# Joint US and CA Mixed-stock Chum Fisheries Sampling 

## Design and Analysis 2016

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#### Abstract

We conducted Genetic Stock Identification (GSI) of 4342 Chum salmon migrating to natal streams through Johnstone Strait (Statistical Areas 12 and 13), along east coast of Vancouver Island (Statistical Areas 14 and 18) and through the San Juan Islands (Statistical Area 7 and 7A) for 2016 using analyses of microsatellite variation. A total of 3100 Chum salmon were analyzed for Canadian fisheries (Areas 12, 13, 14, 18) and 1154 Chum salmon for U.S. fisheries (Area 7-7A).

The analysis of chum salmon sampled in the commercial and test fisheries in Johnstone Strait were mainly from Canadian populations (ranging from 90.2\% to 99.8\%) comprised largely of Fraser River (ranging from $36 . \%$ to $55.5 \%$ ) and Strait of Georgia (east and west sides). The analysis of Chum salmon caught in commercial fisheries in the San Juan Islands were from both Canadian and U.S. origin stocks with a larger contribution of Canadian origin stocks: 99.7\% and 99.8\% in Area 7A and $95.3 \%$ to $99.2 \%$ in Area 7. The Lummi Island reef net fishery had a stock composition with $99.4 \%$ chum of Canadian origin. East coast of Vancouver Island commercial samples (Areas 14 and 18) were largely Canadian contributions ( $95.8 \%$ to $99.6 \%$ ).

Overall the failure to amplify rate was very low ( $0.18 \%$ ) for these samples. In addition, $1.3 \%$ of these samples were excluded from the analysis because the number of loci amplified was below threshold.


## Acknowledgments

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Figure 1. Map of Statistical Areas outlining Chum salmon fishing locations in southern British Columbia 2013-2016

Figure 2. Map of Statistical Areas outlining Chum salmon fishing locations in Puget Sound 2013-2016.

## Introduction

In order to facilitate management responses to Southern Chum stock strength, in accordance with Annex IV, Chapter 6 of the Pacific Salmon Treaty (The Treaty) it is necessary to provide the catch composition in fisheries targeting southern origin Chum salmon (Oncorhynchus keta). This information supports the treaty requirement Section 3 to account for US chum stocks in Canadian fisheries and to account for Canadian chum stocks in US fisheries. This is the first year of an additional four year project to sample and provide Genetic Stock Identification (GSI) on key chum mixed stock fisheries within Canada and the US. This work is replicating previous annual sample collections to obtain uniform and sufficient coverage to meet Treaty requirements.

The main fisheries targeted were Johnstone Strait purse seine and gill net commercial and test fisheries (Area 12 and Area 13-Figure 1), as well as the US commercial purse seine and gill net fisheries occurring in the areas described as San Juan Islands/Point Roberts (SJI/PR) Fishery Management Areas 7 and 7A (Figure 2). In addition, samples were also collected from the Lummi reef net fishery, more terminal east coast of Vancouver Island fisheries in Area 14 and Area 18, and by-catch summer-run Chum in Sockeye fisheries in Area 12. The Strait of Juan de Fuca (SJDF) Fishery Management Areas 4B, 5 and 6C (Figure 2) were sampled and analyzed in 2016 under the Southern Fund project "Chum sampling program for the Strait of Juan de Fuca".

Both Canadian and US Chum salmon populations were grouped into genetically distinguishable groups and must be evaluated for concordance with existing Canadian Conservation Units and Evolutionary Significant Units for conservation management purposes. Besides immediate Treaty obligations, the GSI work is part of the information required for accurate post-season run reconstructions which are essential in evaluating whether domestic management actions were consistent with meeting overall objectives of the Treaty. Run
reconstructions are also important in monitoring the productivity of stocks and assessing the adequacy of current escapement targets and both pre-season forecasting and in-season run assessment techniques. Without this knowledge, managing to achieve Treaty obligations would be difficult and severely limits the assessment of factors influencing stock productivity, which appear to have fluctuated widely in recent years.

