# Skeena Steelhead Conservation Units

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# <u>Summary</u>

- <u>Purpose</u>: Steelhead Conservation Units were defined for the Skeena River using methodology consistent with the Wild Salmon Policy. The process combined *habitat*, life *history* and *molecular genetics* knowledge
- <u>Habitat</u>: To maintain adaptive genetic diversity it is necessary to maintain a diversity of habitats. The diverse watersheds of the Skeena were grouped into terrestrial habitat types consistent with provincial land use planning procedures. The approach also has potential for assisting in achieving the habitat goals of the Wild Salmon Policy.
- <u>Aquatic Classification</u>: Skeena watersheds were classified into Regional hydrology groups. These watershed types, along with gradient, temperature, nutrients, migration difficulty and distance from the ocean are thought to be the physical factors most relevant to the development of critical adaptive traits for salmonids
- Life History Steelhead have the most diverse life history of any of the salmonids. Their absolute dependency on a long period of freshwater rearing (generally 3-5 years) combined with a variable period in salt water (1-3 years with the option of repeat spawning) results in a complex variety of life history strategies. Previous studies (Cox-Rogers 1985) demonstrated clear, genetically-based life history differences among the Zymoetz, Bulkely/Morice, Kispiox, Babine and Sustut populations. This was a small subset of the total number of Skeena steelhead populations but together they accounted for a significant proportion of production and provided representation from lower, middle and upper tributaries.
- <u>Molecular Genetics.</u> Molecular genetics provided new insights into relatedness and the degree of reproductive isolation among steelhead populations. Analysis of Skeena samples using methods consistent with those in the Wild Salmon Policy resulted in the definition of an absolute minimum of 6 main groups within the existing Freshwater Adaptive Zones as follows :(note that all populations were not included in the analysis due to sampling limitations): Lower Skeena (Zymoetz, Lakelse, KitsumKalum); the Middle Skeena subdivided into 3 groups; (Babine), (Kitsequecla/Suskwa) and (Bulkley, Morice/Kitseguecla/Toboggan/Kispiox). The Upper Skeena subdivided into two groups (Mosque/Kluatantan/Lower Sustut) and (Upper Sustut).
- <u>Final Conservation Units</u> Aggregating and analyzing the life history, habitat and molecular genetics data resulted in 11 steelhead Conservation Units (see map below).

#### <u>Conclusion</u>

The analysis provides support for the characterization of steelhead as a species rich in genetic variation as expressed by the complexity of life history types present and utilization of diverse habitat types. It again confirms the Skeena River as one of the few remaining naturally productive and diverse salmonid systems in the world



Proposed Steelhead Conservation Units for the Skeena River. Shading represents stream reach gradients.

# **1. BACKGROUND**

The primary objective of the federal Wild Salmon Policy (WSP 2005) is to safeguard the genetic diversity of wild Pacific salmon. The Department of Fisheries and Oceans (DFO) intends to maintain both genetic and habitat diversity through the management and protection of "Conservation Units" (CUs), where a conservation unit is defined as "a group of wild salmon, sufficiently isolated, that if extirpated, is unlikely to re-establish itself within a human lifetime or in a specified number of salmon generations". According to the policy, stock status and habitat status are both intended to be evaluated at the CU spatial scale.

Among the several challenges facing implementation of the WSP is the omission of steelhead (Oncorhynchus mykiss) as part of the planning framework. Earlier work conducted by the Province of BC (Parkinson, E.A., E. Keeley, E.B. Taylor, S. Pollard and A.F. Tautz. 2005) was not included in the WSP planning, nor was there any significant attempt to harmonize the possible approaches. Consequently, the province used "Conservation unit" as a term to describe aggregations of the steelhead populations listed in the paper for *various* purposes.

In the Provincial application, CUs were a tool to "organize the complexities of intraspecific variation for BC steelhead in a population database that permitted flexibility in how populations are classified for conservation and management purposes." In other words, different aggregations could be used to meet different objectives. <u>It was not intended for the conservation units identified in the paper to be used for mixed stock management purposes (i.e. for application as described in the WSP).</u>

As might be expected, the confusing and inconsistent use of the term CU has added to the already significant difficulties in the management of steelhead bycatch. For the Skeena, two steelhead CUs (winter and summer run) remained as the documented conservation units, even though the federal and provincial objectives in 2005 were dramatically different. It is the view of the authors of the original paper that the use of only two conservation units for the Skeena significantly underestimates the degree of genetic variation requiring protection under the WSP.

Therefore, the purpose of this project is to provide an update of Skeena steelhead population structure that is more consistent with both the federal methodology and the intent of the wild salmon policy. The paper is intended as scientific guidance, not provincial policy per se, and may only be relevant to the Skeena watershed.

# 2. OVERALL APPROACH

The methods in this paper are similar to those used by the federal government in developing the salmon CUs. We attempted to make use of most or all of the available physical and biological information to define both steelhead populations and Conservation Units. Three types of information were used: habitat, life history, and molecular genetics. This combination is now regarded as the preferred approach to the classification and management of genetic variation in salmonids (Waples, 2001).

Prior to developing the Conservation Units themselves, the initial and non-trivial problem was to define the "building blocks" (i.e. "populations") that form the Conservation Units. Fortunately much of the biological information and the criteria for defining steelhead populations were summarized in (Parkinson et al. 2005). The details of the approach will not be repeated here, except to say that the method depends on the provincial Watershed Atlas and the Steelhead Harvest Analysis (SHA). Also, a significant amount of the biophysical data was housed in the EAUBC dataset (MOE) and the province's Land and Data Warehouse (LRDW). The provincial 1:50k dataset was used to define the Freshwater Adaptive Zones (FWAs) in (Holtby and Ciruna 2007).

## 2.1 SPATIAL SCALE

The earlier work was based on the 1:50k federal NTS system but since then the province has completed a 1:20k freshwater atlas (FWA). Since this is now considered the provincial standard, the 1:20k scale was used in this analysis. This meant that much of the work needed to be repeated using the new GIS layers, but a major benefit was that access to all of the other 1:20k layers in the data warehouse was now possible. This included those layers relating to forest harvesting, roads, water licenses etc., all of which would be relevant to work on the habitat (goal 2) part of the WSP. Unfortunately many of the watershed codes and other stream and lake attributes were not consistent, but work is currently underway to cross reference the two systems.

Finally, it should be noted that the work of Gottesfeld and Rabnett (2008) included much of the local and scientific knowledge pertinent to the Skeena watershed and provided an additional comprehensive and detailed description of the River and its resources.

# **3. ANALYSIS UNITS**

For the Skeena, the new freshwater atlas (FWA) contains in excess of 189,000 polygons, each polygon associated with a single streamline. This information was far too detailed to define populations or Conservation Units and an automated method for simplification was required. Since the watershed code (stream identifier) is a hierarchical attribute, it provided a useful tool for aggregating the attributes of individual streamlines and associated polygons. The method of (Parkinson et al. 2005), which consisted of defining significant and accessible tributaries of "large" river systems or the ocean as "populations", was generally followed here. The process is summarized diagrammatically in Fig. 1.



Fig. 1 Diagrammatic representation of the process for developing analysis units for the Skeena Watershed using the Province of BC Freshwater Atlas.

First, all tributaries of the Skeena mainstem had their polygons combined into single polygons or watersheds. This was accomplished by grouping the polygons using the first 10 characters of the watershed code (i.e. the code for the Skeena mainstem plus the code for each tributary mainstem). This calculation captured all watersheds tributary to the Skeena mainstem and their areas (Fig. 2).



Fig 2 First "dissolve" of all tributaries to the Skeena mainstem.

The distribution of areas is approximately log normal (Fig.3), with a median size of between 30-100 hectares. This is below the size where steelhead fisheries are monitored in the Skeena although it is above the minimum size required for persistent steelhead populations (Parkinson et al. 2005, R. Ptolemy pers. com.).



Fig. 3 Log Normal distribution of "dissolved" sizes (in hectares) of polygons forming all tributaries to the Skeena Mainstem.

This initial result was unsatisfactory since the process created a few large polygons and several smaller ones (typical for log normal distributions). To make the units more uniform, a minimum size filter of 100Km2 was imposed. This procedure excluded only one of the "steelhead watersheds" defined in (Parkinson et al. 2005, Appendix 1) and resulted in a significant reduction in size variation of the analysis units. It also produced a set of polygons more easily associated with the data from the steelhead harvest analysis i.e. many were recognized by anglers and managers as "steelhead systems".

A variation from the Parkinson methodology used in this paper was that the "small" (<100 km2) polygons were dissolved into the Skeena mainstem, creating a number of new "mainstem areas" each with its own ecological function and characteristics. The rationale for defining some mainstem sections as "areas" as opposed to "watersheds" is fully described in (Gottesfeld et al. 2008) but simply put, it provided a unit for aggregating small tributaries, side channels and mainstem sections for parts of large watersheds. This

type of ecological feature would normally be ignored in a watershed based analysis, but these mainstem areas perform a critical function in the life history of the species and therefore should be given more consideration in future assessments.

For the large tributary systems (Bulkley, Babine, and Sustut rivers), another iteration of the above process produced the final analysis units shown in Fig. 4. The attributes for the 56 units are summarized in Table 1

Since this method did not take into account information such as known occurrences of steelhead, other salmon or obstructions, it produced more polygons (56 vs 31) than (Parkinson et al. 2005) Most of the omitted watersheds were either small, unproductive or had limited access so that steelhead numbers could be small or nonexistent. On the other hand, it may be that they contain ephemeral populations that, though never sampled, play an undetermined role in the maintenance of genetic variation (e.g. may act as refugia during catastrophic events in the tributaries).



Fig. 4 Analysis units for the Skeena River based on the "automated" method for generating watersheds and mainstem area. Shading represents gradients associated with streamlines.

