

Joint US and CA Mixed-stock Chum Fisheries Sampling Design and Analysis 2015

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Abstract

We conducted Genetic Stock Identification (GSI) of 3316 Chum salmon samples migrating to natal streams through Johnstone Strait (Statistical Areas 12 and 13), West Coast of Vancouver Island –WCVI- (Statistical Areas 25, 123, 126 and 127) and through the San Juan Islands (Statistical Area 7 and 7A) for 2015 using analyses of microsatellite variation. A total of 2996 Chum salmon were analyzed for Canadian fisheries (Areas 12, 13, 25, 123, 126 and 127) and 320 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 (97.9% to 99.1%) comprised largely of Strait of Georgia East (19.1% to 92.0%) and Johnstone Strait (2.1% to 60.8%). The analysis of Chum salmon caught in commercial fisheries in the San Juan Islands where from both Canadian and U.S. origin stocks with a larger contribution of Canadian stocks: 95.0% to 99.7% in Area 7A and 78.0% to 97.5% in Area 7. West Coast Vancouver Island commercial samples were largely Canadian contributions (88.1% to 99.4%) with the exception of Area 126 which had near even proportions (53.8% Canadian vs. 46.2% US stocks).

Compared to previous years US samples comprised only 9.6% of the total samples collected and analyzed. West Coast Vancouver Island samples had a very high GSI failure proportion (35.3%) due to poor sample quality which translated in lack of DNA amplification. Areas 12 and 13 followed with 4.4% failures and US samples with 0.6%.

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TABLE OF CONTENTS

ABSTRACT	II
ACKNOWLEDGMENTS	III
INTRODUCTION	1
MATERIALS AND METHODS	3
RESULTS AND DISCUSSION	7
LITERATURE CITED	8
TABLES	11
Table 1. Sample size of tissue collections for DNA analysis for Chum salmon directed fisheries in 2015. 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.	11
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 2015 fisheries.	12
Table 3. Estimated percentage stock composition of Chum salmon caught in Area 12 and 13 Test and Commercial Fisheries. 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.	1
Table 4. Estimated percentage stock composition of Chum salmon caught in the West Coast of Vancouver Island Areas 123, 126, 127 and 25 Commercial Fisheries in 2015. 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.	2
Table 5. Estimated percentage stock composition of Chum salmon caught in Area 7 and 7A Commercial Fisheries in 2015. 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.	3
FIGURES	4

Figure 1. Map of Statistical Areas outlining Chum salmon fishing locations in southern British Columbia 2013-2015

4

Figure 2. Map of Statistical Areas outlining Chum salmon fishing locations in Puget Sound 2013-2015.

5

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 is the fourth and last year of the project to sample and provide Genetic Stock Identification (GSI) on key chum mixed stock fisheries within Canada and the US, duplicating annual sample collections as closely as possible over the four years to obtain uniform coverage. The main fisheries targeted were Johnstone Strait purse seine and gill net commercial and test fisheries (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. The Strait of Juan de Fuca (SJDF) Fishery Management Areas 4B, 5 and 6C and WCVI are a target area but were not sampled consistently from 2013-2015 (Figure 2). 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. The GSI work is part of the information required for accurate post-season run reconstructions which are essential in evaluating whether management actions were consistent with meeting overall objectives and Treaty obligations. 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.

The Chum 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). The samples were collected from 2996 adult Chum salmon in 2015 captured in test and commercial fisheries from British Columbia Statistical Areas 12, 13, 25, 123, 126 and 127 from Gillnet, Troll and Seine fisheries between May 5 and October 28, 2015. There were 320 Chum salmon captured in commercial fisheries from Washington State Statistical Areas 7 and 7A between September 23 and October 22, 2015. No fish were sampled in 2015 from the Strait of Juan de Fuca (US Areas 4B, 5, and 6C) as originally planned. A duplicate set of tissues that were collected in Canadian waters are available to be analyzed by the U.S. labs.

