1999      | 24.9 | 19.7 | 16.5      | 3.5    | 33.6 | 1.8  | 345    |
| 2000      | 6.8  | 24.4 | 19.0      | 1.6    | 44.5 | 3.7  | 512    |
| 2001      | 6.3  | 8.7  | 33.9      | 2.1    | 47.4 | 1.6  | 378    |
| Mean      |      | 10.0 | 00.0      | 0.0    | 00.0 |      |        |
| 1978–1984 | 11.1 | 19.3 | 38.3      | 3.9    | 22.6 | 4.8  |        |
| 1985–2001 | 7.6  | 14.8 | 21.6      | 3.3    | 47.3 | 5.4  |        |

**Table 1**. Percent contribution of secondary local stocks and secondary groups of local stocks by age structure of Kamchatka River sockeye salmon in the catches of marine trap net for 1978–1984 and 1985–2001.

Note.\* - individuals from "N" group and stock, none differentiated in the catches.

**Table 2.** Abundance ratio (%) by age structure of sockeye salmon escapement from secondary local stocks and groups in the basin of Kamchatka River for 1978–1984 and 1985–2001.

| Vooro     | 0.3  |      |      | 1.3      |       | 2    | .3   |
|-----------|------|------|------|----------|-------|------|------|
| 16013     | С    | В    | E*   | N**      | К     | А    | D    |
| 1985      | 13.2 | 8.9  | 24.4 | 2.6(0.3) | 0.1   | 38.4 | 12.4 |
| 1986      | 22.9 | 16.7 | 18.1 | 2.9(1.3) | 0.1   | 25.9 | 13.4 |
| 1987      | 31.8 | 4.9  | 22.0 | 2.6(0.7) | 0.2   | 28.6 | 9.9  |
| 1988      | 34.5 | 20.1 | 19.8 | 2.7(1.0) | 0.4   | 15.7 | 6.8  |
| 1989      | 31.9 | 8.9  | 23.6 | 3.6(1.4) | 0.2   | 14.1 | 17.7 |
| 1990      | 31.9 | 13.3 | 28.3 | 3.3(0.8) | 0.1   | 13.1 | 10.0 |
| 1991      | 15.3 | 13.3 | 33.7 | 4.0(1.0) | 0.3   | 23.4 | 10.0 |
| 1992      | 10.0 | 5.2  | 14.4 | 2.3(1.0) | m. d. | 57.3 | 10.8 |
| 1993      | 20.4 | 2.7  | 20.2 | 2.0(0.2) | m. d. | 39.9 | 14.8 |
| 1994      | 20.5 | 4.1  | 16.2 | 1.6(0.2) | m. d. | 48.6 | 9.0  |
| 1995      | 7.5  | 4.0  | 25.1 | 2.6(0.4) | m. d. | 48.7 | 12.1 |
| 1996      | 8.5  | 1.5  | 33.2 | 3.5(0.6) | m. d. | 41.3 | 12.0 |
| 1997      | 11.3 | 2.4  | 22.8 | 2.3(0.3) | m. d. | 54.6 | 6.6  |
| 1998      | 16.4 | 4.1  | 41.0 | 3.6(+)   | m. d. | 29.4 | 5.5  |
| 1999      | 18.2 | 4.7  | 42.9 | 3.7(+)   | m. d. | 18.8 | 11.7 |
| 2000      | 15.2 | 4.3  | 53.7 | 5.0(0.3) | m. d. | 14.1 | 7.7  |
| 2001      | 4.8  | 2.7  | 51.1 | 4.4(+)   | m. d. | 22.7 | 14.3 |
| Mean      |      |      |      |          |       |      |      |
| 1970–1984 | 28.0 | 4.9  | 40.8 | 4.4(0.8) | 0.2   | 16.4 | 5.3  |
| 1985–2001 | 18.5 | 7.2  | 28.8 | 3.1      | m. d. | 31.4 | 10.9 |

Note. \* - "N" group individuals are not included; \*\* - united abundance of sockeye salmon "N" stock and "N" group (the portion of the "N" stock is within the parentheses); m. d. - missing data. The sign "+" – less than 0.1%. Average meaning for the period 1985–2001 is not 100% due to missing data on the "K" stock for 1992–2001. Average abundance of sockeye salmon escapement in the basin of Kamchatka River for 1970–1984 – 685,000 and for 1985–2001 – 614,000.

**Fig. 2.** Sockeye salmon abundance of total Kamchatka River basin, 1957-2003: (1) maturation period in the sea before the drift net fishery ; (2) run (coastal catch + escapement); (3) escapement .



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## The Use of Otolith Mass Marking to Estimate Adult Hatchery Sockeye Salmon Returns to the Bolshaya River (Kamchatka)

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Keywords: Sockeye salmon, otolith mark, identification, Bolshaya River

Large releases of hatchery salmon with otolith marks allows for identification of these fishes in marine and coastal catches. Without mass marking it is difficult to estimate hatchery benefits or to evaluate different hatchery strategies (Akinicheva and Rogatnykh 1997). In Russia, mass otolith marking of hatchery salmon is used in the Magadan Region, in Kamchatka and recently in Sakhalin. In Kamchatka it began at Malki and Ozerky hatcheries located within the Bolshaya River basin respectively in 1995 (Vasilkov 1995, 1996) and 1999. In 2002 all Kamchatkan hatcheries were included into the program of mass "otolith" marking. For the period mentioned, in addition to "thermal" marking, we have started to actively use "dry" marking as well; recommendations have been prepared on the procedure and optimal timing of marking, and also (on each salmon species, hatchery and year) a scheme of its realization and structure of marks (Chebanov and Kudzina 2001, 2002). In the recent years the returns of the marked fish to the base reservoirs of these hatcheries have been noted.

The purpose of this study was to estimate the proportion of hatchery sockeye salmon to total returns of the species to Bolshaya River by discovering marked individuals in samples. Work took place in the river estuary and tributaries where hatcheries were located in 2002.

As a result of analysis of the otolith structure of 193 sockeye salmon individuals that returned to the Malki hatchery in 2002, it was found that 93.3% of the fish were of hatchery origin. 5.7% of the fish had marks from 1998 (4-years old, put into incubation in 1997), 86% - marks from 1999 (3-years old, put into incubation in 1998) and 1.6% - mark from 2000 (2-years old, put into incubation in1999). Besides, one fish was of natural origin, and 12 raised doubts about their origin. The individuals were primarily adults from the generation released in 1999, but of 592,300 juveniles only (from 1,198,200), which were transported to Ketkino hatchery for incubation and rearing, and, hence, were not marked (released with average mass of 1g). Therefore, it could be suggested that among 15000 sockeye salmon that returned to Malki hatchery in 2002, 233 individuals were aged 2+, 12,902 - as 3+ (13,900 fishes including unmarked ones) and 855 - as 4+.

