Skeena River Chinook Baseline Sampling 2009

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Executive Summary

A large sampling program was undertaken in 2009 by the Skeena Fisheries Commission to improve the genetic baseline information on Skeena River Chinook. 1712 Chinook were sampled from 14 rivers throughout the Skeena watershed. Most of the sampled fish were fry in their natal rivers. The 2009 sampling has increased the existing baseline from 12 populations in 2008 to 22 populations. The improved data is changing the understanding of wild Chinook populations in the Skeena. The Skeena test fishery sampling has been reanalyzed by Ivan Winther with this improved baseline, procedures for genetic separation of Skeena Chinook have been improved by Terry Beacham and a new dendrogram showing the relationship of Skeena populations has been prepared by John Candy of the DFO molecular genetics laboratory.

Introduction

Salmon are the most conspicuous and most abundant large fishes of the Skeena watershed. They long were the resource that supported the human population of the region. Even in recent decades as food produced in other regions has flooded in, the salmon remain an icon of the Skeena region. *Oncorhynchus tshawytscha,* the Chinook salmon, is the earliest appearing and largest species.

Skeena Chinook salmon are characteristic of large rivers and wander widely in the North Pacific ocean for one to six years before reentering their natal streams to spawn. Chinook from Northern British Columbia rear and feed northward of their home streams and return from the north. They are harvested for the most part in their last year of life on their return migrations to the north coast. On this return migration they are susceptible to interception in troll fisheries in Southeast Alaska and in troll and gill net fisheries of the north coast of BC.

The Pacific Salmon Treaty between Canada and the United States sets exploitation rates for the national fisheries on both sides of the border. The treaty specifies the aggregate national Chinook harvest in transboundary fisheries. Evaluating this aggregate in smaller units of individual rivers is monitored by the treaty designated Chinook Technical Committee (CTC). The CTC is currently engaged in collecting data useful for recognizing and monitoring the escapement of Skeena Chinook with the intention of managing the Skeena for a single Chinook aggregate.

The larger rivers in British Columbia, such as the Fraser and the Nass contain many more-or less separate populations (Holtby and Ciruna 2007). This complex population structure presumably contains much of the genetic diversity of the species. Canada's Wild Salmon Policy adopted in 2005 (Fisheries and Oceans Canada, 2005) is intended to conserve this diversity and hence promote the long-term survival of salmon.

The Skeena Fisheries Commission has been collecting baseline genetic samples for the past three years in order to better define the diversity of Skeena Chinook. In the spring of 2009 the Skeena Watershed Initiative, with the support of the Moore Foundation, funded a large scale effort toward completion of a genetic baseline for Skeena Chinook. The funding was directed toward collection of samples in the lower and middle tributaries of the Skeena. A simultaneous project was carried out for the upper Skeena tributaries. This later project was also supported with Moore Foundation money but funded through the Northwest Institute for Bioregional research. This report discusses the joint results of the 2009 studies.

Chinook salmon are extraordinarily effective homing animals. In the Skeena, eggs are laid in suitable loosely-packed stream gravel in August and September, develop in the gravel

through the winter, and fry emerge in April through June. The fry begin their downstream movement within a few weeks to a few months of emergence. The Chinook occupy feeding stations in cobble-bed habitats often sheltering behind cobbles and boulders and darting out to grab passing food items, often insects. For nearly all Skeena populations the first winter is spent in large rivers, for the most part the Skeena mainstem. Migration to sea occurs during the high turbid flows of the spring snow melt. The single known exception to this pattern are the Ecstall River Chinook that go to sea in their first year.

Nearly all of the adult Chinook return to their stream of birth, in some cases to the same gravel bar where they were born (Quinn, 2005). This extreme fidelity accelerates specialization for the characteristics of each river and presumably results in numerous highly specialized sub-populations. The rate of and extent of evolution in the smaller of these subpopulations are increased by random effects of chance in small populations, called "genetic drift".