In Canadian waters the Johnstone Strait Purse Seine Test Fishery 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. Vessels were sampled as they were encountered at the offload location and 25-30 fish were randomly sampled per vessel. The second commercial purse seine fishery occurred on October 19 following the same sampling requirements as the first fishery, of which a portion was subsampled by area using the fishery catch proportions. 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 & 7A (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 23 (Lummi Island) and continued until October 22 for other

sections. Table 1 summarizes sample collections from both gillnet 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 µl volumes consisting of 0.06 units of Taq polymerase, 1µl of 30ng DNA, 1.5-2.5mM MgCl₂, 1mM 10x buffer, 0.8mM dNTP's, 0.006-0.065µM of labeled forward primer (depending on the locus), 0.4µM unlabeled forward primer, 0.4µM unlabeled reverse primer, and deionized H₂O. PCR was completed on an MJResearch™ DNA Engine™ PCT-200 or a DNA Engine Tetrad™ PCT-225. The amplification profile involved one cycle of 2 min @ 92°C, 30 cycles of 15 sec @ 92°C, 15 sec @ 52-60°C (depending on the locus) and 30 sec @ 72°C, and a final extension for 10 min @ 72°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/mgl-igm>.

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 six commercial and two test fisheries were sampled across six different statistical areas in Canadian waters for a total of 2996 tissue samples collected in 2015. In U.S. waters five commercial fisheries were sampled across three different management areas (Table 1) for a total of 320 tissue samples. Combining U.S. and Canadian waters we analyzed 3316 samples in 2015.

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 2015 (Table 2).

Samples collected from the Canadian Area 12 test fishery consisted of Canadian origin fish (97.9% to 98.0%; Table 3) predominantly from the Johnstone Strait, the Strait of Georgia (east and west sides), and the Fraser River. Samples collected from Area 12 and Area 13 commercial fisheries were also Canadian origin fish (93.4% to 97.4%; Table 3) predominantly from the Fraser River and Strait of Georgia (east and west sides). Areas 123, 126, 127 and 25 gathered by commercial fisheries were predominantly Canadian with the exception of Area 126 which resulted in approximately equal percentages (53.8% Canadian, 46.2% US). The main contributing regions to the West Coast Vancouver Island fisheries were the Fraser River and West Coast Vancouver Island, with the exception of Area 127 composed mainly of Johnstone Strait (62.9%), and Area 126 carrying a significant proportion of Hood Canal (35.8%) (Table 4).

Samples collected from commercial fisheries in U.S. Area 7 also were also largely Canadian origin fish (78.0% to 95.%; 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 (95.0% to 99.7%, Table 5). Lummi Island samples were also dominated by the Fraser River and the Strait of Georgia with 99.7% Canadian allocation. Although Canadian allocations in Juan the Fuca and Areas 7 and 7A have been predominant during 2015, it is important to emphasize that the smaller sample sizes from US fisheries (9.6%) could play a role in the proportions seen in the analyses. Sample failure due to poor quality (e.g. degradation, contamination) translated in lack of DNA amplification. The larger proportion of failures was seen in West Coast Vancouver Island samples (35.3%) followed by 4.4% in Areas 12 and 13 and only 0.6% in US samples.

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Tables

Table 1. Sample size of tissue collections for DNA analysis for Chum salmon directed fisheries in 2015. 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.

Canadian Waters					
Fishery	Gear	# Analysed	#Excluded	#Failed	#Total
Area 12 Comm	seine	488	7	4	499
Area12 Test	gill	20	0	4	24
Area12 Test	seine	1494	26	40	1560
Area13 Comm	seine	494	7	0	501
Area13 Test	seine	163	11	26	200
Area123 Comm	troll	12	0	0	12
Area126 Comm	troll	5	2	3	10
Area127 Comm	troll	88	5	56	149
Area 25	gill	32	1	8	41
Subtotal		2796	59	141	2996

US Waters					
Fishery	Gear	# Analysed	#Excluded	#Failed	#Total
Lummi Island	reef	80	0	0	80
Area 7	gill	4	0	0	4
Area 7A	gill	6	0	0	6
Area 7A	seine	117	1	0	118
Area7	seine	111	0	1	112
Subtotal		318	1	1	320

Total Samples Run	# Analysed	#Excluded	#Failed	#Total
	3115	59	142	3316

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 2015 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, Squakum 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, Collum Cr
North Puget Sound	Skagit R, County Line Cr, Grant Cr, Siberia Cr, Skykomish R, Snohomish R, Stillaguamish 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. 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.