Table 1 Analysis units for Skeena River with associated FAZ, area in hectares, FWA code, percent of Skeena watershed area and cumulative percent (measured as distance upstream)

ID	gt100km2	FAZ	Area(ha)	FWA_Short	sk_pcnt	sk_cum
30	L_Skeena_Main_W	Lower Skeena	138289	400	2.5	2.5
12	Ecstall River	Lower Skeena	148657	400-014636	2.7	5.3
21	Khyex River	Lower Skeena	44185	400-036391	0.8	6.1
39	Scotia River	Lower Skeena	13476	400-056622	0.2	6.3
20	Khtada River	Lower Skeena	15678	400-059865	0.3	6.6
19	Kasiks River	Lower Skeena	26168	400-094444	0.5	7.1
13	Exchamsiks River	Lower Skeena	51420	400-107640	0.9	8.0
17	Gitnadoix River	Lower Skeena	54530	400-116952	1.0	9.1
14	Exstew River	Lower Skeena	45511	400-139009	0.8	9.9
29	L_Skeena_Main_E	Lower Skeena	140839	400-174067	2.6	12.5
31	Lakelse River	Lower Skeena	58285	400-174068	1.1	13.5
55	Zymagotitz River	Lower Skeena	38858	400-185024	0.7	14.3
24	Kitsumkalum River	Lower Skeena	228943	400-195432	4.2	18.5
56	Zymoetz River	Lower Skeena	302611	400-221484	5.6	24.0
26	Kleanza Creek	Lower Skeena	20102	400-234275	0.4	24.4
15	Fiddler Creek	Lower Skeena	17071	400-299006	0.3	24.7
32	Lorne Creek	Lower Skeena	10718	400-303799	0.2	24.9
38	Quill Creek	Lower Skeena	12901	400-315638	0.2	25.1
18	Insect Creek	Lower Skeena	20044	400-326256	0.4	25.5
40	Sedan Creek	Lower Skeena	12114	400-351455	0.2	25.7
33	Middle Skeena	Middle Skeena	130960	400-359642	2.4	28.1
25	Kitwanga River	Middle Skeena	82679	400-365504	1.5	29.7
23	Kitseguecla River	Middle Skeena	71445	400-396037	1.3	31.0
6	Bulkley River	Middle Skeena	279992	400-431358	5.1	36.1
52	Upper Bulkley River	Middle Skeena	231487	400-431358-	4.3	40.4
47	Suskwa River	Middle Skeena	132203	400-431358-079962	2.4	42.8
50	Toboggan Creek	Middle Skeena	12213	400-431358-237852	0.2	43.0
49	Telkwa River	Middle Skeena	120312	400-431358-415251	2.2	45.2
34	Morice River	Middle Skeena	437905	400-431358-585806	8	53.3
22	Kispiox River	Middle Skeena	210066	400-454855	3.9	57.2
42	Shegunia River	Middle Skeena	26184	400-456504	0.5	57.6
2	Babine River	Middle Skeena	180559	400-536025	3.3	61.0
1	Babine Lake	Middle Skeena	632245	400-536025-	11.6	72.6
41	Shedin Creek	Middle Skeena	55643	400-536025-027715	1.0	73.6
43	Shelagyote River	Middle Skeena	57744	400-536025-192976	1.1	74.7
37	Nilkitkwa River	Middle Skeena	82633	400-536025-357066	1.5	76.2
36	Nichyeskwa Creek	Middle Skeena	36155	400-536025-367117	0.7	76.8
10	Deep Canoe Creek	Middle Skeena	16744	400-575120	0.3	77.2
28	Kuldo Creek	Middle Skeena	60225	400-591265	1.1	78.3
54	Upper_Skeena	Upper Skeena	135765	400-615310	2.5	80.8
44	Sicintine River	Upper Skeena	81297	400-637044	1.5	82.3
/	Canyon Creek	Upper Skeena	26429	400-675225	0.5	82.7
45	Slamgeesh River	Upper Skeena	60567	400-706228	1.1	83.9
46	Squingula River	Upper Skeena	69866	400-746513	1.3	85.1
48	Sustut River	Upper Skeena	190959	400-757844	3.5	88.6
53	Upper Sustut River	Upper Skeena	121492	400-757844-	2.2	90.9
4	Bear River	Upper Skeena	45233	400-757844-263905	0.8	91.7
35	ivios que River	Upper Skeena	49935	400-808455	0.9	92.6
51	U_Skeena_HeadWtr	Upper Skeena	157565	400-811559	2.9	95.5
10	FUTT CTEEK	Upper Skeena	10420	400-818//1	0.2	95./
ŏ 11		Upper Skeena	102501	400-852488	0.4	90.1
2	Duu Kiver	Upper Skeena	103581	400-872231	1.9	98.0
3 77	Barker Creek		1107Z	400-070000	0.2	90.Z
27	Currier Creek		0120/	400-030369	1.1	99.3 00 7
9	Poirpos Crock		21000	400-907767	0.4	39./ 100.0
Э	Den nes Creek	opper skeena	10200	400-930143	0.5	100.0

# 4. HABITAT

Our starting point for the steelhead analysis was to assign the analysis units defined above to the current Freshwater Adaptive Zones (FAZ); then determine if habitat, life history and molecular genetic analysis resulted in a finer sub division of those zones into useable Steelhead Conservation units.

The logic of using habitat as a basis for maintaining biological diversity is well established in terrestrial ecosystems. For example, the BEC (Biogeoclimatic) system and the Ministry of Environments' EcoRegional classification have formed the basis for British Columbia's land use planning for decades. The generally accepted view is that when habitats are similar, animal species, even with very different ancestral lineages, will converge in their adaptive characteristics. Therefore, to maintain a natural diversity within species, it is necessary to maintain both ancestral lines and a representative diversity of natural habitats that supports this within-species diversity.

The concept of "representation" has usually been applied to terrestrial ecosystems, but the watershed component of these ecosystems has typically been ignored. This is an odd approach from *ecosystem* biologists, but not surprising given the terrestrial derivation of many conservation biology concepts and the complexity of the interactions. Currently however there is little justification for not using a more integrated approach. Consequently the fundamental method we employed was to intersect the analysis unit boundaries with a number of the physical data sets and look for groupings that were consistent across a number of these data sets.

## 4.1 BIOGEOCLIMATIC CLASSIFICATION

The BEC system (Pojar et al. 1987) has been developed by the Ministry of Forests and has been a cornerstone of land use planning in British Columbia for decades. There are currently 16 BEC zones (Appendix 2) in the province. A zone is generally defined as

"a geographical area (large ecosystem) with a relatively uniform macroclimate, characterized by a mosaic of vegetation, soils and, to a lesser extent, animal life reflecting that climate. Zones are usually named for the potential climatic climax or self-perpetuating vegetation established on mesic (average moisture) sites and zonal (climatically determined) soils. A zone may contain smaller vegetationally and environmentally more uniform ecosystems (subzones) that reflect differences in regional climate, soil moisture, soil nutrient status and environmental disturbance." MCR Edgell, Canadian Encyclopedia While BEC zones were developed as a vegetation planning tool, many of the factors that characterize these BEC zones are also relevant to the classification of watersheds and ultimately to Conservation Units.( i.e. the BEC zone combinations associated with the analysis units would correlate with the physical factors associated with the Freshwater Adaptive Zones).

In conducting the GIS analysis, a number of outcomes were evident. First, each of the analysis units was usually large enough to capture more than a single BEC zone. This was desirable since it provided for the sub-division of watersheds into functional components. A three zone sub-division produced a valley bottom, a side hill and ridge top. These zones have different ecological functions and therefore directly relate to the characterization and evaluation of proper functioning watersheds. Also, since these zones are already in use by land use planners, they provide an existing dataset of familiar indicators. For example, an analysis of the valley bottom BEC Zones may provide a useful indicator of riparian function.

Secondly the BEC zones demonstrate the interaction of at least two physical clines; the west to east decrease in precipitation due to the influence of coastal mountains and the changes in vegetation *within* a watershed from valley bottom to the height of land. These climatic processes, interacting with soil productivity, soil moisture, and disturbance result in the vegetation patterns we see in the Skeena today.

The Skeena contains 8 of British Columbia's 16 BEC zones, (Appendix 2) of which 4 are predominant in the valley bottoms. Starting at the river mouth, the mainstem passes through the Coastal Western Hemlock, (CWH), the Interior Cedar Hemlock (ICH), the Sub Boreal Spruce (SBS) and the Engleman Spruce Sub-Alpine FIr zone (ESSF) at its extreme upper end (Fig. 5).



Fig. 5 Distance Upstream vs elevation showing the approximate locations of the BEC Zones.

These valley bottom zones tend to be associated with predictable zones on the side hills and ridge tops. This leads to a grouping of watersheds based on the patterns of vegetation. The percentage values for each analysis unit are summarized in Table 2.

	WATERSHED	FWA Code	CWH	MH	CMA	ICH	ESSF	BAFA	SBS	SWB
30	L_Skeena_w	400-	52.17	29.78	10.74	7.31				
12	Ecstall River	400-014636	54.49	36.43	9.08					
21	Khyex River	400-036391	39.07	45.60	15.33					
39	Scotia River	400-056622	59.77	35.38	4.86					
20	Khtada River	400-059865	44.45	39.60	15.95					
19	Kasiks River	400-094444	30.19	53.74	16.08					
13	Exchamsiks	400-107640	28.72	46.35	24.93					
17	Gitnadoix River	400-116952	40.04	40.77	19.19					
14	Exstew River	400-139009	25.50	38.85	35.65					
29	L_Skeena_e	400-174067								
31	Lakelse River	400-174068	68.00	25.16	6.84					
55	ZymagotitzRiver	400-185024	36.88	44.88	18.24					
24	Kitsumkalum Riv	400-195432	40.16	35.11	24.73					
56	Zymoetz River	400-221484	21.80	24.57	13.51	2.78	25.15	9.84	2.35	
26	Kleanza Creek	400-234275	36.60	54.11	9.29					
15	Fiddler Creek	400-299006	15.63	63.00	19.29	2.08				
32	Lorne Creek	400-303799	24.74	54.45	18.63	2.18				
38	Quill Creek	400-315638	23.56	52.68	20.25	3.50				
18	Insect Creek	400-326256	20.41	45.90	19.28	2.94		11.46		
40	Sedan Creek	400-351455	20.76	40.23		3.85	1.59	33.57		
33	Middle Skeena	400-359642	5.43	3.24	0.39	55.28	29.31	6.35		
25	Kitwanga River	400-365504	18.30			30.13	44.58	6.99		
23	Kitseguecla River	400-396037	12.17			27.34	48.97	11.47		
6	Bulkley River	400-431358				22.72	27.00	5.84	44.44	
52	Upper Bulkley Riv	400-431358					26.64	0.00	73.36	
47	Suskwa River	400-431358-079962				18.77	48.33	11.36	21.54	
50	Toboggan Creek	400-431358-237852				23.80	22.23	13.80	40.17	
49	Telkwa River	400-431358-415251	4.80			0.09	49.62	15.72	29.76	
34	Morice River	400-431358-585806	5.39		0.10		43.88	11.47	38.67	
22	Kispiox River	400-454855				62.10	31.31	6.59		
42	Shegunia River	400-456504				91.54	7.06	1.40		
2	Babine River	400-536025				8.62	45.04	6.19	40.15	
41	Shedin Creek	400-536025-027715				33.00	34.41	32.59		
43	Shelagyote River	400-536025-192976					51.51	30.51	17.98	
37	Nilkitkwa River	400-536025-357066					55.66	12.33	32.00	
36	Nichyeskwa	400-536025-367117					55.34	9.45	35.22	
1	Babine Lake	400-536025-597113					20.88	0.78	78.34	
10	Deep Canoe Crk	400-575120				33.81	60.14	6.04		
28	Kuldo Creek	400-591265				20.57	65.01	14.42		
53	Upper Skeena	400-615330				17.68	56.30	9.28	16.74	
44	Sicintine River	400-637044				7.79	49.71	37.27	5.24	
7	Canyon Creek	400-675225				20.87	73.08	6.05		
45	Slamgeesh River	400-706228				7.65	63.28	5.69	23.38	
46	Squingula river	400-746513					46.59	26.83	26.58	
48	Sustut River	400-757844					65.23	8.57	17.97	8.23
54	Upper Sustut Rivr	400-757844-					25.18	18.89	0.00	55.93
4	Bear River	400-757844-263905					45.05	5.84	49.11	
35	IVIOSQUE RIVER	400-808455					70.33	12.43	11.13	6.11
51	U_Skeena_hwts	400-811559							<b>0</b> 2 <b>-</b>	
16	Fort Creek	400-818771					62.1	14.1	23.7	
8	Chipmunk Creek	400-852488					55.3	33.6	11.1	
11	Duti River	400-872231					58.1	28.7	5.0	8.2
3	Barker Creek	400-876086					82.7	10.0	7.3	
27	Kiuatantan River	400-898389					80.4	17.8	1.8	
9	Currier Creek	400-907787					84.7	12.0	3.2	
5	Beirnes Creek	400-936143					89.3	10.7		

Table 2 Percentage of each BEC Zone represented in each Skeena watershed analysis unit.