Increasing attention has been applied to biochemical characterization of the genetic differences between salmon populations since the 1990s. The progression has been to ever finer scale genetic characteristics. At first, separation was by analysis of protein differences, called allozymes. By the end of the 1990s attention had shifted to the details of the DNA of the major histocompatibility complex (MHC) and minisatellite components of the chromosomes. In the past decade analysis has been carried out with great success using microsatellite components which are non-coding sections of the chromosome, and in the past few years using single nucleotide polymorphisms (SNPs) of coding and non-coding parts of the chromosome.

The fine scale analyses of chromosomal coding have become highly automated such that analysis for 15 loci in the microsatellite system or 96 SNPs can be carried out overnight at a cost of $10 to $30.

We have worked closely with the Salmon Genetics Laboratory at the Pacific Biological Station which has perfected the use of microsatellite DNA systems for Pacific salmon population separation. Outstanding Chinook studies include Beacham *et al.* 2003a, 2003b, 2006, and 2008 . This genetic determination system is similar to that used for human forensic applications.

Sampling Techniques

Adult Chinook

Adult Chinook were collected on their spawning beds using 6 inch mesh tangle nets. Fish were kept in the water at all times. Two to five scales were taken from each fish for ageing and DNA determination. Collections were made from a representative portion of the spawners. Scales were cleaned and submitted to the Department of Fisheries and Oceans Pacific Biological Station Molecular Genetics Laboratory for microsatellite DNA analysis.

Juvenile Chinook

Chinook juveniles were collected with beach seines 10 to 20 m long x 1.6 m high made with ¼" woven nylon mesh. The nets were simple sheets manipulated with poles at the ends. The lengths could be adjusted by wrapping the net around the pole ends to shorten it. The nets can be manipulated when fishing to form a linear pocket for capturing fry. In boulder reaches of the Suskwa, Shegunia and Gitsegukla Rivers Chinook fry were taken with baited Gee traps baited and left overnight.

Collection sites were selected for being tens of kilometers below known major spawning areas and at least two kilometers above the confluence with the Skeena River. The concern about using juvenile Chinook for population characterization is that if collections are made near the spawning sites, siblings may be aggregated with siblings and other relatives and hence the population would appear to be less variable genetically than it is overall. We make the assumption that after fry have migrated tens of kilometers downstream they have likely stopped and fed more than once and are no longer closely associated with relatives. However, this assumption needs testing.

We observed that juvenile Chinook migrate into small tributary streams to feed on their downstream travels. They may be found in the lowest reaches of streams that appear to be obviously unsuitable for spawning. We therefore took the precaution of collecting juvenile more than two or three kilometres above stream mouths. Rarely, these criteria could not be met, such as at Tantan Creek (the Kluatantan locality in Table 1) which is short (2 km) and joins the Kluatantan River which hosts the upstream Kluayaz juveniles. Juveniles were not collected at Tantan Creek..

Salmonid fry were measured and sorted by species in the lab. Identification of Chinook fry was made in the lab because of the difficulty in separating coho. The separations were based on branchiostegal counts and to a minor degree on colour markings and pattern.

Results

Examination of spawning beds began in late July. The first Chinook appeared August 5, and only in mid-August did they arrive in substantial numbers. Contrary to our expectations, the timing of Chinook moving onto spawning beds was essentially simultaneous at all collected streams ranging from the Kasiks River at the head of tidal influence to the ultimate northern headwaters of the Skeena mainstem. By early September, spawning had ended at all the sites examined. The timing of the major lake outlet Chinook spawning populations such as Morice River is believed to extend a week or two longer in September (Hancock *et al.* 1983). But this does not seem to be the case for one of the three lake outlet populations which according to the Babine River Weir counts of 2009 had the same timing as the populations we collected. We did not collect samples and hence timing information for the early entry stocks of the Upper Bulkley and the Cedar River.

Adult collections were made in six rivers (Table 1). Collections size ranged from 1 to 43 Chinook within a single spawning area. The largest collections were from Slamgeesh River at a site called Gitangwalk two kilometres below the Damshilgwet confluence and at Squingula River about 3 kilometres below Motase Lake. At these sites there were hundreds to thousands of Chinook present in late August.