The data obtained from 2000 to 2002 on the age structure of sockeye salmon in runs to Malki hatchery permitted us to estimate the coefficient of return for some generation (from juveniles released in certain years) (Table 1). As it is clear from this table, the highest return coefficients were from the releases of 1998 and 1999. It should be taken into account, however, that the estimation of return of 1995 and 1996 releases was not complete, and that of 1999 and 2000 releases is still incomplete. Considering the above, the lowest return (among terminated ones) is the return of the 1997 release, and the highest one, evidently, should be the return of the 1999 release (4+ and 5+ fishes have not been included in the return yet).

| Released  | Returns                                                                                  | of this genera                                                                                                                                                                                                        | tion by years                                                                                                                                                                                                                                                                                                            | Total return                                                                                                                                                                                                                                                                                                                                                                                                                               | Return coefficient                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |  |  |  |
|-----------|------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|--|
| juvenile  | 2000                                                                                     | 2001                                                                                                                                                                                                                  | 2002                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                            | released juvenile, %                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |  |  |  |
| 370,100   | 16                                                                                       | -                                                                                                                                                                                                                     | -                                                                                                                                                                                                                                                                                                                        | 16                                                                                                                                                                                                                                                                                                                                                                                                                                         | 0.004                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |  |  |
| 669,500   | 834                                                                                      | 59                                                                                                                                                                                                                    | -                                                                                                                                                                                                                                                                                                                        | 893                                                                                                                                                                                                                                                                                                                                                                                                                                        | 0.13                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |  |  |  |
| 331,700   | 866                                                                                      | 29                                                                                                                                                                                                                    | -                                                                                                                                                                                                                                                                                                                        | 895                                                                                                                                                                                                                                                                                                                                                                                                                                        | 0.27                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |  |  |  |
| 716,700   | 334                                                                                      | 4324                                                                                                                                                                                                                  | 855                                                                                                                                                                                                                                                                                                                      | 5,513                                                                                                                                                                                                                                                                                                                                                                                                                                      | 0.77                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |  |  |  |
| 1,198,200 | -                                                                                        | -                                                                                                                                                                                                                     | 12,902 (13,900)*                                                                                                                                                                                                                                                                                                         | 12,902 (13,900)*                                                                                                                                                                                                                                                                                                                                                                                                                           | 1.08 (1.16)*                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |  |  |  |
| 724,500   | -                                                                                        | -                                                                                                                                                                                                                     | 233                                                                                                                                                                                                                                                                                                                      | 233                                                                                                                                                                                                                                                                                                                                                                                                                                        | 0.03                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |  |  |  |
|           | Released<br>juvenile<br>370,100<br>669,500<br>331,700<br>716,700<br>1,198,200<br>724,500 | Released<br>juvenile         Returns           370,100         16           669,500         834           331,700         866           716,700         334           1,198,200         -           724,500         - | Released<br>juvenile         Returns of this general           2000         2001           370,100         16           669,500         834         59           331,700         866         29           716,700         334         4324           1,198,200         -         -           724,500         -         - | Released<br>juvenile         Returns of this generation by years           2000         2001         2002           370,100         16         -         -           669,500         834         59         -           331,700         866         29         -           716,700         334         4324         855           1,198,200         -         -         12,902 (13,900)*           724,500         -         -         233 | Released<br>juvenile         Returns of this generation by years         Total return           370,100         16         -         -         16           669,500         834         59         -         893           331,700         866         29         -         895           716,700         334         4324         855         5,513           1,198,200         -         -         12,902 (13,900)*         12,902 (13,900)*           724,500         -         -         233         233 |  |  |  |

Table 1. Characteristics of sockeye salmon returns to Malki hatchery

Note. \* - including the return from the generation released with the average mass of 1g.

We also have tried to estimate the ratio between wild and hatchery adult sockeye salmon for the base reservoirs of Malki and Ozerki hatcheries (for Bystraya and Plotnikova Rivers respectively). We realize that this estimation is approximate because reliable information on hatchery fish straying is missing, and poaching in each of the reservoirs

is hard to estimate (though its intensity is most likely the same for both wild and hatchery salmon). Table 2 provides aerial estimates of salmons runs in Bystraya and Plotnikova Rivers (kindly granted by A.V. Maslov) and information about salmon runs to the hatcheries (granted by Sevvostrybvod). The contribution of hatchery fish to reproduction in these reservoirs has been estimated on the basis of these data.

According to the data Table 2, the activity of both hatcheries greatly influences the rate of summer sockeye salmon reproduction in the basins of these reservoirs.

In 2002 the otolith structure was described for 328 fishes caught from July 25 to August 8 within Bolshaya River estuary. Fourteen individuals had the marks of Malki hatchery and 2 individuals had the marks of Ozerki hatchery. These data allowed us to assume that the percent of marked sockeye salmon in the catches in Bolshaya River was approximately 4.88% (Malki hatchery – 4.27%, Ozerki hatchery – 0.61%). Among sockeye salmon of Malki hatchery 13 individuals (93%) had the mark of 1999 (3-years old), 2 individuals (7%) – of 2000 (2-years old). Among Ozerki hatchery fishes one individual (3-years old) had the mark of 1999 and the other (2-years old) - of 2000.

According to the statistics of salmon catches in Bolshaya River for 2002, 83.45 tons of summer sockeye salmon was harvested. The data (calculated by five-day periods) taken together with the information on the percent of marked fishes in samples at a certain date provided us with estimation of the hatchery sockeye salmon harvested (Table 3).

Here one should note that the period of sampling (July 25 – August 8, 2002) was shorter than the entire period of sockeye fishing in Bolshaya River (July 16 – September 5, 2002). We considered it reasonable for estimation of hatchery fish removal for the time until 25 July and after 8 August to use minimal values in available samples (1.63%). With that we estimated the approximate value of hatchery fish removal for corresponding periods as 0.35 tons. So, in total about 4.4 tons of hatchery fish were removed (3.9 tons of Malki and 0.5 tons of Ozerki hatcheries). Here we ignored catches by poachers.

The available data let us make some more conclusions. Knowing the average fish weight in the samples (2.86 kg), we could estimate the number of harvested hatchery sockeye salmon – approximately 1540 pcs (1360 pcs – from Malki hatchery, 180 pcs – from Ozerki hatchery). Among harvested Malki hatchery fish, 1265 individuals were 3-years-old and 95 - 2-years-old. Taking into account the first value in estimation of return coefficient from the release in 1999 increases the coefficient from 1.08 (1.16) to 1.18 (1.24)% (see Table 1). The percentage of harvested Malki hatchery sockeye salmon to its total return to the Bolshaya River (harvest + run to the hatchery) provides us with insight to the intensity of this fishery. The latter turned out rather low – 9.1% only. But, according to the Figure 1 Malki hatchery sockeye salmon was harvested mainly in the beginning of the fishery period. In the other words we may say that a relatively early run of Malki hatchery sockeye salmon to the river to a certain degree

| River      | Total number of<br>spawners in spawning<br>grounds, x10 <sup>3</sup> | Escapement to the hatcheries, x10 <sup>3</sup> | Total escapement,<br>x10 <sup>3</sup> | Hatchery fish contribution, % |
|------------|----------------------------------------------------------------------|------------------------------------------------|---------------------------------------|-------------------------------|
| Bystraya   | 45.1                                                                 | 15.0                                           | 60.1                                  | 25.0                          |
| Plotnikova | 16.8                                                                 | 3.5                                            | 20.3                                  | 17.2                          |

Table 2. The ratio between wild and hatchery summer sockeye salmon in Bystraya and Plotnikova Rivers in 2002.