#### 4.1.1 LOWER SKEENA

The watersheds in the lower Skeena FAZ (Fig. 6) showed relatively high uniformity in both vegetation composition and pattern. The valley bottoms were typically Coastal Western Hemlock (CWH), the side hills Mountain Hemlock (MH) and the ridge tops one of the sub zones of Alpine Tundra (CMA). Only the Zymoetz differed from this pattern, likely because the system was long enough to show some of the characteristics of the Middle Skeena in its upper reaches. The western part of the Lower Skeena could also be distinguished as a hyper maritime zone due to extreme precipitation events. As discussed later, the division of this zone into two Conservation units, "Coastal Winter" and" Coastal Summer" fits well with the ecoregional classification (which has two EcoSections ) and the life history timing (winter/summer).



Fig. 6 BEC Zones of the Lower Skeena FAZ. showing the distribution of CWH(green), Mountain Hemlock(MW) and a form of Alpine Tundra(light green).

#### 4.1.2 MIDDLE SKEENA

In the Middle Skeena FAZ (Fig. 7) the patterns were more complex. The zone is dominated in the south east by the SBS (Sub Boreal Spruce) which covers much of Babine Lake, Upper Bulkley and part of the Morice watersheds. However, a second major grouping of watersheds is represented by the Interior Cedar Hemlock (ICH)-Engleman Spruce Sub Alpine fir (ESSF) zones. These areas are represented by the Kispiox and surrounding watersheds on the North West side of the river, but include downstream portions of some of the systems on the opposite side (e.g. Bulkley, Babine). This creates a major vegetation difference moving west to east that overrides the expected changes with elevation (as one moves upstream). Since the ICH zone in the Skeena is unique among northern watersheds, it is given significant weight in the Conservation Unit analysis. Consequently, the proposed conservation units for the Middle Skeena are the Kispiox, Babine, Morice, Suskwa, Bulkley and Middle Skeena aggregates, but if vegetation were the only criterion, the Middle Skeena would have 2 and possibly 3 additional units.



Fig. 7 Middle Skeena FAZ demonstrating the influence of the ICH (Yellow) zone and SBS (red).

#### 4.1.3 UPPER SKEENA

The Upper Skeena FAZ (Fig. 8) consists of three groupings of BEC zones. The dominant watershed grouping consists of the SBS-ESSF-CMA zone combination, but there is an ICH grouping in the southern part of the zone. The Upper Sustut, Duti, and Mosque are the only systems in the entire Skeena that contain the SWB (Spruce Willow Birch) BEC zone and are indicative of a unique ecotype. The proposed Conservation units are the Upper Sustut, Skeena Headwaters and the Upper Skeena aggregate (Sicintine, Squingula, etc.), although the supporting data for the Upper Skeena/Skeena Headwaters division is weak.



Fig. 8 Upper Skeena FAZ showing the predominance of ESSF and the rare Spruce- Willow Birch Zone of the Upper Sustut (SWB). The overlap of the ICH (yellow) in the North Skeena Mountains is also noteworthy.

A cluster analysis using the data in Table 3 provided a visual summary of the overall relatedness of the analysis units based solely on the percentage of vegetation type . (Fig.9).

The first division in the dendrogram separates out the lower Skeena group (which corresponds closely to the Lower Skeena FAZ) from the rest of the watersheds. The second division separates the Upper Sustut as a unique branch, and so on as one moves down the tree. So for a given level of "relatedness", the number of groups can be determined.

Also, from left to right there is an overall trend of increasing elevation, as well as downstream to upstream. This is not too surprising since the BEC zones are defined largely by elevation but it does provide some valuable structure. For example, other features are the uniqueness of the Kispiox, the tendency for the smaller streams to group together, and the Kitwanga/Kitseguecla association.



Cluster Dendrogram

dist(becp) hclust (\*, "complete")



## 4.2 ECOREGIONAL CLASSIFICATION

While the BEC system successfully groups watersheds based on vegetation type, it is not the only classification system used in land use planning. The EcoRegional classification (Demarchi 1985) has also been used in defining ecosystems for purposes other than forestry (e.g. protected areas, wildlife and biodiversity management). In practice, the land use planning process makes use of both systems, one to define the forestry objectives such as annual allowable cut (AAC) and the other to provide representation targets for the management of forest dependant fish and wildlife species.

The EcoRegional system (Appendix 3) consists of 5 hierarchically organized classes, with each level providing a more detailed description of the province. In this classification, the importance of scale becomes evident. The Ecoprovince is the most closely related in size and number to the FWA units while the EcoSection is closest to analysis unit aggregates.

## 4.2.1 ECOPROVINCES

The Skeena contains 4 of the provinces 11 EcoProvinces. As with most major salmonid river systems, the fundamental physical division is between the coast (Coast and Mountains Ecoprovince) and interior zones: (Northern Boreal, Sub Boreal Interior and Central Interior, Fig. 10).



Fig. 10 Ecoprovince classification superimposed on the BEC zones of the Skeena Basin. Note the transition zone(ICH –yellow) could be considered a convention when assigning it to an Ecoprovince.. There is not a particularly good match between the Skeena FAZ boundaries and the EcoProvinces due in part to the inclusion of the transition systems (ICH) within the Coast and Mountains. In the FAZ system these transition zones are included as part of the Middle Skeena. However, the assignment of the ICH to a particular Ecoprovince was to some degree arbitrary since it is recognized as a transition zone between interior and coast. The important point is that there *is* a transition zone, and that it is currently not included in the FAZ representation. The importance of this zone may be of considerable significance for fish and other fauna since the zone is highly valued in any classification system that includes *ecosystem* values.

## 4.2.2 ECOSECTIONS

The EcoSection (114 marine and terrestrial units provincially, 18 in the Skeena) is the terrestrial scale most commonly used in BC land use planning and we believe is the scale best suited for Conservation Unit analysis. It is the scale used by the Province for hydrological analysis, productive capacity modeling, land use planning and impact assessment (R Ptolemy, pers com). Once again, the choice of scale is relevant not only for the Conservation Unit goal of the WSP, but also to the goals of habitat protection, state-of-environment reporting, biological reference points and ecosystem based management.

Ecosection	FAZ	Ecoprovince	Code
Hecate Lowland	Lower Skeena	Coast and Mnts	HEL
Kimsquit Mountains	Lower Skeena	Coast and Mnts	KIM
Kitimat Ranges	Lower Skeena	Coast and Mnts	KIR
Nass Basin	Lower Skeena	Coast and Mnts	NAB
Nass Mountains	Lower Skeena	Coast and Mnts	NAM
North Coast Fjords	Lower Skeena	Coast and Mnts	NCF
Cranberry Upland	Middle Skeena	Coast and Mnts	CRU
Bulkley Basin	Middle Skeena	<b>Central interior</b>	BUB
Bulkley Ranges	Middle Skeena	<b>Central interior</b>	BUR
Babine Upland	Middle Skeena	Sub Boreal int	BAU
Manson Plateau	Middle Skeena	Sub Boreal int	MAP
Nechako Upland	Middle Skeena	Sub Boreal int	NEU
Southern Skeena Mountains	Middle Skeena	Sub Boreal int	SSM
Northern Omineca Mountains	Upper Skeena	Northern Boreal	NOM
Eastern Skeena Mountains	Upper Skeena	Sub Boreal int	ESM
Northern Skeena Mountains	Upper Skeena	Sub Boreal int	NSM
Southern Boreal Plateau	Upper Skeena	Sub Boreal int	SBP
Southern Omineca Mountains	Upper Skeena	Sub Boreal int	SOM

Table 3EcoSections found in the Skeena River and associated Freshwater AdaptiveZones, EcoProvinces and database Codes.

By their very nature, EcoSection boundaries usually do not coincide with watershed boundaries and therefore GIS intersections usually contain one or more EcoSections (Fig. 11). However mapping errors and scale effects are probably similar to any errors resulting from boundary differences and fortunately in the Skeena, it was usually possible to associate a given analysis unit with a dominant EcoSection (Table 4).



Fig. 11 Intersection of the Skeena EcoSections (colors) with analysis units showing the degree of misalignment of the EcoSections and watershed boundaries

Table 4 Intersection of the Skeena analysis units with BC EcoSections. Values are expressed as percentages of the unit area.

		BAU	BUB	BUR	CRU	ESM	HEL	кім	KIR	MAP	NAB	NAM	NEU NO	CF NOM	NSM	SBP	ѕом	SSM
29	L Skeena W						16		45			37		2				
12	Ecstall						3		97									
21	Khyex						1		99									
39	, Scotia								100									
20	Khtada	_							100	_	_					-		
19	Kasiks								100	_	_					-		
12	Exchamsiks								91			q						
17	Gitnadoix								08			2						
1/	Exctow								20			<u>_</u>		_				
14									Z	_		100			_			
30	L_Skeena_E											100						
31	Lakeise											100		_				
55	Zymagotitz								0			100		_				
24	Kitsumkalum							-	0			100						
56	Zymoetz			23	16			3	0			58						
26	Kleanza											100						
15	Fiddler											100						
32	Lorne											100						
38	Quill											100						
18	Insect											100						
40	Sedan				4							96						
33	M Skeena				50						16	10						25
25	Kitwanga				100							0						
23	Kitseguerla	_		1	99					_	_	0						
6	Bulkley	2	38	13	27					_	0	U						20
52		16	8/	13	21				_	_	0							20
17	C_DURREY	010	-0		1													00
47	JUSKWA	9	40	E 2	7									_				90
50	Toboggan		40	22	10							1						
49	Тегкwа		2 10	60	10			22	0			1	0	_				
34	Morice		10	60				23	0				8	_	27			
22	Kispiox				/						66				27			0
42	Shegunia										/							93
2	Babine Lake	95								1								4
41	Babine	33								3								64
43	Shedin														0			100
37	Shelagyote	5																95
36	Nilkitkwa	32								22								46
1	Nichyeskwa	13																87
10	Upper_Skeena					18					1				52			30
28	Deep Canoe										14				86			
53	Kuldo														100			
44	Sicintine								_						13			87
7	Canvon														100			
45	Slamgeesh	_				61	_	_		_	_				39	-		
46	Squingula					24			_	2								74
40	Bear					10				90								0
40	Suctut					72			_	2				6			10	0
45						50				5				50			- 19	
40						100			_	_				50	0	0	0	
48	O_SKeena_Head					100								7	0	0		
- 54	Iviosque					93								/				
4	FOFT					100								0				
35	Cnipmunk					99								1				
51	Duti					/9								0		21		
16	Barker					100												
8	Kluatantan					100										0		
11	Currier					81									19			
3	Beirnes					41				1					59			

Unlike EcoProvinces, EcoSections have a reasonably good fit within the FAZ units, but this was in part a function of their smaller size. It is noteworthy however that there is a strong association between watersheds and physical features in the EcoSection classification. Therefore an advantage of this system is that the names are less ambiguous than terms such as Upper, Middle and Lower which have different meanings depending on the classification system.