Stock specific data collected in these mixed stock areas will provide the information, deemed necessary by the PSC Joint Chum Technical Committee (Chum TC) and the PSC Southern Panel, to develop management options addressing conservation of stocks of concern while focusing fisheries on stocks of significant abundance. It will also provide a bilaterally agreed method to determine the catch composition on all mixed stock Chum fisheries in Johnstone Strait, US areas 7 and 7A and other border fisheries in accordance with Annex IV, Chapter 6 of the Treaty.

## Materials and Methods

## Collection of DNA Samples and Laboratory Analysis

Caudal punches were taken from sampled fish by sticking tissue on Whatman paper to air dry and DNA was extracted as described by Withler et al. (2000) or placed in vials with nondenatured ethanol preservative. The samples were collected from 4324 adult Chum salmon in 2016 captured in test and commercial fisheries from British Columbia Statistical Areas 12, 13, 14, 18 from Gillnet and Seine fisheries between September 12 and November 2, 2016, 2016. There were 1154 Chum salmon captured for genetic analysis in commercial fisheries from Washington State Statistical Areas 7 and 7A between September 25 and October 26, 2016. Approximately 1000 fish sampled in the Strait of Juan de Fuca (US Areas 4 and 5) test fishery was analyzed under the SF project "Chum sampling program for the Strait of Juan de Fuca". Tissue samples or purified DNA from these collections are available to be analyzed by the U.S. labs at their request.

In Canadian waters fisheries were sampled across a broad range of dates and areas. Fall Chum directed fisheries were sampled in Johnstone Strait (Area 12 and 13), Cowichan terminal area (Area 18) and Mid-Vancouver Island terminal Area (Area 14). The Johnstone Strait Purse Seine Test Fishery (Area 12) was sampled on a weekly basis over a period of 7 weeks. In each week, samples were collected over a 4 day period with approximately 50 fish sampled spread out over the 5-6 sets made each day. A total of 200 tissue samples per week were taken across all weeks. The first commercial purse seine opening in Johnstone Strait (Areas 12 and 13) took place on October $3^{\text {rd }}$. Vessels were sampled as they were encountered at the offload locations and 25-30 fish were randomly sampled per vessel. The catch was sampled between Areas 12 and 13 proportionate to the catch in those areas during the fishery. The second Johnstone Strait commercial purse seine fishery occurred on October 17-18 ${ }^{\text {th }}$ following the same sampling
requirements as the first fishery, of which a portion was subsampled by area using the fishery catch proportions. Samples were collected from the gillnet fleet that was fishing in Area 14 on November $1^{\text {st }}$. In Area 18 the catch form the Oct $27^{\text {th }}$ purse seine opening was sampled and the gillnet catch from November $2^{\text {nd }}$ was sampled. In addition to directed chum fisheries, chum bycatch in the Round Island and Blinkhorn sockeye test fisheries that occurred during the summer months were also sampled. Table 1 summarizes all sample collections by fishery in Canadian waters.

In U.S. waters the chum directed fishery was sampled weekly in Washington Catch Management Areas 7 \& 7 A (San Juan Islands and Point Roberts). Catch Area 7 was split into East and West geographies with a goal of collecting 200 samples by week and area. 7, 7A fisheries began on September 25 (Lummi Island) and continued until October 26 for other sections. Table 1 summarizes sample collections from both reef and purse seine gears for Areas 7 \& 7A.

Once chum salmon genomic DNA was available, surveys of variation at the following 14 microsatellite loci were conducted: Ots3 (Banks et al. 1999), Oke3 (Buchholz et al. 2001), Oki2 (Smith et al. 1998), Oki100 (Beacham et al. 2008b), Ots103 (Nelson and Beacham 1999), Omm1070 (Rexroad et al. 2001), Omy 1011 (Spies et al. 2005), One101, One102, One104, One111, and One114 (Olsen et al. 2000), Ssa419 (Cairney et al. 2000), and OtsG68 (Williamson et al. 2002). Microsatellites were size fractionated in an Applied Biosystems (ABI) 3730 capillary DNA sequencer, and genotypes were scored by GeneMapper software 3.0 (Applied Biosystems, Foster City, CA) using an internal lane sizing standard.