Juvenile collections were made from 12 rivers (Table 1) with collection sizes ranging from 8 to 265. Effective seine net fishing required fishing on cobble bed reaches with a shoreline patch of sandy sediment to beach the net on without many escapes beneath the leading edge of the net. There was great variation in catch per haul ranging from less than one fish per haul with sites emptied in one or two passes to sites where it catches were 5 to 15 Chinook fry per pass and a few minutes of rest would restore fish to the patch fished. Fry density appeared to be a measure of the abundance of spawners on that river, which were similar in rank order to the percentage presence in the Skeena Test Fishery analysis. An adequate sample of Chinook fry was taken at Squingula River in two visits whereas collecting similar numbers of fry from Gitsegukla River was only possible with more than twelve crew-days.

Beach seining juvenile Chinook is not quantitative because many Chinook escape past the ends and the bottom of the net. Some of the best collecting sites were where there were natural obstacles at the end of the net and relatively smooth bottom topography. The Chinook fry appear to hold adjacent to cobbles on the stream bed. We could not collect boulder habitat effectively to determine approximate density of juvenile Chinook , but there does appear to be regular use of such habitat. At Suskwa, Shegunia and Gitsegukla Rivers the catch of Chinook fry with Gee traps in boulder reaches was about one per trap-night.

Based on beach seine catches in cobble-bed reaches, Chinook appeared to be distributed with densities ranging from 0.2/m^2 to 2.0/m^2. Mountain whitefish (*Prosopium williamsoni*) was the next most abundant species see Figure 2). In order of decreasing abundance, juvenile bull trout, steelhead fry, and coho were also caught. The proportion of coho increased if seine passes were made on finer sediment bottoms. There was a strong association of Chinook fry with loose cobbles surfaces. Few or no Chinook were present if the stream bed had more than a few percent sand, especially if fine sediments were abundant enough to embed the cobbles.

Approximately 85% of the salmon specimens brought into the lab for detailed examination were Chinook. The Chinook identifications were ultimately confirmed by DNA analysis. No doubt the high proportion of Chinook in the seine net collections was because of the selection of appropriate stream habitat for sampling. In the few cases where Gee traps were used to collect fry, the proportion of coho was higher.

The state of Chinook knowledge in the Skeena

There are probably fewer than 50 stable Chinook populations in the Skeena. As of 2005 only 10 of these populations had baseline DNA samples >100 individuals (Table 2). This is the minimum number necessary to characterize diversity, and larger samples are desirable. In 2007 Ivan Winther of the DFO Stock Assessment in Prince Rupert and the Skeena Fisheries Commission began expanding the DNA baseline. By the end of 2008 there were 12 populations with collections >100 represented in the DFO Salmon Genetics Laboratory baseline. Our concentrated effort of 2009 increased the number of adequately sampled populations to 22.

In 2009, Moore Foundation funded projects added about 1650 specimens from 14 populations. These included 1557 juveniles and 155 adults. At this point all but a few of the known Chinook populations with more than 100 breeders are sampled. The most conspicuous missing populations are the Khyex River in the lowermost Skeena and the Lakelse River below Terrace. About five other populations need further collecting to enlarge the sample size.

The size of sampled Chinook populations is estimated in Table 2 based on a review of the existing Chinook escapement records, the analysis of the proportions of fish in the Skeena Test Fishery analysis and our observations while collecting the samples for this baseline set. The twenty-two represented populations represent ten large populations identified as having escapements of over 2000 or fluvial bedforms on the spawning grounds that suggest a population of this size (Gottesfeld et al. 2008), eight medium sized populations estimated as having annual escapements of 500 to 2000, and four small populations estimated with escapements of between 20 and 500. Another five populations, of which four are estimated to be small, have as yet only small collections.