Starting from the mouth, the Lower Skeena is subdivided into two EcoSections, with the first 7 watersheds located in the Kitimat /Kimsquit Ranges, while the next 10 watersheds are part of the Nass Mountains. In the Middle Skeena the Kitseguecla and the Kitwanga are the only two watersheds in the Cranberry Upland EcoSection, while the Kispiox is the only system in the Nass Basin EcoSection. As stated previously, the systems often contain parts of other EcoSections, but we usually refer to the dominant EcoSection.

The larger systems such as the Babine and Bulkley encompass more than one EcoSections along their length. The Upper Bulkley is associated with the Bulkley Basin EcoSection while the Morice and Telkwa are part of the Bulkley Ranges Ecosystem. The Babine watershed contains parts of the Babine Uplands, Southern Skeena Mountains and the Manson plateau. Upstream from the mouth of the Babine, the landscape changes again, and becomes associated more with the sub boreal interior characteristics of the province.

In summary, using the EcoSection classification provided a grouping of the Skeena analysis units using 11 EcoSections. The units and their CUs were

- 1. Kitimat Ranges (Coastal Winters)
- 2. Nass Mountains (Coastal Summers)
- 3. Cranberry Uplands (Middle Skeena aggregate)
- 4. Bulkley Ranges (Morice/Telkwa)
- 5. Bulkley Basin (Bulkley)
- 6. Nass Basin (Kispiox)
- 7. Babine uplands (Babine)
- 8. South Skeena Mountains (Suskwa, Upper Skeena)
- 9. North Skeena Mountains (Upper Skeena, Skeena Hdwtrs)
- 10. North Omineca Mountains (Upper Sustut
- 11. East Skeena Mountains (Skeena Hdwtrs)

## 4.3 SUB BASIN CLASSIFICATION

Gottesfeld and Rabnett (2008) provide yet another representation of the Skeena from the perspective of habitat, fish, fisheries and human habitation (Fig 12). This book is more detailed than the material presented here and is a very useful and fairly complete characterization of the Skeena. However it does not contain some of the statistical analysis that might be considered necessary for a discussion of Conservation Unit structure. It does however provide a test of statistical methodology against what many would consider common sense.

In the Sub-Basin system, the Skeena mainstem is a sequence of 5 areas; the Lower Skeena, Middle Skeena South, Middle Skeena North, Upper Skeena and Upper Skeena Headwaters. The only other major river that is divided longitudinally is the Bulkley, which consists of the Lower and Upper Bulkley sub basins. In addition to the mainstem subdivisions, 11 major watersheds are identified. These include the Ecstall, Gitnadoix, Lakelse, Zymoetz, Kalum, Kitwanga, Kispiox, Morice, Babine, Sustut and Bear.



Fig. 12 Sub Basin outlines based on Gottesfeld and Rabnett (2008)

#### 4.4 REGIONAL HYDROLOGY

#### (adapted from Summit Consulting 1995)

While there is a case to be made for terrestrial vegetation being a surrogate for the adaptive factors associated with different fish habitats, it is also true that a number of aquatic variables can be measured directly. Many of these variables form the basis of the province's Ecological Aquatic Unit (EAU) database which in turn assisted in the development of the Freshwater Adaptive Zones. These factors include regional hydrology, regional water chemistry, stream gradient, stream temperature and stream productivity. Similar to terrestrial classifications, these factors can be grouped into categories that contribute to the identification of steelhead Conservation Units.

British Columbia has a diverse hydrology resulting from the complex influence of ocean, mountains, wind, temperature and precipitation. Over the past few decades, there have been several attempts to divide the province into homogeneous hydrologic zones. The most current scheme (MWLAP, 1998) separated the province into 17 homogeneous zones. However, the previous version (Summit Consulting (1995)) had 41 zones and gave a finer description of regional variation. The difference between the two systems was that the current version was based only on fewer selected available data (subject to record length and quality), while the latter combined both the data and expert opinion. We believe the latter is the better approach given the limitations of the data. In both cases however, stream flow data published by Environment Canada were used to determine zone boundaries and characteristics of each zone.

#### Regional Hydrological Zones are defined as

"Streams with similar mean annual runoff, temporal flow and distribution, and peak and low flow characteristics due to homogeneous physiographic and climatic conditions. The degree of homogeneity within a region is a function of scale, so even within a "homogeneous" region, there can be significant variability in hydrologic regime between streams".

Most provincial schemes recognize the division of the province into coastal, interior and interior wetbelt zones. These longitudinal corridors intersect northern, central and southern latitudinal divisions creating a central interior plateau effectively surrounded by mountain ranges. The west-east gradients reflect a decreasing maritime influence while the north-south zones are generally related to decreasing mean annual temperatures.

Pacific weather systems supply large amounts of moisture to coastal regions as orographic uplift increases precipitation (rain or snow generated by a moisture-bearing

air mass being forced over a land surface). As the air masses move over the interior, there is less moisture available for evaporation which further weakens the systems, thereby creating a central interior dry zone. Then, as the systems reach the eastern part of the province, they once again are subjected to the uplift effect of mountain ranges creating an area often referred to as the "interior wetbelt". This is an oversimplification of a complex process, but serves to locate the Skeena at the intersection of a number of these zones, which explains much of the Skeena's complex hydrology.

A number of characteristics of the hydrograph assist in classifying watersheds. Mean annual runoff, expressed as a depth of water over the watershed, is equivalent to the difference between annual precipitation input and evaporative loss. The month with the greatest total runoff is indicative of the type of mechanism (e.g. Rain, rain-on-snow, glacier melt, etc.) which generates the highest flows, while the percentage of the annual runoff within that month gives some indication of the dominance of that peak flow generating mechanism. The percentage of flow within the highest flow month is given as a range, using the highest and lowest percentages from representative hydrometric stations within the zone.

In the Skeena, 10 of the province's 41 hydrological units are represented (Table 5), but only 6 are of any significance (Zones 10-12 and Zones 17-19.) The zonal map (Fig.13) generally demonstrates the west to east gradient in mean annual runoff, but also the importance of some microclimate (e.g. windward, leeward) factors. Zone 17 is nearest the coast followed by Zone 18 (Central Coast Mountains), then Zone12 (Nass Basin). At this point in the Upper Bulkley, Zone 19 (Central Interior Plateau) influences the Upper Bulkley Basin and Bulkley Mountains. As one moves further north, the influence of the Skeena Mountains and the Northern Interior Plateau becomes apparent and the watersheds behave as interior (spring melt) watersheds.

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TABLE 5 Area in hectares of each hydrological zone in the Skeena intersected with each of the analysis units. The systems are ordered from the mouth to the headwaters and distance from the ocean.

					HY	DROLOGI	C ZONE						
FAZ	FWA_CODE	NAME	3	4	10	11	12	16	17	18	19	20	Dzocea
L Skeena	400	Lower Skeena	0	0	0	0	0	5244	104876	168965	44	0	0
	400-014636	Ecstall	0	0	0	0	0	0	148657	0	0	0	9
	400-036391	Khyex	0	0	0	0	0	0	44185	0	0	0	23
	400-056622	Scotia	0	0	0	0	0	0	13476	0	0	0	35
	400-059865	Khtada	0	0	0	0	0	0	15678	0	0	0	37
	400-094444	Kasiks	0	0	0	0	0	0	26168	0	0	0	59
	400-107640	Exchamsiks	0	0	0	0	0	0	51342	78	0	0	67
	400-116952	Gitnadoix	0	0	0	0	0	0	53659	871	0	0	73
	400-139009	Exstew	0	0	0	0	0	0	156	45355	0	0	87
	400-174068	Lakelse	0	0	0	0	0	0	34	58240	11	0	109
	400-185024	Zymagotitz	0	0	0	0	0	0	72	38786	0	0	116
	400-195432	Kitsumkalum	0	0	0	0	0	0	322	228620	0	0	122
	400-221484	Zymoetz	0	0	0	0	0	0	0	263557	39053	0	139
	400-234275	Kleanza	0	0	0	0	0	0	0	20102	0	0	147
	400-299006	Fiddler	0	0	0	0	0	0	0	17071	0	0	187
	400-303799	Lorne	0	0	0	0	0	0	0	10718	0	0	190
	400-315638	Quill	0	0	0	0	0	0	0	12901	0	0	198
	400-326256	Insect	0	0	0	0	0	0	0	20044	0	0	204
	400-351455	Sedan	0	0	0	0	0	0	0	12114	0	0	220
MSkeena	400	Middle Skeena	0	0	16	53201	834	0	0	103060	34	0	221
meneena	400-365504	Kitwanga	0	0	.0	00	49	0	0	82630	0	0	220
	400-396037	Kitsequecla	0	0	0	0	-10	0	0	71207	148	0	249
	400-431358	Linner Bulkley	0	0	492	1361	0	0	0	60434	230101	0	240
	400-431358	Upper Bulkley	0	0	1386	1001	0	0	0	00-04	217705	0	270
	400-431358-070062	Suckwa	0	0	6213	118350	0	0	0	6205	1/35	0	210
	400-431356-079902	Juskwa	0	0	0213	110350	0	0	0	0205	12166	0	292
	400-431356-237652	Toboyyan	0	0	0	0	0	0	0	216	110007	0	207
	400-431356-415251	Mariaa	0	0	0	0	0	0	0	120465	217440	0	387
	400-431356-565	Kiepiex	0	0	0	1250	100707	0	0	20020	317440	0	435
	400-454655	Rispiox	0	0	124660	1250	100/0/	0	0	20030	0	0	285
	400-536025	Babine	0	0	124669	55879	0	0	0	11	0	0	336
	400-536025-027715	Shealn	0	0	94	55550	0	0	0	0	0	0	343
	400-536025-192976	Shelagyote	0	0	5152	52592	0	0	0	0	0	0	386
	400-536025-357066	NIIKItKWA	0	0	82614	19	0	0	0	0	0	0	430
	400-536025-367117	Nichyeskwa	0	0	24781	11375	0	0	0	0	0	0	432
	400-536025-597113	Babine Lake	0	0	616691	4402	0	0	0	0	10453	699	493
U Skeena	400	Upper Skeena	131	73	159100	134020	0	0	0	5	0	0	337
	400-575120	Deep Canoe	0	0	0	16600	144	0	0	0	0	0	361
	400-591265	Kuldo	0	0	0	57383	2842	0	0	0	0	0	371
	400-637044	Sicintine	0	0	62	81235	0	0	0	0	0	0	399
	400-675225	Canyon	0	0	0	26429	0	0	0	0	0	0	423
	400-706228	Slamgeesh	0	0	5680	54887	0	0	0	0	0	0	443
	400-746513	Squingula	0	0	68995	871	0	0	0	0	0	0	468
	400-757844	Upper Sustut	0	3486	118006	89	0	0	0	0	0	0	475
	400-757844	Sustut	0	0	190870	0	0	0	0	0	0	0	475
	400-757844-263905	Bear	0	0	45233	0	0	0	0	0	0	0	507
	400-808455	Mosque	0	1808	48076	50	0	0	0	0	0	0	507
	400-818771	Fort	0	22	10398	0	0	0	0	0	0	0	513
	400-852488	Chipmunk	0	255	19530	0	0	0	0	0	0	0	534
	400-872231	Duti	4214	250	99117	0	0	0	0	0	0	0	547
	400-876086	Barker	0	0	10938	134	0	0	0	0	0	0	549
	400-898389	Kluatantan	223	0	61044	0	0	0	0	0	0	0	563
	400-907787	Currier	0	0	20870	130	0	0	0	0	0	0	569
	400-936143	Beirnes	0	0	16260	46	0	0	0	0	0	0	587

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Fig. 13 Regional hydrology for the Skeena Basin based on gauging data and expert opinion. Numbers refer to the Zones outlined below. Rank is the provincial rank among 41 zones.