In general, polymerase chain (PCR) reactions were conducted in $10 \mu$ l volumes consisting of 0.06 units of Taq polymerase, $1 \mu \mathrm{l}$ of 30 ng DNA, $1.5-2.5 \mathrm{mM} \mathrm{MgCl}{ }_{2}, 1 \mathrm{mM} 10 x$ buffer, 0.8 mM
dNTP's, $0.006-0.065 \mu \mathrm{M}$ of labeled forward primer (depending on the locus), $0.4 \mu \mathrm{M}$ unlabeled forward primer, $0.4 \mu \mathrm{M}$ unlabeled reverse primer, and deionized $\mathrm{H}_{2} \mathrm{O}$. PCR was completed on an MJResearch ${ }^{\text {TM }}$ DNA Engine ${ }^{\text {TM }}$ PCT-200 or a DNA Engine Tetrad ${ }^{\text {TM }}$ PCT-225. The amplification profile involved one cycle of 2 min @ $92^{\circ} \mathrm{C}$, 30 cycles of $15 \mathrm{sec} @ 92^{\circ} \mathrm{C}, 15 \mathrm{sec} @$ $52-60^{\circ} \mathrm{C}$ (depending on the locus) and $30 \mathrm{sec} @ 72^{\circ} \mathrm{C}$, and a final extension for $10 \mathrm{~min} @ 72^{\circ} \mathrm{C}$. Specific PCR conditions for a particular locus could vary from this general outline. Further information on laboratory equipment and techniques is available at the Molecular Genetics Laboratory website at http://www.pac.dfo-mpo.gc.ca/science/facilities-installations/pbs-sbp/mgllgm.

## Baseline Populations

The baseline survey consisted of microsatellite analysis of chum salmon from 130 locations within Canada and the southern US (Table 2). Thirteen regional groupings of populations were identified based on genetic stock structure and the ability to accurately estimate known mixtures on of these groupings (DFO unpublished data). All annual baseline samples available for a specific sample location were combined to estimate population allele frequencies, as was recommended by Waples (1990).

## Estimation of Stock Composition

Analysis of fishery samples was conducted with a Bayesian procedure (BAYES) as outlined by Pella and Masuda (2001). Each locus was assumed to be in Hardy-Weinberg equilibrium, and expected genotypic frequencies were determined from the observed allele frequencies and used as model inputs. For BAYES, the initial FORTRAN-based computer program as outlined by Pella and Masuda (2001) required large amounts of computer analytical time when applied to stock identification problems with a baseline as comprehensive as employed in the current study. Given this limitation, a new version of the program was
developed by our laboratory as a C-based program which is available from the Molecular Genetics Laboratory website (Neaves et al. 2005). In the analysis, ten 20,000-iteration Monte Carlo Markov chains of estimated stock compositions were produced, with initial starting values for each chain set at 0.90 for a particular population which was different for each chain. Estimated stock compositions were estimated when all Monte Carlo Markov chains had converged producing a Gelman-Rubin coefficient $<1.2$ (Pella and Masuda 2001). The last 1,000 iterations from each of the 10 chains were combined, and for each fish the probability of originating from each population in the baseline was determined. These individual probabilities were summed over all fish in the sample, and divided by the number of fish sampled to provide the point estimate of stock composition. Standard deviations of estimated stock compositions were also determined from the last 1,000 iterations from each of the 10 Monte Carlo Markov chains incorporated in the analysis.

## Results and Discussion

A total of seven commercial and one fisheries were sampled across four different statistical areas in Canadian waters for a total of 3,100 tissue samples collected in 2016 (Table 1). This includes 88 chum by-catch tissue samples collected from the Area 12 sockeye test fishery in 2016. As we have seen in previous years, chum caught in the July-August in Johnstone Strait are summer-run populations returning to the southern mainland inlets. GSI allocations are predominantly to Ahnuhati, Orford, Deserted, and Phillips systems from Johnstone Strait and Strait of Georgia east regional groups. In U.S. waters three commercial fisheries were sampled across two different management areas for a total of 1,154 tissue samples. Combining U.S. and Canadian waters we analyzed 4,342 samples in 2016.