Table 3. Estimation of hatchery sockeye salmon harvested in Bolshaya River in 2002.

| Period      | Harvest, tons | Percent of hatchery fish, % | Harvest of hatchery fish, tons |
|-------------|---------------|-----------------------------|--------------------------------|
| 16-20.07    | 0.4           |                             |                                |
| 21-25.07    | 7.63          | 5.45                        | 0.42                           |
| 26.07-01.08 | 11.25         | 12.00                       | 1.35                           |
| 02-05.08    | 38.02         | 4.00                        | 1.90                           |
| 06-10.08    | 13.58         | 1.63                        | 0.37                           |
| 11-15.08    | 3.88          |                             |                                |
| 16-20.08    | 4.03          |                             |                                |
| 21-25.08    | 3.00          |                             |                                |
| 26.08-01.09 | 1.42          |                             |                                |
| 01-05.09    | 0.24          |                             |                                |
| Total:      | 83.45         |                             | 4.04                           |

allowed the fish to avoid a more intensive press of fishery. The reason is probably the use for artificial reproduction in 1998 of sockeye salmon from the earliest runs to Malki hatchery. From the data by Sevvostrybovod the last portion of eggs at that year was set for incubation on September 9 (usually incubation lasts up to the end of the second or even third week of September).

So, the described situation suggests one possible solution of the problem of fishery differentiated removals of individuals of different reproduction types. The fears of some researchers (Reisenbichler and McIntyre 1977; Allendorf and Ryman 1987; Fleming and Gross 1989, 1992) regarding the possibility of "genetic degradation" of salmon stocks with a big percent of hatchery fish may be, in our opinion, partially reduced by efficient temporal management of the fishery, differentiating between the time of spawning migration populations of natural and hatchery origin.

Fig 1. Dynamics of catches summer sockeye salmon in Bolshaya River (by five-day periods) and the percent (in these catches) of marked fish of Malki and Ozerki hatcheries in 2002.



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## The Use of the Method of Mass Marking of Salmon for the Studies of Age Structure of Wild and Hatchery Adult Sockeye Salmon

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Keywords: Sockeye salmon, otoliths, mark, scale, age

Both wild and hatchery sockeye salmon return to the basin of the Bolshaya River. Two hatcheries (Ozerki and Malki) in addition to numerous natural spawning grounds, release from 5 to  $15 \times 10^6$  juvenile sockeye salmon annually. Differentiation of wild and hatchery fish requires accurate age reading based on reliable criteria. It has been found that the age marks in the registering structures, including bones, otoliths and scale, can be created not only as a result of seasonal periodic growth, but by using artificial conditions that cause a delay or acceleration of the growth rate (Mina and Klevezel 1970). A reliable method to test the concordance of the number of annual rings in scales and the real age is to study the scales of fish of known age (Nikolsky 1974). Therefore, for the hatchery fish, the most accurate method of aging is from reading code-bearing otoliths. Thus, the hatchery mass otolith marking (Akinicheva and Rogatnykh, 1996), in our view, can resolve the problem of reading the age from scales. Every year since 1995 mass sockeye salmon marking according to this method has been used in the Malki Hatchery, and has been used since 2000 for the identification of coded fishes. The age of 300 adult sockeye salmon returning to the hatchery in 2000 were based on their otolith codes as the difference between the year of return and the year of juvenile release. Principal differences have been found in the estimation of total (freshwater plus oceanic) age of these fish from the scale structure and their otolith mark. The method of determination of the freshwater and the oceanic growth zones in the otolith has been worked out.

The purpose of this work was to verify the scale-read sockeye salmon ages according to the results of the hatchery otolith mark reading.

The work on the studies of the age structure of wild and hatchery salmon was launched in 2000. The analysis of sockeye salmon returning to Malki Hatchery in 2000 indicated for the first time that the age estimated from scales often did not coincide with the age estimated from the otoliths. Deviation took place in 40% of the cases. The otolith mark method (Akinicheva and Rogatnykh 1999; Chebanov and Kudzina 2000; Kudzina 200, 2001) provided us with the possibility to check both ocean and freshwater periods in the study of sockeye salmon age (Fig.1). The photo was made in incident light. Dark stripes should characterize the growth for the summer period. Three dark rings seen in the center of the otolith (*a*-*d*) characterized the freshwater zone. Oceanic period of sockeye salmon life should be characterized by the d-g parts of the otolith. At the thin section the distance from the center to the otolith edge (a-g) was equal to 1.486mm. The largest increment appeared for the first ocean year (d-e) and was equal to 0.394mm. The distances a-b, b-c, c-d were 0.186, 0.187 and 0.172mm respectively, and the distances e-f and f-g were 0.287 and

**Fig. 1.** Photo of the otolith of 2.3+ sockeye salmon (ocular 10\*\22, objective 2.5). A – otolith center, b – first summer in the river – yearling, c – first annual freshwater ring, d – second annual ring, the end of freshwater period, beginning of migration to the sea, e – first ocean summer, f – second ocean summer, g – third summer, return to the river for spawning.



0.260mm. The otolith itself and its' center (a) had been initiated much earlier compared to the time of embryonic "eyed" stage initiation. The distance a-b should characterize the period from the "eyed" stage to the yearling. The ring b is not annual, and it characterizes the first summer of sockeye salmon growth indicating the time of scale formation. Scale-read age of this fish was 2.3+.

In 2002 the otoliths of 193 adult sockeye salmon from Malki Hatchery were analyzed. It was found that fish in the return, had marks indicating they were mostly released in 1999. All sockeye salmon which otoliths were analyzed immigrated to as yearlings. The otoliths of these fishes bore three ocean zones; maximum growth was noted for the first ocean year (Fig. 2). As the year of juvenile release was known we had estimated the correct age of the fish (3-year-old). Reading the age from the scale was difficult because the scales were collected from prespawning fish in which **Fig. 2.** Photo of the otolith of an age of 0.3+ sockeye salmon returned to the Malki Hatchery in 2002. A – otolith center, b – first summer, migration to the sea, c – first ocean year, d – second ocean year, e – third ocean year.



the scales were partially destroyed including the scale edge (underestimated by one ocean year). In other circumstances, some scales had a ring of sclerites situated close to each other in the center of the scale, and could be mistakenly identified as a freshwater ring. That additional ring appeared in the scale structure of Malki Hatchery fish as a result of special conditions of juvenile rearing in this hatchery (Bugaev et al. 2001).

In 2002 the otoliths and the scales from 328 sockeye salmon were collected within the area of the Bolshaya River outlet. Analysis of the otoliths indicated that 14 fish were of hatchery origin. These were 0.3+ fishes released in 1999 according to the Malki Hatchery code observed. Age parameters in the otolith were similar to the parameters demonstrated in figure 2. In the course of the age reading from scale for these fish by the standard method, there were two age categories subdivided -1.3+(1) and 1.2+(2) (Fig. 3). If we did not know these fish were of hatchery origin, determination of their age from scales would have been incorrect.