Table 1. Specimens Added to the Chinook Microsatellite DNA Baseline in 2009

Chinook DNA specimens 2009		
	Juv	Adult
Khyex		
Kasiks	76	
Exchamsiks	105	
Gitnadoix	185	
Exstew	142	
Zymogotitz	119	
Zymoetz	18	15
Gitsegukla	262	
Suskwa/Harold Price	91	6
Shegunia	65	14
Kuldo	168	
Sicintine	112	
Slamgeesh		49
Squingula	214	56
Kluatantan		15
Nfish	1557	155
Sub-pops sampled	12	6

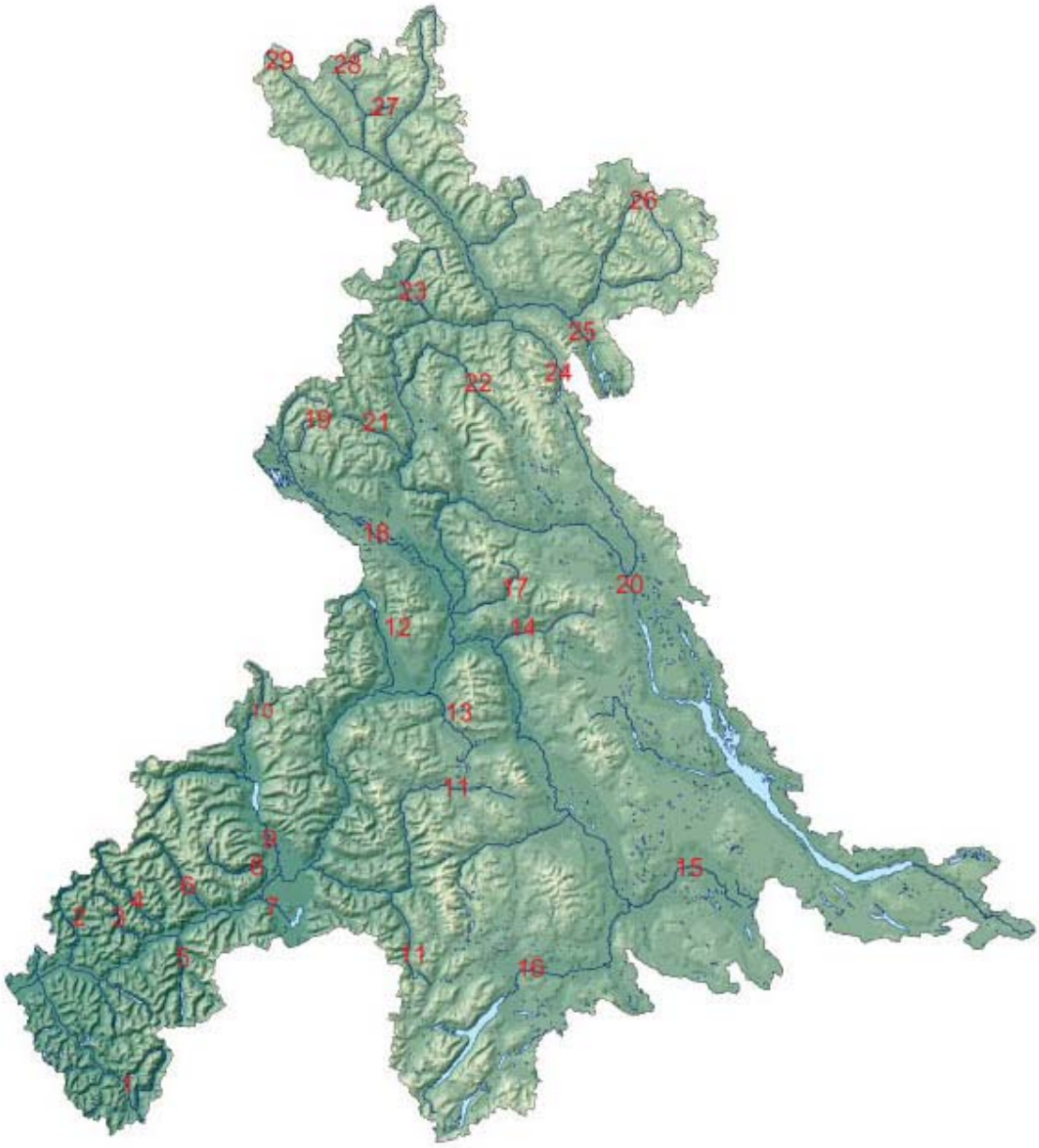

Figure 1. Chinook Baseline collections localities as of 2009. 1 Ecstall River, 2 Khyex River, 3 Kasiks River, 4 Exchamsiks River, 5 Gitnadoix River, 6 Exstew River, 7 Lakelse River, 8 Zymogotitz River, 9 Kalum River, 10 Cedar River, 11 Zymoetz River, 12 Kitwanga River, 13 Gitsegukla River, 14 Suskwa River, 15 Upper Bulkley River, 16 Morice River, 17 Shegunia River, 18 Kispiox River, 19 Sweetin River, 20 Babine River, 21 Kuldo Creek, 22 Sicintine River, 23 Slamgeesh River, 24 Squingula River, 25 Bear River, 26 Sustut River, 27 Tantan Creek (Kluatantan), 28 Kluayaz Creek, 29 Kluakaz & Otsi Creeks

Applications of this research

The improvement of the Skeena DNA baseline has changed the understanding of Skeena Chinook and has led to several changes. The new data has encouraged the improvement of the techniques for separating Skeena Chinook populations. An ongoing reanalysis of the population genetics of Skeena Chinook, is expected to be used for evaluation of the effectiveness of sampling juvenile salmon and been applied to interpretation of the Skeena Test Fishery.

Change in technique for sampling Chinook populations

It became clear early in the 2009 field season that insufficient adult Chinook could be collected to complete the baseline in less than five years. We therefore switched to collecting juveniles where it seemed justifiable. We took care to sample well downstream of the spawning areas, frequently in the most downstream area of suitable habitat while taking care to be at least three kilometres above the mouth of the river.

Sibling analysis of juvenile samples