The Skeena has relatively high annual unit runoff nearest the coast, falling off on the leeward side of the Coast Mountains. Highest daily and monthly flows on the coast (Zone 17) are associated with fall and winter rainfall, often accompanied by melting snow. Storage of snow does occur at the higher elevation but spring snowmelt does not produce the highest annual peak flows. The peak flow month in this zone accounts for only 17% of the total annual runoff, which is approximately half that of snowmelt-dominated zones (Table 6). Six of the 10 zones in Table 5 account for the vast majority of the watershed.

		Mean Ann	ual Runoff	annual pea	100 km2	Yearly Distribution			
zone	no obs	(mm) rank		(m3/s)	rank	peak month	% o	f ru	noff
17	3	2144*	2	105	2	May,June	32	-	16
18	22	1354	12	20	20	June,July	29	-	17
19	27	268	36	6	39	May,June	43	-	21
12	2	1709*	11	36	14	June	22	-	23
11	3	2045*	6	43	13	July	27	-	22
10	9	393	30	46	11	May,June	35	-	21

Table 6 Annual runoff, annual peak flow, timing and intensity (% of runoff) of the peak.

\* limited data

Of equal significance to fish values are the characteristics of the low flow periods during both summer and winter periods (Table 7).

Table 7 Skeena low flow characteristics indicating month, % of MAD, summer 7 day low day low flow and annual 7 day low flow.

zone	No Stat	Mean Mo	nthly flow		Mean 7 day low				
		in Lowest	Month	(	(m3/sec / 100 km2				
		Month	% MAD	Jun-Sept	Rank	Annual			
10	8	Mar	22	0.612	28	0.234			
11	3	Feb	10	5.856	2	0.439			
12	2	Feb	12	4.118	5	0.39			
17	2	Feb	24	4.702	3	0.756			
18	18	Mar	22	2.758	7	0.662			
19	14	Feb	26	0.462	31	0.112			

While these tables summarize information derived from expert hydrologists, an additional and more detailed discussion of the Skeena hydrology is provided in Appendix 3 The appendix describes a single gauging site within each Ecosection and may be a better approach than averaging a number of stations spread over a larger area, but only partially inside the Skeena basin.

The conclusion that the Skeena contains at least 6 hydrological zones argues again for an increase in the number of Conservation Units.

#### 4.5 HABITAT SUMMARY

The overwhelming conclusion from an examination of habitat classification systems is that even with the most conservative classification, the Skeena contains a greater number of habitat types than the 2 currently identified. The second major point is that there is general agreement among the different systems around the number of possible habitat units (5-11).

In the BEC zone system, at least 5 types of watersheds exist based solely on the vegetation associated with the riparian zone at the mouth of each river. If the additional vegetation components (e.g. side hills, ridge tops) are added to the classification, the number of watershed types increases to 8.

The BEC cluster analysis separates the Skeena into approximately 12 branches above a given separation distance. Note that the Upper Sustut and the Kispiox was differentiated from the Middle and Upper Skeena, and that the Kitwanga and Kitseguecla form a branch at the same bifurcation level as the Kispiox. The ability to further classify watersheds into groups is limited, probably by many systems sharing similar vegetation and elevations in their upper reaches (e.g. the Babine and Bulkley).

In the EcoRegion analysis, the Skeena contains 3 EcoProvinces and 18 EcoSections. When compared to the FAZ units, the lower Skeena FAZ matches the Coast and Mountain EcoProvince reasonably well, but the Sub Boreal Interior does not fit with the Middle and Upper FAZ units. This strongly suggests that the further subdivision of these units is appropriate.

The EcoSection level does provide a workable scale with a relatively small number (11) of habitat types. As noted above, the EcoSections tend to be associated with fairly homogeneous physiographic features. The units, starting from the mouth are the Kitimat Ranges, Nass Ranges, Cranberry Uplands, Nass Basin, Bulkley Ranges, Bulkley Basin, Babine Uplands and the North, East, and South Skeena Ranges.

Finally, in the Sub basin system there are 11 major watersheds along with 5 subdivisions of the mainstem, and two sub divisions of the Bulkley. These most closely approximate the EcoSection framework but with different names and slightly different boundaries. Also, it is possible to include most of the sub basins within the smaller number of EcoSection units proposed.

Data for life history and molecular genetics is not as complete as the habitat data, so much of the following analysis will focus on the consistency of those data sets with the proposed 11 units suggested by habitat groupings.

# **5. LIFE HISTORY**

The life history of steelhead is the most complex of any of the salmonids and for decades this phenotypic variation has been of academic and management interest. For example, the consistent yearly differences in a number of population attributes can serve as a means of stock identification. Cox-Rogers (1985) demonstrated significant differences in scale pattern, growth, age composition, and size at age (all of which have a demonstrated genetic basis) for 5 major Skeena rivers (Zymoetz, Kispiox, Babine, Bulkley, Sustut) He also demonstrated morphometric differences in the juveniles of 3 of the 5 major Skeena populations. (Bulkley/ Morice, Kispiox, Zymoetz,) After a detailed analysis he concluded that "This variability confirms the subdivision of Skeena River steelhead into discrete stocks and suggests that stock discreteness is an adaptive property of the species that has arisen through natural selection."

While this work related specifically to stock discrimination, the results are very relevant to the definition of steelhead Conservation Units. Even with the passage of 25 years, his thesis still makes a compelling case for the recognition and protection of the high level of genetically-based phenotypic variation evident in this species.

Of the several theoretical combinations of freshwater and saltwater age strategies available to steelhead, 6 were found frequently, and 6 occasionally in the Skeena(Cox-Rogers 1985). However, when combined with variations in fecundity, run timing, repeat spawning, and the possibility of remaining in fresh water as residuals, it was apparent that steelhead demonstrate a sophisticated range of adaptive responses at a broad scale. At the same time, this approach to a survival strategy makes it difficult to determine which life history patterns are of sufficient importance to merit Conservation Unit status. Of course, some of these patterns may be the result of environmental rather than genetic factors (e.g. smolt age is primarily a function of stream temperature) but since most phenotypes are the result of the interaction of genetic and environmental factors.(e.g growth rates are a heritable trait) it is reasonable to conclude that there is a correlation between phenotypic and genotypic diversity.

#### **5.1 SCALE METHODS**

Updated scale collections in the Ministry of Environment database were used in this analysis, but the significant problem of determining the correct stock assignment for individuals in a given sample still remained. For example, the Bulkley River samples could include fish originating from the Suskwa River, Toboggan Creek or the Morice River. Additionally, steelhead use of Upper Bulkley River and various tributaries such as Buck Creek is well known; parr are known to migrate from spawning locations into the mainstem Bulkley River prior to smoltification. The same applies to the Sustut River where samples taken in the lower Sustut River could include fish that were enroute to the Upper Sustut.

A different complication exists for the Kalum and Lakelse rivers, where the scale collection dates and locations suggest a mixture of summer and winter steelhead (fish collected or angled could be either summer or winter fish). However, this is only a problem for the systems containing winter run fish. It is important to note that all of these sources of error would tend to *obscure differences when they existed, rather than creating differences when they did not*. Therefore the analysis using the current data would likely result in a more conservative estimate of genetic variation than what actually exists.

A final complication was that the available records indicated that until the mid-1990s all adult steelhead scales in the regional MOE office were processed and read by the same MOE staff. Readers were familiar with the bio-physical conditions in the watersheds and had experience in sampling juvenile steelhead from many of them. This gave reasonable assurance that the freshwater life history portion of the scales was understood and interpreted as accurately as possible.

From the late 1990s forward MOE began contracting out scale reading. The interpretations provided by the contractor of freshwater age tended toward shorter freshwater residence prior to smoltification. This was especially noticeable for the most recent years' Upper Sustut River scales. Unless indicated otherwise, the average smolt age calculations for individual stocks in the database include all adult scale interpretations regardless of the year of collection and the scale reader.

#### 5.2 OCEAN LIFE HISTORY

Body size is among the most important and visible adaptations of steelhead to their environment. For Skeena populations with sufficient sample size, an analysis of variance was performed (R aov) on factors influencing the length of returning adults (Table 8).

Table 8 Analysis of variance for all Skeena steelhead scales in the MOE scale data base (after removing outliers) examining the effects of gender, ocean age and watershed.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)					
Sex	1	1921483	1921483	539	< 2.2e-16 ***					
AnnOc1	1	42490976	42490976	11911	< 2.2e-16 ***					
Water	10	4500509	450051	126	< 2.2e-16 ***					
Residuals	4679	16691842	3567							
aov(formula = Length ~ Sex + AnnOc1 + Water) '										

Not surprisingly, size at ocean age on return (AnnOc1), watershed, and gender were all significant factors. The analysis demonstrated; 1) the importance of ocean age in determining the length of returning spawners 2) differences between populations and 3) support for a separate analysis for males and females.

Of course, for the purpose of using life history as a factor in defining Conservation units, it is the *difference in life history strategies between populations* that is of most interest. As noted above, the amount of phenotypic variation in Skeena steelhead is extensive, so the problem becomes more one of grouping rather than differentiating stocks. Examination of the box-whisker plot and the ANOVA results suggest some possible ways to proceed (Fig. 14)..

First, it is clear that the ocean age 4 fish were smaller than expected based on a linear extrapolation of the previous age increments and results from this group should be treated cautiously given the small sample size. Nonetheless, 4+ fish are predominately males (75%) and appear to be limited to a subset of rivers (17 of 29 were either from the Kispiox or Kalum rivers). While the percentage of the population of these older fish was small, their significance to the sport fishery and perhaps to the population's life history strategy should not be ignored. Repeat spawners were not included in the analysis.