**Fig. 3.** Photo of 0.3+ sockeye salmon scale (the fish bears Malki Hatchery mark in the otolith), *a*, *b*, *c*, *d* – the zones of sclerites situated close to each other.



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## DNA Analysis Increases the Utility of Other Stock Identification Methods in Sockeye Fisheries Management

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Keywords: Stock Identification, scale pattern analysis, sockeye, microsatellites

Fraser River sockeye return mainly as 4-year olds to coastal waters in one of four run-timing groups: Early Stuart, Early Summer run, Summer run, and Late run. The run-timing groups comprise many independently spawning populations of sockeye in the Fraser River. The characteristic run-timing of these populations relative to one another enables direction of higher exploitation rates toward healthy stocks and reduced fishing on weaker ones. Scale patterns reflect different growing conditions among lakes, and these patterns have been applied successfully to distinguish among Fraser sockeye stocks and runs in the past (Henry 1961). Scale patterns among some major Summer run and Late run stocks have converged recently, thereby reducing the utility of scales in stock identification. Compounding this problem is a concurrent reduction in the availability of scales from 3-year olds on the spawning grounds. In-season management on the basis of scale-based stock identification is therefore hindered because predictions of scale patterns are uncertain for returning 4-year olds. Post-season and in-season estimates of stock composition can differ considerably as a result.

Analysis of DNA provides accurate and precise estimates of stock composition. Because DNA-based analyses are not afflicted by the problems recently affecting scale-based analyses, DNA was used extensively for Fraser sockeye management in 2002 (Beacham et al. in press). Data provided via DNA are extremely informative but genetic analyses must be judiciously applied due to financial limitations. Scales are examined for purposes other than stock identification (such as estimation of age composition and evaluation of growing environments in lakes) and are not expensive to analyse, so we investigated the possibility of increasing their effectiveness in stock identification by the use of matched DNA analyses.

Our scale analysis procedure is outlined in Gable and Cox-Rogers (1993). Briefly, we examine the zone of freshwater growth of scales from sockeye sampled from mixed-stock fisheries and compare, via linear discriminant functions, four measured variables to standards based on equivalent data from scales sampled on the spawning grounds. Composition of the standards is influenced by run-strength forecasts. Genotypes of sampled fish were compared to a 47 population baseline including 15 genetic loci (see Beacham et al. in press). An individual sockeye's scale data were matched to its DNA-based stock of origin using Geneclass (Cornuet et al. 1999). Other data, including length, were also matched. Stock-specific lengths obtained from the spawning grounds are collected too late for in-season management, and they differ from lengths in fisheries because of wear and sexual maturation. We developed in-season scale standards from DNA-based identification of individuals and compared results to estimates that would otherwise have been available in-season. We compared marine lengths, scale resorption, and texture of gonads among stocks to evaluate the possibility of applying in-season DNA analyses to generate stock identification models using those variables.

Some stocks are not easily distinguished using scales and so the number of stocks included in the baseline for scale analyses is limited according to the strength and timing of runs. Stocks that rear in the same lake can have different migration timing and, whereas these are likely indistinguishable using scales, DNA analyses can resolve their relative abundances. Knowing proportions of these stocks allows better interpretation of scale results and can be used to describe run progression. For example, in-season estimation of the precise timing of the Summer run using only scale data and estimated abundance is difficult. Components of the Summer run are identifiable using DNA, however, and because these also have characteristic relative timing they can provide independent information on whether the Summer run is half or three quarters complete. This information can be used to predict appropriate baselines for scale analyses of samples in subsequent weeks.

Use of DNA can help scale analyses not only by suggesting the incorporation of appropriate stocks in baselines but also by improving the accuracy of the scale characteristics estimated for baseline stocks. Stock identification analyses based on scale standards generated using matched DNA were similar to post-season results generated using scale standards obtained from the spawning grounds. In contrast, scale standards from pre-season predictions yielded models whose results differed strongly from those obtained in the post-season. Only one season has been examined, but the results observed indicate that updating scale standards via matching DNA samples collected inseason can potentially improve in-season results from scale models.

Length is another variable that could be estimated on an age- and stock-specific basis using in-season DNA analyses. In 2002, post-orbit to fork distance in Summer run sockeye was less than in Late run sockeye in general (P < 0.0001 for both males and females), but the dominant stock groups in the Summer run and Late run did not differ greatly. Because males tend to be longer than females from the same stock (combined P < 0.0001), separate discriminant function models should be constructed for males and females, which would unfortunately increase sample size requirements. Nevertheless, including length in stock identification models can apparently be useful in some years for discriminating some stocks.

We evaluated other characteristics that change over time which might distinguish Summer run and Late run sockeye, and we found significant differences in scale resorption (P < 0.001) and gonad texture (P < 0.005 for both males and females) among the dominant stocks in each run. Scale resorption appears particularly promising: over 80% of Summer run sockeye tested had resorbed scales, whereas only 50% of Late run sockeye from the same samples had scales that were significantly resorbed.

These results demonstrate how DNA-based stock identification can improve existing stock identification techniques and be used in the development of new ones. The benefits of such an approach are not limited to the management of Fraser River sockeye.

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## Evaluating the Efficacy of Probabilistic Neural Networks to Determine Stock Structure in Sockeye Salmon Using Fourier Transformed Luminance Profiles of Scale Circuli

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Keywords: Salmon, stock separation, fish scales, neural networks, Fourier analysis

Patterns of circuli groupings within scales are used to determine the stock structure of sockeye salmon (*Oncorhynchus nerka*). The methodology typically employed involves using trained scale readers to interpret and manually measure circuli spacing patterns. These measurements are used as input into Linear Discriminant function Analysis (LDA) to determine stock structure. This pilot study introduces a new technique, probabilistic neural networks, to evaluate scale patterns for stock composition. We compare the method directly to LDA by using the same measurement data as input. We then explore Fourier analysis of luminance profiles of the scale images as an objective means to classify scale patterns. The samples used in the pilot study are from two Canadian stocks and one Alaskan stock encountered in South-east Alaskan fisheries. Correctly identifying these stocks has been a challenging problem for fisheries management.

The probabilistic neural networks (PNNs) used for this study were implemented using proprietary software (Ward Systems Neuroshell®). PNNs are intrinsic classification models and are known for their ability to quickly train (Masters 1993). The PNN categorised the frequency transformed luminescence profiles from scales into one of three output categories, each representing a discrete stock. The PNN provides a probability density function of stock-membership as an output where the most probable stock identification is classified in the output vector as the element with the highest value. A 'sphere of influence' weighting function, a multi-variate extension of Parzen's method (Masters 1995), is used to map the inputs to their respective output. The width of the 'sphere of influence' is determined by a scaling parameter that varies between input variables. As there is no objective method for determining the size of this scaling parameter for each input element.