Ruth Withler of the Pacific Biological Station has agreed to analyze the juvenile samples to check on the abundance of siblings in the collections. Siblings are recognizable in that they share 50% of their genes. Should it turn out that the juvenile collections are well-mixed, in the future collections of Chinook can be made efficiently at much reduced cost. The abundance of siblings is also an indication of the effective population size (P_e) and holds promise for future work on population size estimation. The sibling analysis work has yet to be carried out, but the general success in sorting out Chinook populations suggests that there is not a serious problem with sibling sampling.

Improvements in Skeena salmon genetics

The existence of a larger set of Skeena populations encouraged Terry Beacham of the salmon genetics laboratory to add three additional microsatellite alleles to the existing set of 12 alleles to improve the interpretation of Skeena populations. This new data has been used in the creation of a new dendrogram of Chinook populations and the PORGS analysis of the relatedness of these populations by John Candy similar to the existing analysis of the west coast of Vancouver Island populations (Candy *et al.* 2009).

Reinterpretation of the Skeena Test Fishery by Ivan Winther

The Skeena Test Fishery collections were extended to begin earlier in 2009 than in previous years and to better sample the Chinook runs. If the baseline is of high enough quality and enough specimens are collected and analyzed, the test fishery results can be used to estimate the escapement of the larger Skeena stocks. This was attempted by Ivan Winther, in his January 2010 report, with the estimated escapements compared to the available data on escapements based on the mark and recapture estimate for the Kalum River, the fence count for the Kitwanga River, the partial fence counts for the Babine River and the Sustut River and several visual estimates. The Kalum River mark and recapture estimate was assumed to be accurate and other populations were estimated by relative proportion of the total. In

general the results of the genetic estimates yield estimates of escapement larger than those provided by other methods. This is similar to results from earlier analyses with grossly incomplete genetic baselines. We anticipate that better results will follow from further improvements in the baseline collections and in genetic technique. A reanalysis using the expanded set of microsatellite loci and the improved baseline collection is currently in progress.

