Gitksan Watershed Authorities

2007 DNA Collection Report

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1.0 Introduction

In 2003 the Gitksan Watershed Authorities (GWA) initiated a project to collect genetic (tissue) samples for DNA analysis from chum and pink salmon in selected streams throughout the Gitxsan territories. Chum DNA collection continued in 2004 where the project was expanded to include chinook from selected streams. In 2005 the tissue sample collection continued but on an opportunistic level and included stream type sockeye. In 2006 the Alaska Department of Fish and Game contracted the GWA to collect 100 axillary process samples from sockeye salmon in 3 streams. In 2007 two more locations were added for dna collection, Kluakaz Creek and Kluayaz Creek both near the headwaters of the Skeena.

From the individual tissue samples and their spawning streams, DNA analysis will provide a 'genetic map' that displays the genetic diversity of the wild stocks throughout the Skeena. In establishing genetic profiles, the commercially valuable species/stock can be identified to the watershed and/or stream, therefore improving harvest management. The profiles would also help with preserving information about the genetic diversity of all species.

2.0 Methods

The streams are either walked or floated using inflatable rafts. Capture methods vary between angling, using dip nets, and the use of a tangle net (4.5" mesh). The capture method used depended on stream type, size, water conditions, and the number of fish in a certain area. All fish were handled with care, in order to minimize lethal or sub-lethal impacts from sampling, and released back to the stream alive.

When the tangle net was used, fish were removed and held in dip nets until sampled. Fish captured with fishing rods or dip nets were processed immediately. Using a hole-punch one tissue sample was taken from the operculum of each fish. Tissue samples from the individual streams were pooled in vials consisting of 95% ethanol with a maximum of 50 samples per vial. Further process details are included in Appendix 1.

The axillary process spine is located above the pelvic fin. The spine is removed using dog nail clippers; one axillary is cut per fish. This process is detailed in Appendix 2.

Data collected and recorded are as follows: sample date, time, stream name, crew names, capture method, vial #, fork length (cm), girth (cm), sex, level of maturity (immature, mature, spawned out, dead pitch), scales (book #, scale #'s), and waypoints. Waypoints were taken using a handheld GPS.

Two hundred tissue samples from each stream for each species is considered adequate to accurately describe the genetic variation of each population (Brian Spilsted, DFO North Coast Stock Assessment, personal communication 2003).

3.0 Results

In 2008 the DNA collection efforts continued on the following streams: Kispiox River (Chum, Sockeye), Nangeese River (chum, sockeye), Date Creek (chum), Skeena River at the Halliday Campground (chum, sockeye), Slamgeesh River (chinook), Skeena River at Kluakaz Creek (Skeena headwaters), and Kluayaz Creek & Kluayaz Lake (Upper end of Kluatantan River). 30 samples were collected from Kluakaz Creek and 86 samples were collected from Kluayaz Creek. Refer to table 1 for a summary of all tissue sample collections from 2002-2007. Refer to table 2 for sample dates and sample locations (UTM's).

Table 1. Summary of all dna collection from 2002-2007.

		# of samples collected by year						
Stream Name	**Species**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**Total**
Kispiox River	Chinook	0	0	62	0	28	0	**90**
	Chum	0	0	13	25	7	0	**45**
	Sockeye	0	0	0	176	0	0	**176**
	Pink	0	201	0	0	0	0	**201**
Nangeese River	Chum	0	96	5	18	17	7	**143**
	Sockeye	30	0	0	38	49	26	**143**
Sweetin River	Chinook	0	0	46	9	0	0	**55**
Suskwa River	Chinook	0	0	21	3	0	0	**24**
Slamgeesh River	Chinook	0	0	32	4	10	13	**59**
	Sockeye	0	0	0	0	100	0	**100**
	Pink	0	0	0	1	0	0	**1**
Skeena River (Halliday Campground)	Chum	0	0	5	18	2	4	**29**
	Sockeye	0	0	0	29	26	13	**68**
Date Creek	Chum	0	13	13	42	3	16	**87**
Lower Club Creek	Sockeye	0	0	0	0	108	0	**108**
Oweegee Creek	Chinook	0	0	1	0	0	0	**1**
Tantan Cr. & Kluatantan Lk.	Chinook	0	0	0	0	7	0	**7**
Kluayaz Cr. & Kluayaz Lk.	Chinook	0	0	0	0	0	86	**86**
Skeena R. at Kluakaz	Chinook	0	0	0	0	0	30	**30**
Fiddler Creek	Chum	0	0	6	1	0	0	**7**
Bulkley River	Pink	0	200	0	0	0	0	**200**
Morice River	Pink	0	0	0	200	0	0	**200**
Total Collection	Chinook	0	0	162	16	45	129	**352**
	Sockeye	30	0	0	243	283	39	**595**
	Chum	0	109	42	104	29	27	**311**
	Pink	0	401	0	201	0	0	**602**
						Total # of all Samples		**1860**

Table 2. 2007 DNA sample dates and locations.