A total of 599 scale samples were obtained as acetate impressions from US and Canada sampling projects by ADFG in 2002. These include 200 samples from nine areas within Alaska, 199 samples from the Nass River system and 200 from the Skeena River system in Canada. They were analysed as mixed stock samples with LDA following established procedures that use scale measurement data to estimate stock composition of commercial catches (Bloomquist et al. 2002). Digital images of the scales (8 bit, 14.8 M pixels) were obtained with a digital microfiche system designed for scale analysis (Hagen et al. 2001) and transmitted electronically in JPEG 2000 format along with the scale measurement data and the associated sampling data (area of capture, length, age and sex) to CAF laboratory in Australia. No information was available on the sex of the Skeena River samples.

To create datasets for PNN analysis in this study, the images were reduced to 1.1M pixels using Leadtools File Converter<sup>TM</sup> and selected for further analysis where the impression was suitable for extraction of pixel data. Subsequently, the number of samples used for analysis was 199, 199 and 191 (n = 589) for the Alaskan, Nass River and Skeena River area of capture respectively. Optimas<sup>TM</sup> image analysis software was to draw six transects from the focus of each scale, radiating in approximate equal steps around the posterior region of the scale (Fig. 1). From each transect, luminance profiles were extracted from the first 128 pixels or sampling points (Fig. 2). This distance covered the period of freshwater growth, but did not cover the first marine annulus as did the measurement data.

The grey scale values in the profiles were collected as complex numbers using the Discrete Fast Fourier Transformation, (FFT, Equation 1). To minimise spectral leakage from the FFT, it was necessary to window the data. This was accomplished using the Welch window (Equation 2, Fig. 3). This is needed as the FFT assumes the time domain sample is periodic, and is captured over an integral number of periods (the end of the series implicitly wraps to the beginning). This is not the case with transect data.

**Fig. 1.** Acetate scale impression from an Alaskan sockeye salmon (AK0212U0001) sampled from Hugh Smith Lake (490 mm mid-eye to fork length, 3). Ideal location of transects shown (T1-T6). The number postscripts represent the marking sequence. Transect location varied by up to approximately 10 degrees from the ideal transect locations. Mark {A} represents the approximate length of 128 pixels from the biological center of the scale.



**Fig. 2.** Extracted Luminance profile (T1) from a sockeye salmon (AK0212U0001) scale acetate press sampled from Hugh Smith Lake (490 mm mid-eye to fork length,  $\Diamond$ ).



The data was transformed into the time domain using the inverse FFT (Equation 3). The pixel values were multiplied by the Welch window and transformed back to the frequency domain. The power (harmonics) of the Fourier series was calculated as the absolute value of the complex number (Equation 4). Since reconstruction of the original luminance profile from the transect which was adequately described using 21 complex numbers (Fig. 4), the power from the first 21 complex numbers was used as inputs to the network (Fig. 5). Network models were tested using harmonics from single transects and combinations of transects; both with and without length and sex data (Table 1). Sex data was treated as categorical inputs with 1=male, 2=female and 3=unknown.

Individual data sets, which consisted of either a single array or combinations of arrays of harmonics were randomly divided into three sets; these were the training set (60%), test set (20%) and validation set (20%). The training set was used for model minimisation, the test set was used to determine when training was complete and the validation set was used as an unseen data set to evaluate the model. Results are presented for the validation sets. The original measurement data set (n = 599) was also randomly divided using the same techniques that were used for the neural network classification of harmonics. These results were used as a comparison for the results obtained using LDA, and from those obtained from the transect data.

The results from the neural network classification using the measurement data showed an overall correct classification of 0.84, 0.84 and 0.86 for the Alaskan, Nass and Skeena stocks, respectively. This was essentially the same result as the LDA analysis that showed an overall correct classification of 0.84 for the entire data set. The classification accuracy using harmonics was less than when using the measurement data and appeared to be variable

depending on the choice of transects and whether length and sex were included (Table 1). In general, the addition of biological data increased classification rates. For instance, the highest classification rates achieved without biological data were from the Nass River (0.80); while the highest classification rates from the Nass River stock with the addition of biological data was 0.89. High classification rates for the Skeena River may be an artefact of unknown sex and subsequently requires further investigation.

This pilot study demonstrates the utility of neural networks for handling difficult classification problems and as a tool for developing new scale analysis approaches for stock separation. We found the method to be flexible: it did not require the explicit construction of algorithms to develop classification models and could be adapted readily to new datasets. In this study PNN was comparable to LDA when using same dataset and it allowed us to explore harmonics of the luminance profiles as a new dataset – one which could be obtained with a significant savings in labour. While the use of harmonics will require further investigation – such as using longer profiles to cover the first marine growth and combining it with measurement and sampling data – we believe it could lead to a more accurate and cost effective mechanism to determine stock relationships.

**Equation 1.** Discrete Fast Fourier transform used to transform the signal data from individual greyscale values from the time (t) domain to the frequency domain (f). Note return of complex number in the form of (a+bi).

$$H(f) = \sum_{t=0}^{n-1} h(t) \cos(2\pi f t) + i \sum_{t=0}^{n-1} h(t) \sin(2\pi f t)$$

Equation 2. Welch data window. Used to "pull" both ends of the luminance values close to zero.

$$w_i = 1 - \left(\frac{1 - 0.5(n - 1)}{0.5(n + 1)}\right)^2$$

**Equation 3.** Inverse Discrete Fast Fourier transform used to transform the signal data from the frequency (*f*) domain to the time domain (*t*).

$$h(t) = \frac{1}{n} \sum_{f=0}^{n-1} H(f) \cos(2\pi f t) + i \frac{1}{n} \sum_{f=0}^{n-1} H(f) \sin(2\pi f t)$$

**Equation 4.** The power (harmonics) of the Fourier series was calculated as the absolute value |z| of the complex number.

$$|z| = \sqrt{a^2 + bi^2}$$

Fig. 3. Welch window function over the range 0–128.



**Fig. 4.** Original Luminance profile after application of Welch window (blue series) with reconstructed profile using 21 complex numbers from the Fourier series (pink series) from T1 - AK0212U0001.