Interpretation of dendrogram of 29 March 2010

The new dendrogram of Skeena Chinook populations produced by John Candy (Figure 6) clarifies the relationship of the Skeena Chinook populations. The horizontal distance units in this dendrogram reflects the overall degree of genetic differentiation of the breeding populations. They are F_{st} values presented as the proportion of intrapopulation genetic variation compared to the combined population genetic variation. There is a strong geographic component to this dendrogram. The population grouping supported by this diagram are as follows.

Lower Skeena

The rivers tributary to the Skeena from the Zymoetz River downstream to the Kasiks River form a compact grouping. The Kalum River population is near the base of the classification for the rivers downstream of Terrace. The Rivers below the Kalum are especially tightly clumped. As far as is known these Chinook have similar life history patterns, they are all river type Chinook that spend their first year in the river environment and they return to the Skeena from late June to early August. These Chinook move into their spawning rivers in August and spawn from late August to early September. The Kalum stock differ from the other lower Skeena stocks in that it is dominated by six-year old returning fish whereas the other Skeena stocks are dominated by five-year old fish.

Middle Skeena

The Middle Skeena Chinook stocks form a compact group with roots in the Kispiox and/or Kitwanga stocks. As far as is known these Chinook have similar life history patterns, they are all river type Chinook that spend their first year in the river environment and they return to the Skeena from late June to early August. These Chinook move into their spawning rivers in August and spawn from late August to early September. According to this dendrogram The Harold Price Creek and Suskwa River samples are not significantly different. They have now been combined in the Skeena baseline and are considered a single stock elsewhere in this report.

Upper Skeena

The Upper Skeena Chinook stocks form a compact group with roots in the Slamgeesh stock. As far as is known these Chinook have similar life history patterns, they are all river type Chinook that spend their first year in the river environment and they return to the Skeena from late June to early August. These Chinook move into their spawning rivers in August and spawn in mid to late August. According to this dendrogram the Kluakaz Creek and Otsi Creek samples are not significantly different. The two localities are both in the northern

headwater reach of the Skeena at alluvial fans about 15 km apart. They have now been combined in the Skeena baseline and are considered a single stock elsewhere in this report.

Early Run Stocks

The early Bulkley River Chinook spawn above Houston in the upper Bulkley River. They are an early returning stock that passes through Tyee in May and June and is complete by the time the bulk of Skeena Chinook arrive. Upstream passage of these Chinook in the upper Bulkley River is restricted to high flow conditions at Bulkley Falls. The Cedar River Chinook spawn in northern tributaries of Kalum Lake. They also have early timing entering the Skeena in May and peak in early June. The third stock that sorts out with the early Chinook is Sicintine River. This stock was not formerly represented in the Skeena baseline. There are relatively few Sicintine River Chinook in the 2009 Skeena Test Fishery analysis but all of the fish (N=9) observed were in June, and mostly in early June.

Ecstall River

The most differentiated Skeena Chinook population are the Ecstall river Chinook with an F_{st} value of 0.049. They are the only Skeena population that is ocean rearing, that is the fry leave in their first summer to rear on the coast. The fry probably do not migrate north to the Gulf of Alaska as the river type Chinook do, but remain in the coastal environment. In 2007 we collected a single immature Ecstall River Chinook in Prince Rupert harbour in mid winter. All other Chinook in the small collection of winter resident Chinook were from southern British Columbia and Puget Sound where this life history type is the dominant one.

Large Lake Outlet

The three large upriver Chinook stocks are very similar and may be a distinct genetic unit. They occupy spawning habitats below major lakes and may spawn later (into September) than other Skeena stocks. The lake outlet spawning habitat may provide a more moderate winter environment for egg development.