The southern British Columbia/Washington Chum salmon baseline consisting of fourteen microsatellite markers, a subset of the Pacific Rim baseline for Chum salmon ranging from Japan, across the North Pacific (including the Yukon River) to the southern range limit of Chum salmon in the Columbia River (Beacham et al. 2008; Beacham et al. 2008b) was used to determine the compositions of the fishery samples taken in 2016 (Table 2).

Samples collected from the Canadian Area 12 test fishery consisted of Canadian origin fish $(90.2 \%$ to $99.8 \%$; Table 3) predominantly from the Fraser River, with large contributions the Strait of Georgia (east and west sides). The US, Puget Sound portions of the test fishery catch in Johnstone Strait typically increase in the late October samples which has been reported in previous years. Samples collected from Area 12 and Area 13 commercial fisheries were also Canadian origin fish (98.9\% to 99.2.4\%; Table 3) predominantly from the Fraser River and Strait of Georgia (east and west sides). The main contribution to the west side of the Strait of Georgia fisheries (Area 14 and 18) were mainly composed of Canadian origin fish ( $95.8 \%$ to $99.6 \%$ ). Bycatch from Area 12 sockeye test fisheries were also dominated by Canadian origin fish (91.5\% and 99.1\%; Table 4).

Samples collected from commercial fisheries in U.S. Area 7 also were also largely Canadian origin fish (95.3\% to 99.2\%; Table 5) again predominately Fraser River and Strait of Georgia stocks. The US Area 7A samples exhibited the same trend with the majority of samples allocating to Canadian stocks (99.2\% and 99.7\%). Lummi Island samples were also dominated by the Fraser River and the Strait of Georgia with 99.4\% Canadian allocation.

Sample failure due to tissue quality (e.g. degradation, contamination) will result in absent or poor DNA amplification. Overall failure rate was extremely low, with only two fish failing from the Canadian samples and six from the US samples resulting in an overall failure rate of $0.18 \%$. Samples were excluded from the analysis if the number of loci fall below a threshold of <9 of 14 available markers. Both the Canadian and US samples had 28 individuals which fell below this threshold and were not used in the analysis. The overall exclusion rate was still low at $1.3 \%$.

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## Tables

Table 1. Sample size of tissue collections for DNA analysis for Chum salmon directed fisheries in 2016. Samples analyzed are the number that was effectively analyzed by the GSI program. Samples excluded are those that were included in the analyses but did not provide sufficient information for genetic stock identification. Samples that failed are those that did not amplify due to poor quality and therefore did not make it to the analyses.


Table 2. Baseline of 130 sample sites/populations by regional genetic groups used to estimate stock composition of Chum salmon from southern British Columbia and Washington State in 2016 fisheries.