**Fig. 5.** Harmonics from T1 - AK0212U0001. The first 21 were used as inputs to the neural network.



Table 1. Neural network inputs used for classification of scales for the three areas. Number of inputs in braces represent number of inputs including length and sex.

|                    |                      | Number    | Without Biological data |        | With   | length and | d sex  |        |
|--------------------|----------------------|-----------|-------------------------|--------|--------|------------|--------|--------|
|                    | Input                | of inputs | Alaska                  | Nass   | Skeena | Alaska     | Nass   | Skeena |
| Single transects   | Transect 1           | 21 (23)   | 0.6667                  | 0.6098 | 0.3243 | 0.8333     | 0.7778 | 1      |
|                    | Transect 2           | 21 (23)   | 0.2564                  | 0.6341 | 0.2703 | 0.6389     | 0.8444 | 1      |
|                    | Transect 3           | 21 (23)   | 0.2564                  | 0.5122 | 0.4595 | 0.6667     | 0.8222 | 0.9722 |
|                    | Transect 4           | 21 (23)   | 0.5385                  | 0.4878 | 0.4595 | 0.8056     | 0.6667 | 1      |
|                    | Transect 5           | 21 (23)   | 0.5128                  | 0.5366 | 0.3784 | 0.6944     | 0.6667 | 0.9722 |
|                    | Transect 6           | 21 (23)   | 0.2564                  | 0.6341 | 0.2703 | 0.6111     | 0.8    | 1      |
| Multiple Transects | Transect 1,6         | 42 (44)   | 0.4615                  | 0.6098 | 0.2703 | 0.8333     | 0.8222 | 0.9444 |
|                    | Transect 2,5         | 42 (44)   | 0.4872                  | 0.6341 | 0.5676 | 0.6389     | 0.8222 | 0.9444 |
|                    | Transect 3,4         | 42 (44)   | 0.4103                  | 0.8049 | 0.3243 | 0.75       | 0.7778 | 1      |
|                    | Transect 3,4,5       | 63 (65)   | 0.4103                  | 0.8049 | 0.3243 | 0.5833     | 0.8444 | 0.9167 |
|                    | Transect 1,2,3,4,5,6 | 126 (128) | 0.5128                  | 0.7561 | 0.2973 | 0.5833     | 0.8889 | 0.6944 |

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## Population Structure and History of Steelhead Trout in California

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Keywords: Steelhead, genetics, microsatellites, phylogeography, bottlenecks

Steelhead trout (*Oncorhynchus mykiss*) are the most widespread of the anadromous salmonids. In the western continental United States, 14 Evolutionarily Significant Units (ESUs) have been delineated on the basis of genetic, geographic and ecological variation. Of these 14 western ESUs, 11 have been listed as protected under the US Endangered Species Act (ESA). In California, there are six ESUs, 5 on the coast and 1 in the Central Valley. All but the Klamath Mountain Province ESU is listed, with the Southern California ESU classified as "Endangered" and the others "Threatened".

Here, we investigate genetic population structure and demographic history of steelhead trout in coastal California using multilocus genetic data. We use size variation at highly variable microsatellite loci from populations at 62 sites from 41 basins, covering almost the entire range of the species in coastal California. The list of sites is found in Table 1. Results of phylogeographic analyses and assignment tests are described and the partitioning of variation at the tributary, river basin and ESU level is examined.

| Table 1. Populations sar | npled in this study. | Populations are listed     | south to north. I   | Ho is observed I | heterozygosity.  | No of alleles is |
|--------------------------|----------------------|----------------------------|---------------------|------------------|------------------|------------------|
| the mean across 18 loci. | M is the mean M ra   | atio = no. of alleles/(ran | nge in allele size- | +1). *=Significa | nt values of the | M-ratio.         |

| Watershed        | Ho    | No. of alleles | М      | Watershed      | Но    | No. of alleles | М      |
|------------------|-------|----------------|--------|----------------|-------|----------------|--------|
| Santa Ynez-Hilt  | 0.640 | 10.5           | 0.683* | Big River      | 0.648 | 12.3           | 0.702* |
| Santa Ynez-Sal   | 0.512 | 14.1           | 0.623* | Noyo           | 0.687 | 11.7           | 0.721* |
| Chorro Creek     | 0.687 | 5.8            | 0.629* | Noyo-Kass      | 0.756 | 12.7           | 0.733  |
| San Simeon Ck    | 0.722 | 9.0            | 0.710* | Noyo-LNFk      | 0.722 | 12.8           | 0.696* |
| WIIIow Creek     | 0.666 | 12.1           | 0.674* | Noyo-SFk       | 0.709 | 11.5           | 0.653* |
| Big Creek        | 0.697 | 10.7           | 0.760  | Pudding Creek  | 0.647 | 8.2            | 0.663* |
| Big Sur River    | 0.705 | 11.6           | 0.709* | TenMile-LtNFk  | 0.704 | 10.8           | 0.667* |
| Carmel River     | 0.696 | 8.1            | 0.715* | TenMile-LSFk   | 0.654 | 10.1           | 0.697* |
| Pajaro River     | 0.686 | 12.3           | 0.652* | TenMile-Redwd  | 0.682 | 11.5           | 0.708* |
| SLor-Bear        | 0.608 | 11.6           | 0.625* | TenMile-Smith  | 0.711 | 10.9           | 0.709* |
| SLor-Boulder     | 0.717 | 9.5            | 0.679* | Wages Creek    | 0.726 | 12.7           | 0.719* |
| SLor-CarbCk      | 0.673 | 8.2            | 0.636* | Usal Creek     | 0.685 | 12.9           | 0.725* |
| SLor-Zayante     | 0.724 | 10.2           | 0.679* | Big-Lost Coast | 0.703 | 10.4           | 0.698* |
| Scott Creek      | 0.662 | 11.8           | 0.625* | Mattole River  | 0.689 | 10.5           | 0.705* |
| Waddell Creek    | 0.667 | 7.8            | 0.685* | Bear River     | 0.646 | 12.3           | 0.749  |
| Pescadero        | 0.685 | 9.1            | 0.643* | Eel-Hollowtree | 0.697 | 11.7           | 0.730* |
| San Pedro        | 0.692 | 8.1            | 0.668* | Eel-Indian     | 0.557 | 7.0            | 0.722  |
| LTrancos-SF Bay  | 0.631 | 9.8            | 0.637* | Eel-Lawrence   | 0.612 | 13.3           | 0.795  |
| Miller Ck-SF Bay | 0.636 | 8.2            | 0.645* | Eel-Willits    | 0.687 | 10.5           | 0.655* |
| Redwood Creek    | 0.699 | 13.0           | 0.741  | Freshwater Ck  | 0.675 | 11.0           | 0.753  |
| Lagunitas        | 0.691 | 13.8           | 0.737  | Mad-BlueSlide  | 0.657 | 10.8           | 0.695* |
| Lagunitas-Olema  | 0.697 | 12.0           | 0.698* | Mad-Canon      | 0.717 | 10.1           | 0.725* |
| Lagunitas-Bline  | 0.665 | 9.2            | 0.623* | Mad-Sullivan   | 0.707 | 7.1            | 0.591* |
| Walker Ck        | 0.679 | 9.4            | 0.674* | Redwd-LostMan  | 0.691 | 11.6           | 0.711* |
| Russian River    | 0.705 | 9.2            | 0.694* | Redwd-Panther  | 0.727 | 12.8           | 0.705* |
| Gualala River    | 0.664 | 8.4            | 0.720* | Redwd-Prairie  | 0.648 | 9.5            | 0.720* |
| Garcia River     | 0.645 | 9.9            | 0.726* | Klamath-Blue   | 0.727 | 12.7           | 0.794  |
| Elk Creek        | 0.698 | 10.2           | 0.704* | Klamath-Hunter | 0.614 | 10.3           | 0.694* |
| Navarro River    | 0.724 | 13.5           | 0.809  | Klamath-HLinto | 0.749 | 11.9           | 0.792  |
| Big Salmon       | 0.690 | 12.5           | 0.708* | Wilson Creek   | 0.664 | 12.0           | 0.819  |
| Albion River     | 0.692 | 8.5            | 0.648* | Smith River    | 0.751 | 11.7           | 0.801  |

Samples were collected from June to October 2001 by electrofishing. Small tissue clips were taken non-lethally from caudal fins of juvenile trout and dried on blotter paper. Samples were collected from five habitat units per site and only fish believed to represent young of the year were included in the analyses, in attempts to minimize the number of related individuals in the sample and include only one cohort. Steelhead are iteroparous and can have a highly variable life history strategy. When they are not anadromous, they are called rainbow trout. At present, all fish found in water bodies with ocean access are classified as steelhead, and those above barriers to anadromy as rainbow trout. We thus treat all sampled fish as steelhead, though we can not be certain that they are anadromous.