GWA Chum/Chinnok/Sockeye Adult DNA Samples - 2007					
Date	Location	Easting	Northing	Species	# of samples
12-Sep-07	Nangeese River	541344	6172213	Chum	7
17-Sep-07	Date Creek	582934	6138267	Chum	16
24-Sep-07	Halliday Slough	584121	6137006	Chum	4
16-Aug-07	Upper Skeena at Kluakaz	520818	6329342	Chinook	30
22-Aug-07	Kluayaz R. & Kluayaz Lk.	548749	6318841	Chinook	86
28-Aug-07	Slamgeesh River	566539	6250737	Chinook	13
11-Sep-07	Nangeese River	541344	6172213	Sockeye	26
24-Sep-07	Halliday Slough	584121	6137006	Sockeye	13

Total Chum DNA	27
Total Chinook DNA	129
Total Sockeye DNA	39
Total # of samples	195

4.0 Conclusion

The collection of tissue samples will continue on an opportunistic basis until the goal of 200 samples per species per stream is met. The goal of 200 samples has been met in the Kispiox, Bulkley, and Morice Rivers for pink salmon.

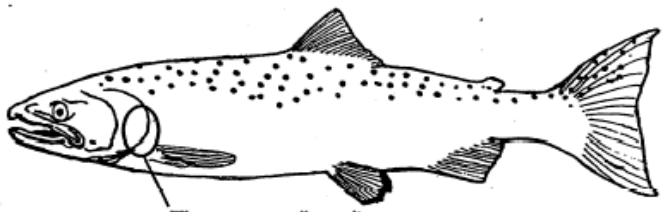
5.0 References

Spilsted, Brian 2003 personal communication DFO North Coast Stock Assessment

Appendix 1. DNA Sampling Protocol

DNA SAMPLING PROTOCOL FOR DFO.
Sampling instructions for fresh tissue.

- Use a common paper punch to take a tissue sample from the operculum of the fish (see below) taking only one punch per fish.
- Place punch in a sample bottle containing 95% non- denatured ethanol solution. Do not overload the vials because the DNA will degrade. Vials should contain no more than 25% tissue to 75% ethanol.
- Label each bottle with geographic location, species, date and sampler. It is important that this information be included for the sample to be useful to us.
- Labels should be attached to the outside of the vials. Cover the labels with clear tape to ensure the writing does not dissolve with preservative or use solvent resistant markers.



Appendix 2. DNA Sampling Protocol

Sampling Non-lethal Finfish Tissues for DNA Analyses

Alaska Department of Fish and Game, Gene Conservation Lab, Anchorage

I. General Information

We use axillary process samples from individual fish to determine the genetic characteristics and profile of a particular run or stock of fish. This is a non-lethal method of collecting tissue samples from adult fish for genetic analysis. The most important thing to remember in collecting samples is that **only quality tissue samples give quality results**. If sampling from carcasses: tissues need to be as "fresh" and as cold as possible and recently moribund, do not sample from fungal fins.

Sample preservative: **Ethanol (ETOH) preserves tissues for later DNA extraction without having to store frozen tissues (Avoid extended contact with skin).**

These sampling instructions are written for bulk method (N=100/125ml or N=200/250ml) bottle per site.

How to *collect* the axillary process tissues:

- Preservative used: Isopropanol/Methanol/Ethanol (ETOH).
- Wipe dry the axillary process "spine" prior to sampling to avoid getting excess water or fish slime in the 125/250ml bottle (see attached print out of axillary process).
- Clip off the axillary "spine" using dog nail clippers or scissors to get roughly a ½ - **1" inch max**. piece and/or about the size of a small fingernail.
- Place this piece into the sample bottle **(place only one piece of axillary from each fish)**.
- Repeat procedure for **up to 100 or 200 individual fish** per bottle (dependant on bottle size). This is the limit for proper preservation of axillary tissue.
- Record on pre-printed label: Sample location, date samples are collected, number of fish sampled, species and field notes.
- After 24 hours; **"refresh"** step - pour out the alcohol from the sampling container and pour in fresh alcohol to assure proper preservation. If collections are under 100 fish and/or collected over time (~ 7-10 days) don't worry about refresh until collection is complete.
- Freezing not required, store sample bottle in cool location for good tissue quality.