| Region | Populations |
| :---: | :---: |
| Johnstone Strait | Heydon Cr, Klinaklini R, Ahta R, Viner Sound, Waump Cr, Nimpkish R, Kakweiken R, Glendale Cr, Ahnuhati Cr, Mackenzie Sound, Phillips R, Viner/Scott Cove |
| Strait of Georgia East | Tzoonie Cr, Cheakamus R, Sliammon R, Mamquam R, Wortley Cr, Squamish R, Indian R, Theodosia R, Southgate R, Algard Cr, Orford R, Shovelnose R, Mashiter Cr, Stawamus R, Homathko R, Kwalate Cr, Lang Cr, Deserted Cr, Myrtle Cr, Snake Cr, Anderson Cr |
| Strait of Georgia West | Goldstream R, Cowichan R, Nanaimo R, Chemainus R, Puntledge R, Qualicum R, Little Qualicum R, Campbell R, Cold Cr, Englishman R |
| West Coast Vancouver Island | Smith Cr, Kirby Cr, Demaniel R, Nitinat R, Hathaway Cr, Petattum Cr , Goodspeed, R, Cayeghle Cr, Colonial R, Sugsaw, Cr, Nahmint R, Hoiss Cr, Black Cr, Parks R, Tsowwin_R, Kaouk R, Sucwoa R, Canton R, Little Toquart R, Tranquil Cr, Salmon Cr, Bedwell R, Warner Bay, Burman Cr, Sooke R |
| Fraser River | Silverdale Cr, Squawkum Cr, Wahleach Cr, Chilliwack R, Chehalis R, Stave R, Alouette R, Vedder R, Harrison R, Inch Cr, Lower Lillooet R, Norrish-Worth Cr, North Alouette R, Widgeon Slough, Kawkawa Cr, Blaney Cr, Chilqua Cr, Serpentine R, Kanaka Cr, Worth Cr , Hopedale Cr, Hicks Cr, Harrison Lake, Peach Cr, Sweltzer Cr, Nathan Cr, McIntyre Cr, Street Cr, Railroad, Cr, Silverhope Cr |
| North Puget Sound | Skagit R, County Line Cr, Grant Cr, Siberia Cr, Skykomish R, Snohomish R, Stilllaguamish R, Sauk R |
| South Puget Sound | Kennedy Cr, Minter Cr, Nisqually R, Mill Cr, Skookum Cr, Puyallup R, South Prairie Cr |
| Juan de Fuca/ Hood Canal Summer | Salmon R, Big Quilcene R |
| Coastal Washington | Ellsworth Cr, Bitter Cr, Quinault R, Satsop R |
| Nooksack | Nooksack R |
| Tulalip | Tulalip R |
| Central Puget Sound | Green R, Grovers Cr |
| Juan de Fuca/ Hood Canal Fall | Elwha R, Hoodsport, Spencer Cr, Big Mission Cr, Dewatto R, Hamma Hamma R, Big Beef Cr |

Table 3. Estimated percentage stock composition of Chum salmon caught in Area 12 and 13 Test and Commercial Fisheries in 2016. Stock compositions were estimated using 14 microsatellite loci and the baseline outlined in Table 2. Number of fish excluded because of their inability to provide sufficient information for genetic stock identification in parentheses. Samples that failed due to lack of amplification are not included in these analyses (see Table 1 for more details). Standard error of the estimated stock composition is in parentheses.


Table 4. Estimated percentage stock composition of Chum salmon caught in the east coast of Vancouver Island Areas 14, 18 chum directed commercial fisheries and by-catch in the Area 12 sockeye fishery in 2016 . Stock compositions were estimated using 14 microsatellite loci and the baseline outlined in Table 1. Number of fish excluded because of their inability to provide sufficient information for genetic stock identification in parentheses. Samples that failed due to lack of amplification are not included in these analyses (see Table 1 for more details). Standard error of the estimated stock composition is in parentheses.


Table 5. Estimated percentage stock composition of Chum salmon caught in Area 7 and 7A Commercial Fisheries in 2016. Stock compositions were estimated using 14 microsatellite loci and the baseline outlined in Table 1. Number of fish excluded because of their inability to provide sufficient information for genetic stock identification in parentheses. Samples that failed due to lack of amplification are not included in these analyses (see Table 1 for more details). Standard error of the estimated stock composition is in parentheses.