An average of 66 fish per site was analyzed with 18 microsatellite loci. They include genes with a wide range of variability (from 4 to 64 alleles) that were originally isolated in several species, both to minimize ascertainment biases. Genomic DNA was extracted from the dried fin clips using a semiautomated protocol. Genotypic data was generated via the PCR with fluorescent primers and electrophoresis was done on automated sequencers. Two people performed all allele calls independently and any discrepancies were resolved by mutual agreement.

We found substantial genetic variation in coastal California steelhead with a total of 540 alleles, as defined by number of repeats, at the 18 microsatellite loci assayed. The mean number of alleles per locus varied from 5.8 in Chorro Ck. to 13.8 in Lagunitas Ck (Table 1). Heterozygosity varied from 0.557 in Indian Ck (Eel River) to 0.756 in Kass Ck. (Noyo River). We also examined the population genetic data for evidence of recent reductions in population size, or bottlenecks, using the M-ratio method of Garza and Williamson (2001). In general, we found a trend of increasing evidence for bottlenecks from north to south, with no significant tests among the 5 non-ESA protected populations from the Klamath ESU (Table 1). We also examined independence of populations through use of tests of genic population differentiation (Raymond and Rousset 1995). Every pairwise comparison of samples from our study yielded a highly significant test.

To evaluate the relationships between sites, we calculated several population-based measures of genetic distance and population subdivision. These include Fst, the standardized variance in allele frequencies among sites, and Cavalli-Sforza and Edward's (1967; CSE) chord distance.

Fst was transformed as Fst/(1-Fst) and regressed on geographic distance as measured by rivermouth distance plus stream miles. The relationship was highly significant, with  $R^2 = 0.204$ . Thus, there is a strong signal that migration is dependent on distance, leading to a pattern that has classically been called isolation by distance, with geographic distance alone explaining about 20% of the genetic variation in the samples. This dependence of population structure on geographic distance was also evident in the matrices of genetic distance and the trees constructed with them. The consensus tree for 1,000 bootstrap replicates with CSE distances and the neighbor joining (NJ) tree-building algorithm is found in Fig. 1. The concordance with geography is very high, with the topology almost entirely consistent with geographic proximity. The CSE/NJ tree is also characterized by concordance with geography, and also by long terminal branch lengths.

**Fig. 1.** Bootstrap consensus tree for 62 steelhead populations. Genetic data were bootstrapped 1,000 times, CSE chord distances calculated and neighbor-joining trees constructed. The majority-rule tree consensus tree is shown with internal branches representing the number of times that grouping was found.



These long terminal branches are reflected in the power of assignment tests to assign individuals to their population of origin. We found that 76% of individuals were correctly assigned to their population of origin with our dataset. When assignment to another site within the same basin was not considered an error, this accuracy rose to over 80%. The frequency with which individuals were misassigned across ESU boundaries was well over 90%. It should be noted that this assignment accuracy is probably affected by the presence of related individuals in our sample and would thus decrease slightly with individuals from other year classes. However, the high assignment accuracies indicate that genetic data can be used to accurately assign individuals to river of origin, even on small spatial scales, and that a combination of limited migration and local adaptation is shaping steelhead population genetic structure in coastal California.

In summary, we have examined multilocus genetic data from 62 populations of steelhead trout in all five of the coastal California ESUs. The results of our work indicate that population structure of steelhead trout in coastal California has been largely unaffected by hatcheries and remains influenced primarily by migration, which is dependent on distance. The significant relationship between geographic and genetic distance, as well as the high concordance of geography with genealogy, are indicative of this. The long terminal branch lengths and high assignment accuracies indicate that, while migration is important, drift and local adaptation likely contribute to the differentiation between all populations in our study.

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## Workshop Review

Over the past several decades the biomass of Pacific salmon has shown significant fluctuations, and the pattern of these fluctuations differs among species as well as local stocks. Recent attention has focused on how the ocean environment and variable marine ecosystems affect these fluctuations and the abundance and distribution of salmon stocks. Information on the oceanic migration pattern and marine survival of individual stocks is essential for understanding the population dynamics of these species. Further, despite hatchery releases over the last decades, biological interactions between wild and hatchery fish in the ocean are poorly understood.

Stock-specific biological information has been provided by various stock identification techniques including tags, parasites, scale patterns, and genetic marks. The Workshop explored where and how these techniques are applied, what types of information are being generated, and what directions are desirable in the future. A consistent theme of the Workshop was the need for cooperation and collaboration to develop comprehensive and transferable methods and maximize research opportunities.

Contributed papers were organized around species. A synopsis of the presented work by species follows:

#### Chum salmon

Significant efforts have been directed towards gathering stock-specific information on chum salmon. Genetic databases have been particularly useful in this species. An extensive allozyme baseline was cooperatively developed and shared by the NPAFC Parties to determine stock components of mixtures of stocks at various life stages in coastal and high seas areas of the Bering Sea and Pacific Ocean. The oceanic migration pattern of Japanese chum salmon throughout their entire marine life cycle was estimated using this baseline. Stock-specific distribution and migration of juvenile and adult chum salmon of North American stocks in the shelf of eastern Bering Sea and Gulf of Alaska were also investigated using the allozyme baseline. Variation in the mtDNA control region and microsatellite loci are also under investigation. Japanese and United States laboratories are investigating DNA microarray and analysis of single nucleotide polymorphisms (SNPs) as rapid and easily-standardized methods of stock identification. In addition, mass otolith marking has been useful to determine hatchery origins, and a combination of genetic and otolith marks has been used in understanding biological interactions between hatchery and wild fish.

### Chinook salmon

Historically scale pattern analyses were used extensively to estimate the composition of mixed-stock fisheries for chinook salmon in the Pacific Northwest and Bering Sea. More recently an extensive allozyme baseline was developed cooperatively among the NPAFC Parties. The allozyme baseline has been used extensively to monitor nearshore migration patterns of juvenile and adult chinook salmon. Currently multiple laboratories are developing comprehensive microsatellite and SNP databases for populations around the Pacific Rim. A large effort is currently being coordinated by the Pacific Salmon Commission to standardize a set of microsatellite loci and develop a comprehensive baseline within the Commission's area of interest.