The stock groupings presented above can be proposed as six "natural" conservation units based on genetics. One additional conservation unit might be the separation of the Kalum Chinook into it's own CU because of the difference in age structure. These proposed conservation units are similar to those proposed by Holtby in 2008 and distributed at least within the DFO and apparently used for the 2009 Science Advisory report "Framework for Implementation of the Wild Salmon Policy" (CSAS 2009). The Lower Skeena, Middle Skeena, and Upper Skeena units of Holtby 2008 are retained but the included populations have changed somewhat. The characterization of the middle Skeena CU has changed somewhat. A separate CU for Gitnadoix, suggested by Holtby, is rejected as with better data the Gitnadoix population is placed firmly within the Lower Skeena unit. Separate conservation units for the upper Bulkley and the Cedar river are combined into a Skeena Early Run unit. A conservation unit for large lake outlet populations is restricted to the three large lake populations below the Morice lake, Babine lake and Bear Lake. Overall this genetics based proposal represents a minor reduction of CUs from nine in CSAS 2009 to six or seven.

Acknowledgements

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Table 2. Baseline DNA samples of Skeena Chinook Spawning Populations in the DFO molecular Genetics Laboratory.

Region Name	Pop Name	Collect Year	2005 SampleSize	2008 SampleSize	2009 SampleSize	Rel Pop Size
Skeena Upper	Bear	1991 1995 1996 2005	177	182	182	L
Skeena Upper	Kluakaz_Otsi Cr	2007 2008 2009		106	199	M
Skeena Upper	Kluatantan	2006 2009		18	21	S
Skeena Upper	Kluayaz_Cr	2007 2008 2009		127	150	L
Skeena Upper	Kuldo	2008 2009		1	170	M
Skeena Upper	Sicintine	2009			112	M
Skeena Upper	Slamgeesh	2004 2005 2006 2007 2008 2009	34	81	129	L
Skeena Upper	Squingula	2008 2009		1	271	L
Skeena Upper	Sustut	1995 1996 1999 2001 2002 2003 2005 2006	416	519	519	L
Skeena Babine	Babine	1994 1995 1996	266	266	266	L
Skeena Bulkley	Bulkley_sp	1991 1992 1994 1995 1996 1998 1999	585	588	588	M
Skeena Bulkley	Morice	1991 1995 1996	228	228	228	L
Skeena Bulkley	Suskwa	2004 2005 2009	21	22	109	S
Skeena Mid	Gitsegukla	2009			260	S
Skeena Mid	Kispiox	1979 1985 1989 1991 1995 2004 2006 2008	153	197	197	L
Skeena Mid	Kitwanga	1991 1996 2002 2003	240	288	288	L
Skeena Mid	Shegunia	2009			79	S
Skeena Mid	Sweetin	2004 2005 2008	44	64	64	S
Skeena Lower	Cedar_sp	1996	116	116	116	M
Skeena Lower	Ecstall	1995 2000 2001 2002 2003	293	293	293	M
Skeena Lower	Exchamsiks	1995 2009	11	9	116	S
Skeena Lower	Exstew-juv	2009		0	140	M
Skeena Lower	Gitnadoix	1995 2002 2003 2009	66	56	268	M
Skeena Lower	Kasiks	2009		0	61	S
Skeena Lower	L_Kalum	1991 1995 1996 1998 2001 2009	647	420	791	L
Skeena Lower	Zymoetz	1995 2003 2004 2009	29	26	61	M
Skeena Lower	Zymogotitz	2006, 2009		1	120	S
		Sample size with N>100	10	12	22	

Sample size source J. Candy Mar 2009.

Figure 2. Beach seine for juvenile Chinook. Typical habitat is in the higher energy reach of the distant channel.

Figure 3. Chinook, larger robust specimens and mountain whitefish, smaller more gracile forms.

Figure 4. Scale sampling of Chinook at Tantan Creek (Kluatantan).

Figure 5. Tangle net fished at Gitangwalk on the Slamgeesh River.