| Year | 2016 |  | 2016 |  |  | 2016 |  |  | 2016 |  |  | 2016 |  |  | 2016 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Julian date | 269-274 |  | 284-287 |  |  | 291-293 |  |  | 284-287 |  |  | 291-293 |  |  | 300 |  |  |
| Gear | reef |  | seine |  |  | seine |  |  | seine |  |  | seine |  |  | seine |  |  |
| Stat Area | Lummilsland |  | Area7A |  |  | Area7A |  |  | Area7 |  |  | Area7 |  |  | Area7 |  |  |
| Fishery Type | Week 40 |  | Week42 |  |  | Week43 |  |  | Week42 |  |  | Week43 |  |  | Week44 |  |  |
| Dates | Sept25-Sept27 |  | Oct10-Oct13 |  |  | Oct17-Oct19 |  |  | Oct10-Oct13 |  |  | Oct17-Oct19 |  |  | 26-Oct |  |  |
| sample Size | 87(0) |  | 151(3) |  |  | 185(4) |  |  | 333(12) |  |  | 257(7) |  |  | 107(2) |  |  |
| Region | Estimate SD |  | Estimate |  |  | Estimate |  |  | Estimate | SD |  | Estimate |  |  | Estimate |  |  |
| Johnstone Strait | 0.5 | (1.5) | 0.1 |  | (0.5) | 2.7 |  | (4.5) | 0.9 |  | (1.6) | 4.7 |  | (4.3) | 0.5 |  | (1.4) |
| Strait of Georgia East (F) | 3.0 | (3.4) | 22.5 |  | (4.5) | 15.4 |  | (4.2) | 9.6 |  | (3.4) | 1.7 |  | (2.5) | 39.4 |  | (7.7) |
| Strait of Georgia West (F) | 1.3 | (2.5) | 1.9 |  | (3.2) | 11.0 |  | (4.7) | 31.4 |  | (4.4) | 33.5 |  | (5.0) | 7.0 |  | (5.8) |
| Fraser River (F) | 92.4 | (4.6) | 75.0 |  | (4.3) | 69.5 |  | (4.9) | 54.7 |  | (3.9) | 53.9 |  | (4.6) | 48.0 |  | (6.4) |
| West Coast Vancouver I(F) | 2.2 | (3.0) | 0.3 |  | (0.8) | 1.0 |  | (1.4) | 2.6 |  | (1.9) | 2.5 |  | (2.1) | 0.3 |  | (1.2) |
| North Puget Sound (F) | 0.4 | (1.5) | 0.1 |  | (0.5) | 0.1 |  | (0.6) | 0.1 |  | (0.4) | 0.3 |  | (0.9) | 0.2 |  | (0.9) |
| Central Puget Central (F) | 0.0 | (0.2) | 0.0 |  | (0.1) | 0.0 |  | (0.1) | 0.0 |  | (0.1) | 0.0 |  | (0.1) | 0.0 |  | (0.3) |
| South Puget Sound (F-W) | 0.0 | (0.4) | 0.0 |  | (0.2) | 0.0 |  | (0.2) | 0.6 |  | (1.1) | 1.6 |  | (2.7) | 0.1 |  | (0.5) |
| Hood Canal (S) | 0.0 | (0.1) | 0.0 |  | (0.1) | 0.1 |  | (0.4) | 0.0 |  | (0.0) | 0.0 |  | (0.1) | 0.0 |  | (0.1) |
| Hood Canal (F) | 0.1 | (0.6) | 0.1 |  | (0.4) | 0.0 |  | (0.2) | 0.0 |  | (0.2) | 1.7 |  | (1.6) | 4.4 |  | (2.9) |
| Juan de Fuca (F) | 0.0 | (0.2) | 0.0 |  | (0.1) | 0.0 |  | (0.1) | 0.0 |  | (0.1) | 0.0 |  | (0.2) | 0.0 |  | (0.1) |
| Coastal Washington (F) | 0.0 | (0.3) | 0.0 |  | (0.2) | 0.0 |  | (0.1) | 0.0 |  | (0.1) | 0.0 |  | (0.1) | 0.0 |  | (0.3) |
| Country |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Canada | 99.4 | (1.7) | 99.8 |  | (0.7) | 99.7 |  | (0.9) | 99.2 |  | (1.2) | 96.3 |  | (2.5) | 95.3 |  | (3.2) |
| US | 0.6 | (1.7) | 0.2 |  | (0.7) | 0.3 |  | (0.9) | 0.8 |  | (1.2) | 3.7 |  | (2.5) | 4.7 |  | (3.2) |

Figures
Figure 1. Map of Statistical Areas outlining Chum salmon fishing locations in southern British Columbia 2013-2016


Figure 2. Map of Statistical Areas outlining Chum salmon fishing locations in Puget Sound 2013-2016.