#### Skeena Steelhead Length vs Ocean age

Fig. 14 Box whisker plots of all data used in the ANOVA table. (Age is shortened so that 1 represents 1+ etc.).

Differences in mean length of spawners can be accounted for in a number of ways: 1) fish of the same age could grow at different rates 2) populations could have different age structures, or 3) differences could result from variable annual survival and growth combined with the multiple brood years that comprise any given sampling year. In addition, size selection in the gill net fishery and variable harvest rates add to the known sources of variability.

From this point we proceeded by treating the male and female populations separately given that the differences in mean size between males and females was likely of minor consequence in defining Conservation Units. By using this approach, patterns and conclusions applying to both sexes within a river, but differing between rivers, might be given more weight.

## 5.2.1 MALE OCEAN LENGTH AT AGE

As a simplification of the detailed analysis provided by Cox Rogers and as a result of the limitations of the available dataset, we looked at the average length across all ages of returning male adults for each Skeena population. Body size has a known adaptive role (e.g. correlates with fecundity) and local knowledge supported the premise that populations in the Skeena show a consistent and wide range in body size.

Table 9 provides a summary of the attributes of the various populations of the Skeena for which sufficient numbers of samples were collected. Mean length for a given population was calculated from all observations across all ages of return (row means). Column means were calculated as the mean of the cell means in each row.

Water	Ocean Age									
_	1	2	3	4	mean	s.d.	n	rank		
Babine	602.4	810.8	958.2	984	854.2	132.3	374	9		
Bulkley	575.4	790	904.2	NA	665.3	125.2	249	2		
Kalum	593.7	720.8	831.8	908	745.5	118.9	162	4		
Kispiox	617.9	836.1	975.4	998.2	862.2	135.7	206	10		
Lakelse	610	763.2	831	880.3	799.9	83.9	83	7		
Morice	579.3	780.7	899.1	NA	643.7	112.5	315	1		
Suskwa	642.2	803	896.9	NA	796.5	108.1	78	6		
Sustut	636.1	825	932.8	959	865.2	82.4	312	11		
Toboggan	551	771.1	889	NA	707.7	119.6	117	3		
U_Sustus	552.5	787.3	879.3	943.3	832.9	71.8	119	8		
Zymoetz _	593.8	803.3	901.1	NA	751.0	121.6	153	5		
Mean	595.8	790.1	899.9	945.5	774.9	110.2				

Table 9 Mean male length at age for each population(cells), mean male length(row means) and mean length at age(column means) ranked by mean size (smallest to largest).

There was a large range (>220mm) in the mean size of individual populations. The Morice population was the smallest (643.7 mm) while the largest was the Sustut(865.2mm). A one-way ANOVA using only the male data was conducted to reconfirm that significant differences existed among Skeena populations. A Tukey HSD multiple comparison analysis was used to determine which populations contributed to these significant differences (Appendix 4).

Since we were interested in grouping the populations by life history (in this case body length) we examined which populations did not differ statistically from the largest (Sustut) and smallest (Morice). This resulted in a group of "Large" fish containing the Sustut,, Babine and Kispiox and a "Small" group consisting only of Morice fish. The Bulkley is very close to significance and probably could be included in the "small" group. However, it is also quite likely that the Bulkley samples contained a significant number of Morice fish in addition to other individuals from numerous tributary populations so the actual composition of the Bulkley is in doubt.

A coincidental but perhaps useful distinction is that "small" male populations are those below 700mm whereas the "large" populations are greater than 800 mm (Fig 15).



#### Mean Lengths of male Skeena Steelhead

Fig. 15 Mean lengths of Male Skeena River steelhead grouped into small, medium and large categories.

#### 5.2.2 AGE STRUCTURE OF MALE POPULATIONS

To examine age structure, the scale data observations were aggregated by river system and classified according to average ocean age of return (Table 10).

Table 10 Number and percentage of observations by age class for male steelhead.

		no of obs					Percenta	age of obs		_mean age
	1	2	3	4	total	1	2	3	4	
Babine	44	158	170	2	374	11.76	42.25	45.45	0.53	2.35
Bulkley	149	92	8		249	59.84	36.95	3.21	0.00	1.43
Kalum	23	80	54	5	162	14.20	49.38	33.33	3.09	2.25
Kispiox	29	94	77	6	206	14.08	45.63	37.38	2.91	2.29
Lakelse	1	37	42	3	83	1.20	44.58	50.60	3.61	2.57
Morice	219	88	8		315	69.52	27.94	2.54	0.00	1.33
Suskwa	16	40	22		78	20.51	51.28	28.21	0.00	2.08
Sustut	8	174	128	2	312	2.56	55.77	41.03	0.64	2.40
Toboggan	38	71	8		117	32.48	60.68	6.84	0.00	1.74
U_Sustut	2	55	59	3	119	1.68	46.22	49.58	2.52	2.53
Zymoetz	47	87	19		153	30.72	56.86	12.42	0.00	1.82

Plotting the mean length against mean age for each population indicated that for Skeena males there was a good relationship between the mean size at return and the number of years at sea (Fig 16).



g. 16 Regression of mean age at return (m\_agem) vs mean length of male steelhead (m\_lengm) (mm) with associated residuals and outliers.

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This of course is not surprising, and if age structure were the only determining factor then the regression would be a straight line, essentially repeating the information in Figure 15. However, examination of the residuals provides some possible additional information The largest residuals (possible outliners) are points 3, 4 and 5 and are associated with the Lakelese, Kalum and Kispiox Rivers. Removing these outliers generated a more robust regression with slope 185.34 (mm), intercept 401.29 and adjusted R<sup>2</sup> of 0.94.

As stated in the methods, the Lakelse and Kalum rivers are systems that contain winter runs that could not be separated out in the analysis. Therefore it may be possible that there may be other difference between winter and summer runs in addition to the obvious difference in timing.

Finally, the presence of the occasional large 4+ ocean residency in some systems may be a distinct life history attribute in its own right as well as contributing to the reputation of river as a sport fishery.

## 5.2.3 MALE GROWTH RATE IN THE OCEAN

The other obvious factor contributing to variation in mean spawner length is growth rate. Specifically, differences in spawner length are the result of age structure reduced or enhanced by stock specific growth rates. From an examination of the residuals one would predict different growth rates in the Kalum, Kispiox and Lakelse systems. Although any proper analysis was limited by the lack of data on individual fish, large differences should be detectable for each population by comparing the transformed length at age (cube root to account for the size effect) in a regression analysis.

Figure 17 distinguishes faster from slower growing fish by the slope of the regression line. The slower growing fish were in fact the Lakelse and Kalum but the Kispiox did not demonstrate the fast growth expected. Consequently, the reason for its status as an outlier remains unclear although the many tributaries with different physical attributes may be part of the explanation.

In summary, male populations with a large proportion of young fish also appear to have the fastest growth rates, and conversely populations with older fish tend to have slower growth. Again, this could be a function of the relationship between size and growth (smaller fish grow faster, even if their intrinsic growth rate is the same) but this should have been accounted to some degree by the length transformation.



Fig. 17 Regressions of transformed length at age for male steelhead of the Skeena River.

Water	Intercept	Х	rank	(untransformed data)
Lakelse	620.9121	69.75255	1	
U_Sustus	580.2563	99.88872	2	
Kalum	491.2498	112.859	3	
Sustut	590.4173	114.6308	4	
Suskwa	537.6762	124.6189	5	
Kispiox	507.2264	154.9097	6	
Babine	465.4427	165.5985	7	
Zymoetz	445.3388	168.2488	8	
Morice	392.9623	188.4818	9	
Toboggan	370.7211	193.2619	10	
Bulkley	380.9459	198.3011	11	

#### 5.2.4 FEMALE OCEAN LENGTHS

The above process was repeated for the female data by first grouping the various populations into small medium and large populations. We examined the female data for mean size at return in the same manner as for males (Table 11).

_	1	2	3	4	mean	sd	nobs	rank
Babine	576.7	767.2	864.0	NA	760.6	75.2	269	7
Bulkley	565.8	742.2	864.5	NA	677.4	102.2	624	2
Kalum	598.8	724.9	768.2	903.5	746.7	75.3	169	6
Kispiox	580.6	777.0	860.3	991.0	770.3	77.9	262	8
Lakelse	698.3	746.9	795.1	911.0	775.8	68.7	98	9
Morice	567.8	734.2	NA	NA	657.2	96.5	365	1
Suskwa	647.3	775.7	858.4	NA	784.2	62.6	95	11
Sustut	597.0	770.3	847.9	840.0	778.8	45.0	471	10
Toboggan	585.7	705.4	812.6	NA	700.6	55.0	136	3
U_Sustut	NA	734.3	807.7	NA	746.1	42.0	174	5
Zymoetz _	603.5	751.1	843.4	NA	745.2	57.0	173	4
mean	602.1	748.1	832.2	911.4				

Table 11 Mean female length at age for each population(cells), mean female length(row means) and mean female length at age(column means) ranked by overall mean size

The first observation was that females had a much smaller size range (127mm) than males(220), but even so, significant differences still occurred in the ANOVA. The Tukey HSD provided a grouping of large, medium and small populations as for the males. In the "small" category, the results were the same as for the male analysis i.e. the Morice and possibly the Bulkley were in the small group. However, the "large" group consisted of the Suskwa, Kispiox, Sustut, Lakelse and possibly the Babine. However if there is a real "winter run effect" from the Kalum and Lakelse, it is probably prudent to remove these systems from the comparisons. If that is done, the "large" group retains the Sustut, Kispiox, and Babine, but added the Suskwa (Fig 18). Longer term observations by anglers would support this.





Fig. 18 Mean lengths of Female Skeena River Steelhead grouped into small, medium and large classes

#### 5.2.5 AGE STRUCTURE OF FEMALE SPAWNERS

The female age population data reflects the male data to a significant degree but there are also some differences. The two oldest populations are Lakelse and Kalum, while the youngest are the Bulkley and Morice (Table 12).

Table 12 Number and percentage of observations by watershed and age group with average age for each female population.

	_	n	o of obs	ervatio	ons		_	F	)S			
ID	Water	1	2	3	4 t	otal	_	1	2	3	4	Avg Age
	1 Babine	20	228	21	0	269		7.43	84.76	7.81	0.00	2.0
	2 Bulkley	116	194	2	0	312		37.18	62.18	0.64	0.00	1.6
	3 Kalum	6	73	86	4	169		3.55	43.20	50.89	2.37	2.5
	4 Kispiox	18	224	19	1	262		6.87	85.50	7.25	0.38	2.0
	5 Lakelse	4	36	56	2	98		4.08	36.73	57.14	2.04	2.6
	6 Morice	169	196	0	0	365		46.30	53.70	0.00	0.00	1.5
	7 Suskwa	4	75	16	0	95		4.21	78.95	16.84	0.00	2.1
	8 Sustut	1	416	53	1	471		0.21	88.32	11.25	0.21	2.1
	9 Toboggan	9	123	4	0	136		6.62	90.44	2.94	0.00	2.0
	10 U_Sustus	0	146	28	0	174		0.00	83.91	16.09	0.00	2.2
	11 Zymoetz	10	158	5	0	173		5.78	91.33	2.89	0.00	2.0

Repeating the regression /residual approach for the females did not mirror the male results. Points 4, 7 and 9 were identified as the outliers, so that only the Kispiox appeared to be included in both the male and female outliers (Fig.19).