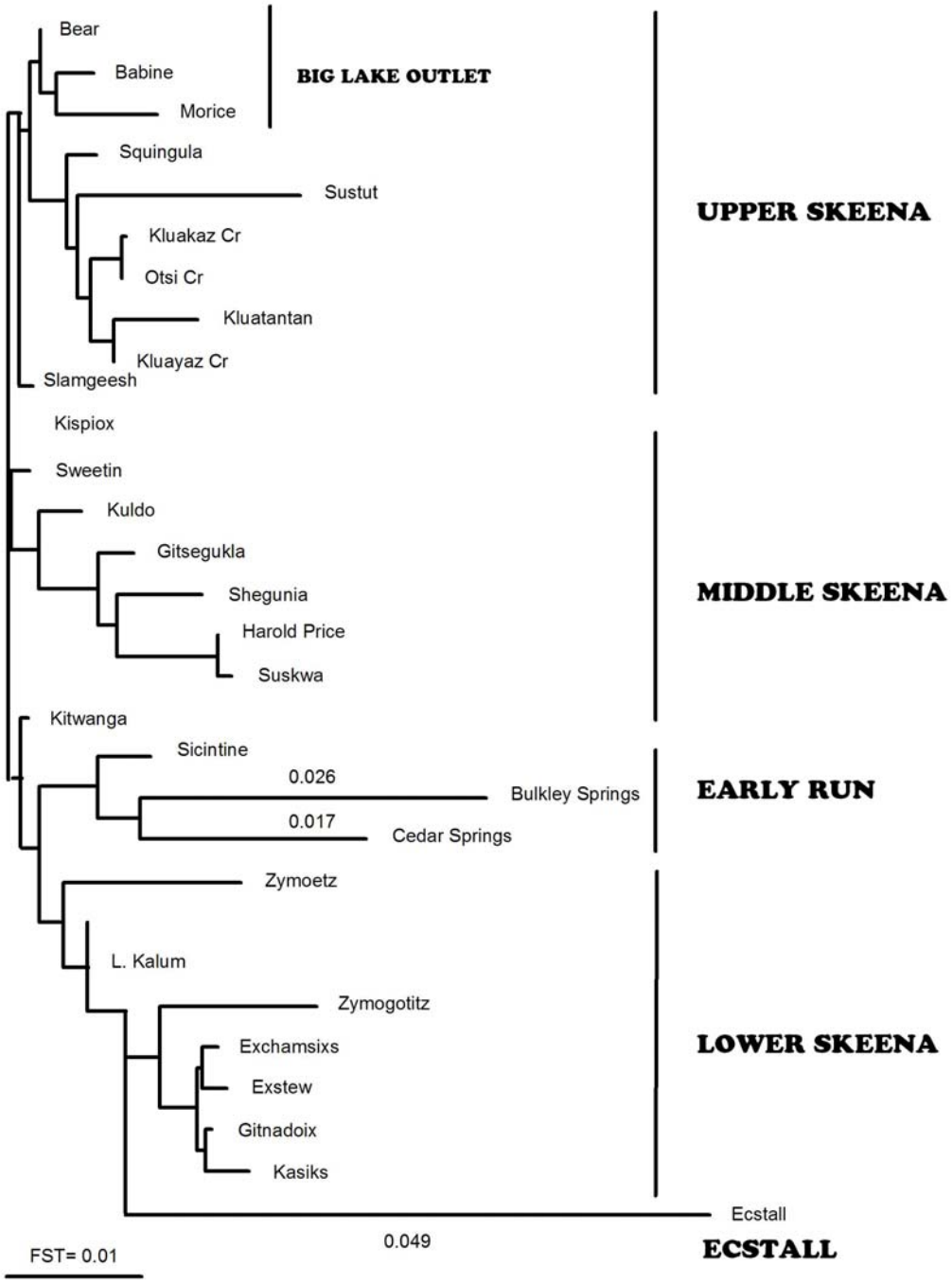

Figure 6. Dendrogram of Skeena Chinook populations as of March 30, 2010. The horizontal distances are proportional to interpopulation F_{ST} values. Prepared by J. Candy DFO Salmon Genetics Laboratory

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