Year	2015		2015		2015		2015		2015	
Julian date	278-292		211-225		217-301		278-292		218-224	
Gear	seine		gill		seine		seine		seine	
Stat Area	Area12Comm		Area12TF		Area12TF		Area13Comm		Area13TF	
Fishery Type	Week41-43		Week31-33		Week32-44		Week41-43		Week32-33	
Dates	Oct05-Oct19		Jul30-Aug13		Aug05-Oct28		Oct05-Oct19		Aug06-Aug12	
sample Size	488(7)		20(0)		1494(26)		494(7)		163(11)	
Region	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Johnstone Strait	1.8	(2.2)	60.8	(16.2)	5.7	(1.7)	1.4	(2.8)	2.1	(1.9)
Strait of Georgia East (F)	17.0	(3.7)	19.1	(13.5)	27.1	(2.4)	22.9	(4.0)	92.0	(3.8)
Strait of Georgia West (F)	40.7	(4.2)	0.8	(3.4)	30.0	(2.6)	37.4	(4.5)	1.2	(2.3)
West Coast Vancouver I(F)	0.2	(0.4)	12.9	(13.0)	0.2	(0.4)	0.3	(0.7)	0.5	(1.0)
Fraser River (F)	37.9	(3.3)	4.3	(7.4)	35.1	(1.8)	31.4	(3.1)	3.4	(2.8)
North Puget Sound (F)	2.0	(2.3)	1.1	(3.8)	1.2	(0.7)	1.6	(2.0)	0.4	(1.0)
Central Puget Central (F)	0.1	(0.2)	0.0	(0.8)	0.0	(0.1)	0.6	(0.6)	0.0	(0.1)
South Puget Sound (F-W)	0.3	(0.6)	0.1	(1.3)	0.1	(0.2)	1.2	(1.2)	0.0	(0.3)
Hood Canal (S)	0.0	(0.1)	0.0	(0.7)	0.1	(0.2)	0.0	(0.1)	0.0	(0.2)
Hood Canal (F)	0.1	(0.3)	0.8	(3.7)	0.6	(0.4)	2.6	(1.2)	0.5	(0.9)
Juan de Fuca (F)	0.0	(0.1)	0.0	(0.5)	0.0	(0.0)	0.0	(0.1)	0.0	(0.1)
Coastal Washington (F)	0.0	(0.1)	0.0	(0.9)	0.0	(0.0)	0.6	(0.5)	0.0	(0.1)
Country										
Canada	97.4	(2.4)	97.9	(5.7)	98.0	(0.8)	93.4	(2.4)	99.1	(1.4)
US	2.6	(2.4)	2.1	(5.7)	2.0	(0.8)	6.6	(2.4)	0.9	(1.4)