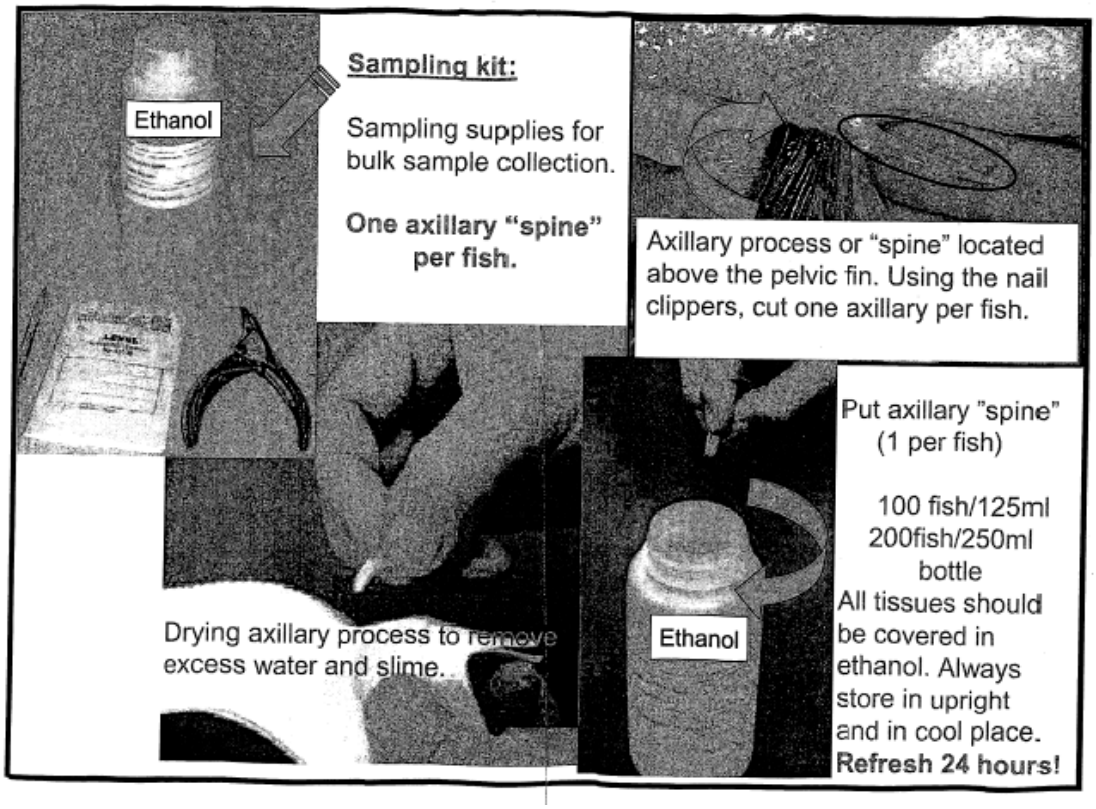
Return to ADFG Anchorage office:

ADF&G – Genetics
333 Raspberry Road
Anchorage, Alaska 99518

Lab staff: 1-907-267-2247
Judy Berger: 1-907-267-2175
Shipping code:

Supplies included with sampling kit:

1. (1) - Dog toe nail clippers and/or scissors: used to cut a portion of **one** axillary process.
2. 125 ml or 250 ml wide mouth bottle(s) for ETOH.
3. Pencil
4. Sampling instructions


Ethanol
Sampling kit:
Sampling supplies for bulk sample collection.
One axillary "spine" per fish.
Axillary process or "spine" located above the pelvic fin. Using the nail clippers, cut one axillary per fish.
Drying axillary process to remove excess water and slime.
Ethanol
Put axillary "spine" (1 per fish)
100 fish/125ml
200fish/250ml
bottle
All tissues should be covered in ethanol. Always store in upright and in cool place.
Refresh 24 hours!