Fig. 19 Length age plots for female Skeena steelhead and residuals from fitted line

#### 5.2.6 FEMALE OCEAN GROWTH RATES

While the residuals did not produce a consistent pattern when compared with the males, the slopes of the regression lines did identify what appeared to be slower growing stocks (Fig 20). These included the Kalum, Lakelese and Upper Sustut and possibly the lower Sustut.

A more complex analysis such as fitting a Von Bertalanffy curve could have been performed but the results would have been more difficult to interpret and likely not have added substantially to the overall conclusions.



Fig. 20 Female regression	of length^.333	versus age.
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Water	Intercept	slope	rank	(Untransformed data)
Lakelse	637.81	53.65	1	
Kalum	584.85	64.20	2	
U_Sustut	587.61	73.36	3	
Sustut	617.01	76.52	4	
Suskwa	584.76	93.81	5	
Toboggan	473.53	115.66	6	
Zymoetz	492.05	128.45	7	
Kispiox	497.04	135.87	8	
Babine	474.61	142.71	9	
Morice	401.45	166.40	10	
Bulkley	392.28	174.45	11	

## 5.3 LIFE HISTORY and CONSERVATION UNITS

The current Wild Salmon Program recognizes the separation of summer and winter run fish into different Conservation units (Holtby and Ciruna 2007). This results in the subdivision of the Lower Skeena FAZ into two CUs, Coastal winters and Coastal Summers. This division is consistent with the EcoSection classifications of "Kitimat Range" and "Nass Mountains", along with a possible "winter run "effect on the life history parameters. The Coastal summer CU (Nass Mountains) represents a transition zone where a number of river systems contain both summer and winter fish e.g. Kalum, Lakelese and possibly the Zymoetz. This mixture of summer and winters confounds any further analysis in this zone since the proportion of each timing group in the sample is unknown. However, the outlier status of the Kalum and Lakelse suggest a difference not only in timing, but in other genetically based attributes as well.

In the Middle Skeena FAZ, several different pieces of evidence support subdivision. First, the work of Cox Rogers (1985) clearly separates the Babine, Bulkley/Morice and Kispiox based on discriminant function analysis. Like the Lower Skeena, each of these systems is associated with one or more EcoSections, and in the Middle Skeena, a different vegetation (BEC) zone. Morphometric characteristics of the Morice and Kispiox juveniles were also shown to be significantly different from each other (Cox-Rogers 1985), as were their habitats (Appendix 3).

The dramatic age structure difference of the Morice (Table 10) from the other tributaries of the Bulkley (Suskwa, Toboggan) argues for a single Morice Conservation Unit. However the lack of information from the other Upper Bulkley tributaries (Telkwa, Buck Creek) makes the determination of possible aggregates problematic. Further, the issue is not resolved by other classification systems. The Sub basin classification includes the Morice in an "Upper Bulkley" group, while the EcoSection analysis combines the Telkwa and Morice in a "Bulkley Ranges" EcoSection, with the Upper Bulkley found in the Bulkley Basin EcoSection.

The same type of problem exists for the Suskwa. It too is in a separate EcoSection from the rest of the Bulkley (South Skeena Mountains) and has a life history more typical of "Large Fish" systems. From a habitat perspective it is more closely associated with the Babine than with the Bulkley and a differentiation of this system is also suggested by the molecular genetics (see molecular genetics section); but note that it is not grouped with the Babine, but rather oddly with the Kiseguela.

In the Upper Skeena FAZ the life histories are characterized by adaptations to colder temperatures, winter icing conditions and lake refugia and older smolt ages (R. Ptolemy,

data on file). This is signaled by the emergence of the Spruce-Willow-Birch BEC Zone and of smolts up to 5 years in age. However, a problem with determining average smolt age for each system is that very often the first annulus is not evident in these colder systems because the fry are too small to form scales or annuli prior to winter. This error is likely variable among years and between systems thereby confounding the analysis. However, the Upper Sustut is distinguished from other Skeena systems in all data categories and requires Conservation Unit status.

# **6. MOLECULAR GENETICS**

The purpose of this section is to characterize the degree of genetic isolation of the various populations in the Skeena and group them into statistical aggregations. Similar to Holtby and Ciruna (2007), we based this assessment on microsatellite DNA loci variation, but while Holtby and Ciruna(2007) developed genetic population classifications using unrooted neighbour-joining trees, we added a Bayesian approach to identify genetic groups. The branching methodology used by Holtby and Ciruna (2007) placed considerable weight on bifurcation location and chord length of the tree branches. These in turn rely on the bottom-up approach to clustering that is associated with the creation of phylogenetic trees which may not necessarily reflect a true topology.

The relatively new Bayesian Analysis of Population Structure (BAPS) does not lose information nor does it rely on averaging used in traditional approaches. Rather it allows for combining multi-locus data into a single probability model (Corander et al. 2003). Furthermore, it makes no *a priori* assumptions about population structure; it considers all group combinations equally likely (Corander et al. 2003). Finally, although many measures of population differentiation are available, they often require conditioning on a known population structure (summarized in Corander et al. 2003), and quantifying uncertainty requires re-sampling methods which can often be problematic. A possible disadvantage of the Bayes system is that attempts to minimize the number of groups which may result a very conservative description of genetic structure.

(Waples et al. 2001) noted that steelhead trout are similar to Chinook salmon (*O. tshawytscha*) in that they contain high degrees of heterozygosity and moderate levels of genetic differentiation among populations. In particular, a strong relationship was observed between genetic variation and life history diversity for these species. Exposure to different freshwater habitats and migratory routes, combined with strong homing

behavior, result in the accumulation of adaptive variation and genetic differentiation over time.

Although some variation is simply a response to the environment (i.e. phenotypic plasticity), many to most of the attributes we are interested have a demonstrable genetic basis (Taylor 1991; Hard and Hershberger 1995). Steelhead trout and Chinook salmon, along with Sockeye salmon (O. nerka), also have the broadest geographic range which is reflected in the greatest genetic and life history diversity (Waples et al. 2001). Given the above, we might expect to see similar patterns in diversity resulting in similar numbers of major groups or conservation units for steelhead trout and chinook salmon.

#### **6.1 MATERIALS AND METHODS**

#### Sampling

A total of 2,718 adult and juvenile steelhead trout have been sampled (via blood, fin clips and scales) from assumed natal streams in the Skeena River watershed from 1991 to 2009 for genetic stock identification purposes (see Beacham et al.. 2000). The older samples were collected from returning adult spawners, usually during late summer and fall months to ensure (a) steelhead trout, not resident rainbow trout were analyzed; and (b) samples represented spawner populations. However, given conservation concerns and the difficulty of accessing adult spawners for small remote tributaries (spawners tend to wait until spring to access such systems), recent sampling has focused on juveniles (parr) except where adults can be opportunistically sampled (i.e. fence and recreational fishing guides).

The samples represent 16 rivers within the Skeena River watershed, all of which are known to support summer-run spawners. These include 10 tributaries to the Skeena River mainstem (Zymoetz, Lakelse, Bulkley, Babine, Kitwanga, Kispiox, Kitsequecla, Kitsumkalum, Sustut and Kluatantan rivers), three tributaries to the Bulkley River (Morice, Suskwa, Toboggan), and two mainstem sections of the upper Skeena River mainstem (at Mosque and Kluatantan confluences). In addition, the Sustut River is represented by two distinct reaches (upper and lower).

While these sampled locations represent only a fraction of the 56 analysis (or populations) units for this study, they represent the summer-run component in each of the lower, mid and upper sections of the Skeena River with reasonable coverage. In most cases, rivers are represented by collections spanning more than a single year, and sample sizes ranged from 7 to 239 per year (Table 13).

River	91	92	93	94	95	96	97	98	99	00	01	03	04	05	09	Grand Total	Comments
Babine	19	18			38	31	28	128								262	likely all from upper 50 km of mainstem, all adults
Bulkley					7	36	20									63	all adults collected in fall and spring, not sure of origin, a composite of various tributaries, especially Morice, Bulkley gets very small upstream of Morice
Kispiox		20			28			34				46				128	adults from mainstem
Kitsguecla								13						239		252	juveniles (except 13?) from mainstem, >0+ to avoid family effects
KitsumKal um													157	41		198	adults, likely all summer from volunteer guides
Kitwanga											140					140	no winter, all adults
Kluatantan														74		74	almost all juveniles
U. Skeena @														74		74	from the Skeena mainstem at confluence with Kluatantan, all
Kiuatanan							40					50		75		4 47	juveniles
L_Sustut							13					59		75		147	all adults from lower mainstem but may be from Bear River, 2005 sample juveniles?
Lakelse													79			79	adults, could be summer or winter, from guides
Morice	20	41			15			46				65	75			262	adult collections by MOE, early samples from Moricetown mark- recapture
U. Skeena @ Mosque														69		69	all juveniles, from Skeena mainstem at confluence with Mosque River
Suskwa															207	207	all juveniles, collected far upstream
U. Sustut		13		48		50	71	50	92	100	100					524	all adults from fence
Toboggan							••	128								128	all adults
Zymoetz			16		19		38		38							111	all adults, could be summer or winter, from guide volunteers
Grand Total	39	92	16	48	107	117	170	399	130	100	240	170	311	572	207	2718	

Table 13. Summary of all steelhead tissue samples collected from Skeena-origin populations. Note that both juvenile and adults included. Comments were provided b M. Beere (Ministry of Environment, Skeena Region, May 18, 2010).

Original analyses screened for variation at eight moderately to highly variable nuclear microsatellite DNA loci (see Beacham et al. 1999 and 2000 for details on laboratory techniques to extract, amplify and visualize allelic variation). Since then, six additional microsatellite DNA loci have been added to the suite of loci screened. Thus, microsatellite variation was quantified for 14 loci (Table 14). Laboratory analyses were conducted at the Pacific Biological Station. This included a statistical analysis to test relatedness in recently collected juvenile samples for the upper Skeena River by examining the distribution of genotypes for potential full-sibling and half-sibling family groups using Colony 2.0 (Jones and Wang 2009). A lower Sustut River sample collected in 2005 appeared to represent a few, large full-sibling groups and was excluded from analyses.

samples of steelhead trout for the Skeena River drainage.
<u>Microsatellite Number of total alleles Citation</u>

Table 14. Original list of loci screened and total numbers of alleles observed across

Witchosutchite	Number of total afferes	Citation
Ogo4	18	Olsen et al. (1998)
Oke4	14	Buchholz et al. (2001)
Oki10	22	Smith et al. (1998)
Omm1008	19	Rexroad (2002)
Omm1037	28	Rexroad (2002)
Omm1276	19	Rexroad (20020
Omm5140	9	Coulibaly (2005)
Omy325	29	O'Connell et al. (1997)
One111	14	Olsen et al. (2000)
		-

#### 6.1.1 WITHIN RIVER VARIATION

Departures from Hardy–Weinberg expectations (HWE) and linkage disequilibrium (LD) were tested using Fisher's exact test where P-values were estimated for each locus within each sample year for each river using the Markov chain method in Genepop 4.0 (Rousset 2008). This evaluation is useful to test for further sub-structuring within samples (i.e. not a single randomly breeding unit) and for loci that are physically linked. Only samples with a minimum size of 20 were assessed. Where samples were collected in more than one year for a particular river, temporal stability of allelic frequencies was evaluated by calculating the F<sub>st</sub> estimator  $\Theta$  (Weir and Cockerham 1984) in Genetix 4.05 (Belkhir et al.. 2004), and the significance of differences between years was assessed using re-sampling methods, based on 1,000 permutations.