Table 4. Estimated percentage stock composition of Chum salmon caught in the West Coast of Vancouver Island Areas 123, 126, 127 and 25 Commercial Fisheries in 2015. 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	2015		2015		2015		2015	
Julian date	272-272		129		125-274		282	
Gear	troll		troll		troll		gill	
Stat Area	Area123Com		Area126Com		Area127Com		Area25Com	
Fishery Type	WCVI		WCVI		WCVI		WCVI	
Dates	Sep29		May9		May5-Oct1		Oct 9	
sample Size	12(0)		5(2)		88(5)		32(1)	
Region	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Johnstone Strait	1.5	(4.9)	4.5	(12.6)	62.9	(8.1)	2.5	(4.1)
Strait of Georgia East (F)	5.9	(10.6)	9.9	(18.5)	0.9	(2.0)	0.9	(2.6)
Strait of Georgia West (F)	0.4	(3.2)	10.6	(18.6)	0.4	(1.3)	1.1	(3.0)
West Coast Vancouver I(F)	47.0	(18.5)	10.4	(18.2)	13.2	(4.7)	94.4	(6.4)
Fraser River (F)	33.4	(18.6)	18.3	(23.8)	11.0	(5.7)	0.5	(2.3)
North Puget Sound (F)	9.4	(13.4)	9.4	(16.6)	0.1	(0.7)	0.1	(1.1)
Central Puget Central (F)	0.0	(1.3)	0.1	(2.7)	0.1	(0.4)	0.0	(0.4)
South Puget Sound (F-W)	1.5	(5.0)	0.4	(4.1)	1.5	(1.5)	0.0	(0.5)
Hood Canal (S)	0.0	(0.9)	0.5	(4.0)	0.0	(0.1)	0.0	(0.3)
Hood Canal (F)	1.0	(5.1)	35.8	(24.9)	7.4	(3.5)	0.0	(0.7)
Juan de Fuca (F)	0.0	(0.7)	0.0	(1.5)	0.0	(0.1)	0.0	(0.4)
Coastal Washington (F)	0.0	(1.4)	0.1	(3.2)	2.5	(2.3)	0.6	(1.7)
Country								
Canada	88.1	(14.4)	53.8	(28.2)	88.4	(4.3)	99.4	(2.3)
US	11.9	(14.4)	46.2	(28.2)	11.6	(4.3)	0.6	(2.3)

Table 5. Estimated percentage stock composition of Chum salmon caught in Area 7 and 7A Commercial Fisheries in 2015. 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	2015		2015		2015		2015		2015	
Julian date	269-270		295		295		285-295		285-295	
Gear	reef		gill		gill		seine		seine	
Stat Area	Lummil Island		Area7		Area7A		Area7APS		Area7PS	
Fishery Type	Area7_7A		Week43		Week43		Week42-43		Week42-43	
Dates	Sept23-Sep27		Oct22		Oct22		Oct12-Oct22		Oct12-Oct22	
sample Size	80(0)		4(0)		6(0)		117(1)		111(0)	
Region	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Johnstone Strait	0.3	(1.1)	0.2	(6.9)	0.3	(3.9)	0.5	(2.0)	0.4	(1.5)
Strait of Georgia East (F)	4.4	(4.5)	0.7	(8.3)	36.7	(21.0)	8.2	(4.6)	9.9	(6.2)
Strait of Georgia West (F)	2.8	(4.0)	92.0	(21.1)	2.6	(10.3)	3.7	(5.2)	41.2	(7.8)
West Coast Vancouver I(F)	6.4	(4.3)	3.0	(13.2)	3.9	(11.2)	0.3	(0.9)	2.4	(2.9)
Fraser River (F)	85.8	(6.0)	1.6	(9.9)	51.5	(24.7)	87.0	(4.5)	24.1	(6.0)
North Puget Sound (F)	0.2	(0.8)	1.1	(7.5)	1.7	(7.4)	0.2	(0.8)	21.3	(6.1)
Central Puget Central (F)	0.0	(0.3)	1.2	(5.9)	0.0	(1.5)	0.0	(0.2)	0.0	(0.2)
South Puget Sound (F-W)	0.1	(0.6)	0.1	(3.3)	2.6	(7.5)	0.0	(0.3)	0.1	(0.4)
Hood Canal (S)	0.0	(0.1)	0.0	(2.0)	0.0	(1.6)	0.0	(0.1)	0.0	(0.1)
Hood Canal (F)	0.0	(0.4)	0.2	(4.3)	0.8	(5.2)	0.0	(0.3)	0.3	(1.1)
Juan de Fuca (F)	0.0	(0.1)	0.0	(1.7)	0.0	(1.1)	0.0	(0.2)	0.3	(1.1)
Coastal Washington (F)	0.0	(0.4)	0.1	(3.7)	0.0	(2.1)	0.0	(0.2)	0.0	(0.2)
Country										
Canada	99.7	(1.2)	97.5	(11.7)	95.0	(12.0)	99.7	(0.9)	78.0	(6.1)
US	0.3	(1.2)	2.6	(11.7)	5.0	(12.0)	0.3	(0.9)	22.0	(6.1)