### Coho salmon

Coho salmon have recently been surveyed for MHC and microsatellite loci. These genetic analyses suggest that there are clear stock-specific differences in the migratory behavior of the species. Development of standardized and comprehensive genetic databases for coho salmon has not yet become a priority among laboratories.

#### Sockeye salmon

Scale pattern analysis has been used extensively to separate eastern and western Kamchatka stocks of sockeye salmon in mixture fisheries within the Russian EEZ as well as stocks in freshwater. Expansion of these analyses to determine the continental origins of high-seas fish has not been successful because of similar scale structures among Asian and North American stocks. Baselines of allozyme, microsatellite, and SNP markers are under development to determine the composition of sockeye stock mixtures caught in the Bering Sea. The current baseline, composed of Alaskan and Russian stocks, can precisely estimate the stock origins among and within major regions of the Pacific Rim.

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#### Pink salmon

Pink salmon is the most abundant species among Pacific salmon and is essential to North Pacific ecosystems. Although extensive allozyme data have were collected for pink salmon across the Pacific Rim, standardized baselines have not been used extensively as has occurred in chum, sockeye and chinook salmon. The rigid two-year life cycle has produced reproductively isolated brood lines with large genetic differences, essentially requiring two independent baselines and analyses for this species. North American and Asian stocks are currently being examined for allozyme loci, and simulations are being done on even- and odd-year data sets to determine the regional groupings for mixed-stock analyses of coastal and high-seas pink salmon.

#### Statistical Analysis

For genetic data, various statistical analyses have recently been developed that are particularly appropriate for hypervariable microsatellite data with large numbers of alleles. These analyses are targeted at improving compositional estimates, dealing with incomplete baseline coverage, evaluating the relative information content among loci, and assessing the ability of the database to identify individuals to stock-of-origin. For scale data, probabilistic neural networks have been developed for handling difficult classification problems and as a tool for developing new scale analyses for stock separation.

#### Discussion

During the panel discussion a number of points emerged including the following:

- Significant information on migration patterns of Pacific salmon is emerging from the stock identification work to identify such factors as feeding areas, migration of juveniles, and extent of seasonal feeding competition. However, winter patterns are still poorly understood.
- It is important for future studies to examine the ocean carrying capacity and the interactions between hatchery and wild fish on the high-seas.
- Molecular techniques must be coordinated and standardized to develop comprehensive baselines, to extend research funds, and to maximize limited high-seas research opportunities.
- The question under consideration must first be identified before an appropriate method for stock identification is chosen.
- Sampling opportunities exist in the bycatch fisheries, and the composition of the bycatch can potentially be used as a forecasting tool.
- Significant resources have been spent on salmonid research in the last fifty years, but the legacy of that research is unclear. In the future, effort may be placed on developing mega-databases with emphasis on other species in addition to salmonids.
- Korea has recently joined the NPAFC and looks forward to future collaborations and assistance from the other NPAFC Parties.
- Collaboration of NPAFC scientists is an excellent example of international cooperation, and NPAFC successes should be communicated to policy-makers throughout the Pacific Rim.

#### Conclusions

The workshop demonstrated that information on stock identification is essential to our understanding to the marine ecosystem, potential effects of global warming, as well as the roles of hatchery releases. Significant progress has been made in stock identification, and techniques are changing rapidly with the emergence of new technologies in genetics, tagging, and microchemistry. Coordination and scientific exchanges among Pacific Rim nations is essential to insure continued progress, reduce unnecessary duplication, and maximize research opportunities.

Lisa Seeb Shigehiko Urawa Co-chairs of the Workshop Organizing Committee
## **APPENDIX 1**

## List of Participants

| Beacham, Terry<br>Beamish, Richard<br>Irvine, Jim<br>Jones, Russ<br>Kristianson, Gerry<br>Latham, Stephen<br>Trudel, Marc<br>Withler, Ruth                                                | Garza, John Carlos<br>Glazebrook, Catriona<br>Guthrie, Charles<br>Habicht, Chris<br>Hatch, Doug<br>Hawkins, Sharon<br>Helle, Jack<br>Heard, William<br>Jacobson, Kym                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
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| Agler, Bev<br>Armstrong, Janet<br>Augerot, Xanthippe<br>Banks, Michael<br>Bellinger, Renee<br>Blankenship, Scott<br>Crane, Penelope<br>Davis, Nancy<br>Flannery, Blair<br>Francis, Robert | NASCO Secretariat:<br>NPAFC Secretariat:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Windsor, Malcolm<br>Fedorenko, Vladimir<br>Kondo, Yoshikiyo<br>McGrann-Pavlovoc, Denise<br>Morris, Wakako<br>Holloway, Robyn<br>Graham, Ikumi<br>Lee, Migyong<br>Wasserman, Michael                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|                                                                                                                                                                                           | Beacham, Terry<br>Beamish, Richard<br>Irvine, Jim<br>Jones, Russ<br>Kristianson, Gerry<br>Latham, Stephen<br>Trudel, Marc<br>Withler, Ruth<br>Abe, Syuiti<br>Kaeriyama, Masahide<br>Moriya, Shogo<br>Nagasawa, Toru<br>Nagata, Mitsuhiro<br>Ueda, Hiroshi<br>Uoya, Toshinori<br>Urawa, Shigehiko<br>Kang, Sukyung<br>Oh, Bong Se<br>Park, Jung Youn<br>Seong, Ki Baik<br>Bugaev, Alexander<br>Gritsenko, Oleg<br>Karpenko, Vladimir<br>Klovatch, Natalia<br>Temnykh, Olga<br>Varnavskaya, Natalia<br>Volobuev, Vladimir<br>Volvenko, Igor<br>Agler, Bev<br>Armstrong, Janet<br>Augerot, Xanthippe<br>Banks, Michael<br>Bellinger, Renee<br>Blankenship, Scott<br>Crane, Penelope<br>Davis, Nancy<br>Flannery, Blair<br>Francis, Robert | Beacham, Terry Garz<br>Beamish, Richard Glaz<br>Irvine, Jim Guth<br>Jones, Russ Habi<br>Kristianson, Gerry Hatc<br>Latham, Stephen Haw<br>Trudel, Marc Hell<br>Withler, Ruth Hear<br>Jaco<br>Klein<br>Abe, Syuiti Konn<br>Kaeriyama, Masahide McN<br>Moriya, Shogo Morz<br>Nagasawa, Toru Mye<br>Nagata, Mitsuhiro Naru<br>Ueda, Hiroshi Olse<br>Uoya, Toshinori Orsi,<br>Urawa, Shigehiko Partt<br>Warava, Shigehiko Partt<br>Bugaev, Alexander Ulm<br>Gritsenko, Oleg Volk<br>Karpenko, Vladimir Wall<br>Klovatch, Natalia Wen<br>Temykh, Olga Whit<br>Varnavskaya, Natalia Wihi<br>Varnavskaya, Natalia Wihi<br>Bellinger, Renee<br>Blankenship, Scott<br>Crane, Penelope<br>Davis, Nancy<br>Flannery, Blair<br>Francis, Robert |

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