Figures

Figure 1. Map of Statistical Areas outlining Chum salmon fishing locations in southern British Columbia 2013-2015

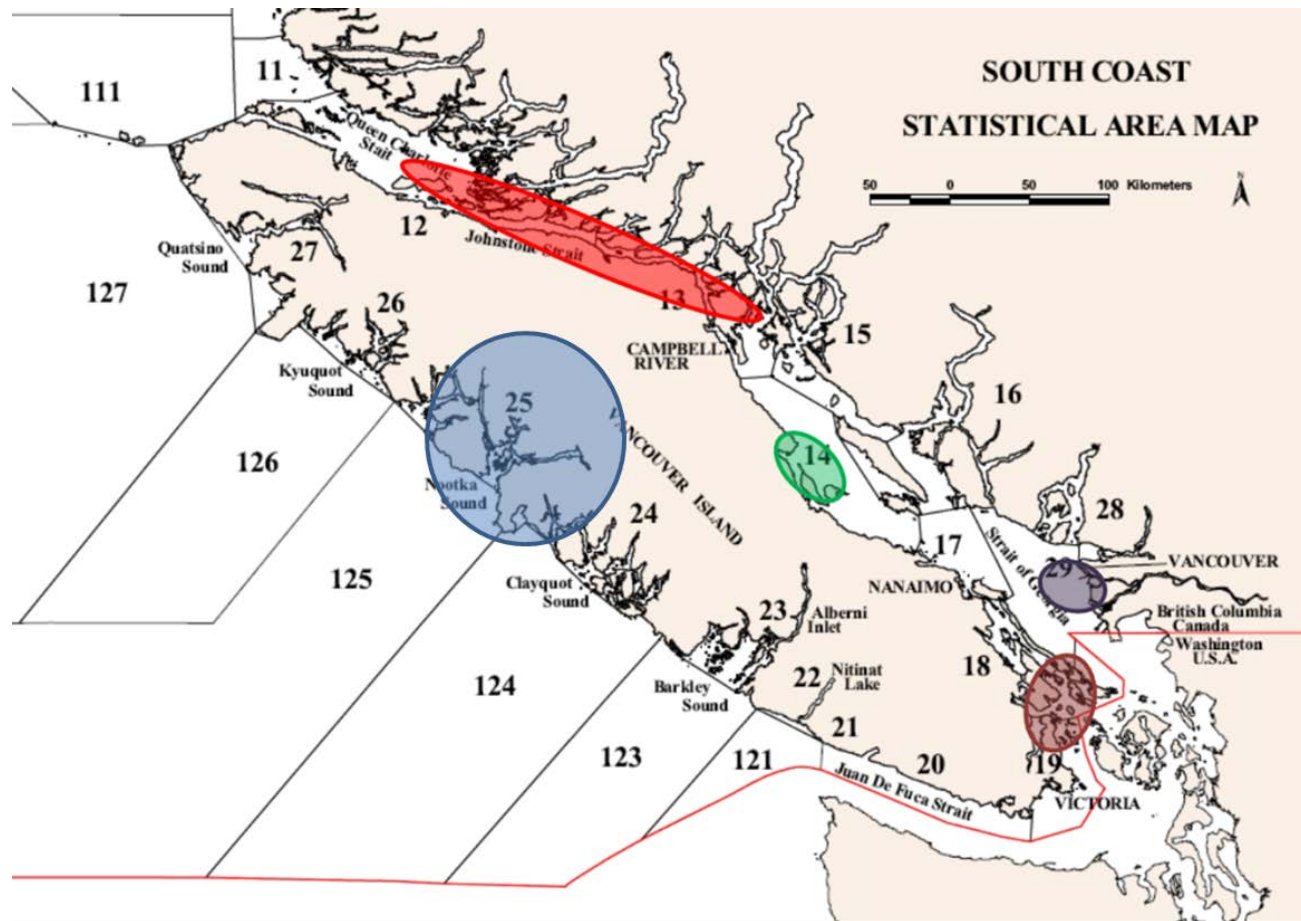


Figure 2. Map of Statistical Areas outlining Chum salmon fishing locations in Puget Sound 2013-2015.