Genetix 4.05 was used to assess the significance of differences between the two mainstem upper Skeena River samples collected from the confluence of the Mosque River and the Kluatantan River to determine if these two locations could be pooled to represent a single upper Skeena River mainstem sample. In all statistical tests, P-values (initial level of significance set at 0.05) were corrected using the sequential Bonferroni adjustment (Rice 1989).

## **6.1.2 GENETIC POPULATION STRUCTURE**

Allelic frequencies for combined years for each river were tabulated using Genepop 4.0 to provide an overview of variation among rivers. Population structure at a landscape level was assessed using a mixture model based on Bayesian predictive classification theory available in the statistical software package BAPS (Bayesian Analysis of Population Structure, Corander et al. 2008). This analysis determines the probability, based on observed allele frequencies of the pre-grouped data, that underlying allele frequencies differ and determines the most probable number of genetic clusters without reference to any *a priori* designation of structure based on sample locality. All sampled years were maintained separately given that significant differences observed for some loci between some years.

A traditional analysis of molecular variance (AMOVA) was also performed following Excoffier et al.. (1992) to consider hierarchical partitioning of microsatellite variation: (1) between years within samples within rivers relative to that among rivers; and, (2) within and among the clusters identified in the BAPS analysis, using the statistical package ARLEQUIN 3.1 (Excoffier et al. 2006).

To illustrate genetic relationships among samples and rivers, genetic distances among sampled years were estimated using Cavalli-Sforza and Edward's chord distance bootstrapped (1,000 times) to produce an un-rooted neighbour-joining tree in Phylip (Felsenstein 2009).

Finally, to visualize the distribution of among- sample variation, the microsatellite DNA allele frequency data were subjected to a factorial component analysis (FCA) using Genetix 4.05 (Belkir et al.. 2004). Genetix was also undertaken to generate pairwise  $F_{st}$  values (a measure of genetic differentiation ranging from 0 (panmixia) to 1 (complete separation) between all pairs of samples. In this way,  $F_{st}$  values between years within rivers could be compared to samples among rivers.

#### 6.2 RESULTS

#### **6.2.1 GENETIC VARIATION WITHIN RIVERS**

Once adjusted for 14 multiple tests per sample, 29 significant deviations from HWE were observed within samples. There was no evidence of significant deviations associated with a particular locus, and in most cases, only a single locus deviated within a particular sample with the following exceptions: Babine River 1995 (three loci), Kitsumkalum River (three loci), Morice River 1992 (seven loci), Suskwa River (eight loci), suggesting that further population structuring may exist within these samples. The Morice River 1992 sample had deficiencies in heterozygotes for several of these loci.

With respect to linkage equilibrium (LD), 90 possible loci pair combinations were tested for each sample. No particular locus pair was consistently out of equilibrium, and most samples were in equilibrium for all loci pairs with the following exceptions: Kitseguecla River 2005 had 21/90 combinations in linkage disequilibrium, Kluatantan River 2005 had 20/90, lower Sustut River 2003 2/90, Morice River 1992 >50/90, Morice River 1998 1/90, Suskwa River >50/90, upper Skeena River at Mosque 17/90 and upper Sustut River 2000 1/90.

Estimates of F<sub>st</sub> were not significantly different from zero among temporal samples for several rivers including Babine, Bulkley, Kispiox or Kitsumkalum rivers, indicating temporal stability. F<sub>st</sub> values were significantly greater than zero for the following temporal comparisons: lower Sustut River (1997 versus 2003), Morice River (1992 versus 1998, 2003, 2004), upper Sustut River (1996 versus 1999, 2000, 2001 and 1997 versus 1999, 2000, 2001, 1998 versu 2000), and Zymoetz River (1995 versus 1994) (see Appendix 6).

In considering differences between the two locations sampled for the upper Skeena mainstem (i.e., at Mosque River and Kluatantan River confluence), the  $F_{st}$  estimator was significantly greater than zero (Appendix 6).

## 6.2.2 LANDSCAPE LEVEL GENETIC STRUCTURE

In general, pairwise  $F_{st}$  estimates within rivers between years were either not significantly greater than 0 or were relatively small (i.e., < 0.01) whereas comparisons among rivers were generally significantly greater than 0 (Appendix 6). Allelic frequencies and numbers of alleles for rivers (combined for years) are summarized in Appendix 7. Average numbers of alleles varied from 10.8 for Kluatantan River to 13.9 for Babine River but were largely associated with total sample size. Private alleles were observed in several instances, but not consistently for particular populations; in all cases, frequencies for these alleles were very low (i.e., < 0.01).

#### 6.2.2.1 CLUSTER ANALYSES

Six population genetic clusters were consistently identified within the Skeena River watershed with a probability of 1 using BAPS (Table 15).

Table 15. Genetic Groups in steelhead trout within the Skeena River based on BAPS analysis.

Genetic Group	Location	Region
1	Kitsumkalum, Lakelse, Zymoetz	Lower Skeena
2	Babine	Middle Skeena
3	Bulkley, Kispiox, Kitwanga, Morice,	Middle Skeena/Bulkley
	Toboggan	
4	Kitseguecla, Suskwa	Middle Skeena/Lower
		Bulkley
5	Upper Sustut	Upper Skeena
6	Kluatantan, Upper Skeena, Lower Sustut	Upper Skeena

Each cluster included regionally proximate rivers, and all samples across years were also captured within clusters. In most cases, tributaries of rivers clustered together with one exception. The Suskwa (a Bulkley tributary) and the Kitseguecla River did not cluster with their parental streams but instead with each other. In contrast, two other nearby mid-Skeena mainstem tributaries, the Kispiox and Kitwanga rivers, did cluster with the Bulkley-Morice. Most of these groups were also strongly supported by high bootstrap values on the consensus branches of the un-rooted neighbour-joining tree produced from genetic distances (Fig.21).

In addition to the dendrogram, the EcoSection associated with each sample was identified with a unique color and the results superimposed on the branches of the dendrogram.



Fig. 21 Dendrogram of genetic distance for Skeena microsatellite DNA samples with EcoSection associations superimposed.

The results form a logical pattern except for the smaller middle Skeena watersheds and Toboggan Creek. The Toboggan Creek results are almost certainly the result of either misread scales or some other artifact. This was suggested by the significant number of one year old smolts in the data that would be impossible under natural conditions and inconsistent with known size at age juvenile data or known growth season length.

#### 6.2.2.2 AMOVA

An AMOVA pooling years within rivers confirmed that a significant and greater proportion of variation was attributed to variation among the BAPS groups (1.37%) versus variation among rivers within the BAPS groups (0.95%) (Table 17).

Table 17 Analysis of molecular variance(AMOVA) partitioning genetic variance among individual steelhead trout, among rivers within groups identified by BAPS and among gBAPS groups (Note that years were pooled for rivers with more than one sample year).

Source of Variation	D.F.	% of Variation	P-Value
Among BAPS groups	5	1.37	<0.0001
Among rivers within I	BAPS 10	0.95	<0.0001
groups			
Among individuals	5416	97.68	<0.0001

Results of an additional AMOVA indicated that of the total allele frequency variation observed within the Skeena River drainage, 97.9% was attributable to variation within samples and 2.1% was attributable to variation among locations (p = 0.000). No significant variation was attributed to temporal differences within locations across sample years, suggesting that, while temporal variation may be significant in some cases (based on pairwise Fst tests), it is relatively insignificant in considering spatial differences across the study area (Table 18).

Table 18. Analysis of molecular variance(AMOVA) partitioning genetic variance among individual steelhead trout samples, among years within rivers, and among rivers.

Among Groups	15	558.591	0.1068	Va	2.23
Among pops in groups	20	84.419	-0.00448	Vb	-0.09
within pops	5130	24056.202	4.68932	Vc	97.86
Totals	5165	24699.212	4.79165		

Significance tests (1023 permutations)

Fixation	FSC	-0.00096	Vb and FSC : P(rand. val > obs. val) = 0.9697
	FCT	0.02229	Va and FCT : $P(rand. val > obs. val) = 0.000$
	FST	0.02136	Vc and FST : P(rand. val < obs. val) = 0.000

## **6.2.3 FACTORIAL CORRESPONDENCE ANALYSIS**

The FCA summarized approximately 37% of the total allele frequency variation across three axes (Fig. 22) and generally supported the genetic groups identified by the cluster analyses. However, there were also some differences. In the FCA there were groups identified; the Lower and Upper Skeena groups remained as in the other approaches while the Middle Skeena again proved problematic. In this case the Morice appeared to be the population most distinct from the others in the Middle Skeena, which was supported in the life history and habitat analysis.



Fig.22. Factorial Correspondence Analysis of Skeena River steelhead trout samples generated using Genetix.

#### 6.3 GENETICS DISCUSSION

Clearly, significant population structure exists within the Skeena River watershed for steelhead trout. F<sub>st</sub> values and AMOVA results indicated that temporal variation tended to be relatively small to insignificant, whereas significant variation existed among samples from different rivers. Genetic similarity was clearly associated with geographic proximity of rivers with several genetic groups consistently identified. In particular, the upper Sustut River and lower Skeena River (Zymoetz, Kitsumkalum and Lakelse rivers) were most differentiated from all others. At a minimum, the six genetic groups identified by the BAPS analysis should be used in combination with the ecotypic CUs to establish comprehensive CUs. Maintaining winter and summer run populations in separate CUs as proposed for chinook salmon by Holtby and Ciruna (2007) and for steelhead trout in the US (Waples et al. 2001) would result in a minimum of seven preliminary CUs .

Additional characterization of analysis units using adaptive variation particularly for the proposed middle Skeena CUs provided further resolution of these CUs. The tributaries are very difficult to characterize genetically as different life stages use different systems (e.g. spawning versus rearing tributaries) and migration of parr from smaller tributaries into larger mainstem habitats made representative tissue sampling challenging. Thus, the genetic variation measured for this area may not reflect the breadth of population structure here. Furthermore, it is thought that until fairly recently (i.e., within a few 1000 years), significant exchange occurred between Nass and Skeena rivers' watersheds, as well as between these watersheds and those in the interior Fraser River tributaries (McPhail and Lindsay 1986). A coast wide analysis (in prep) supports this idea with some mid- and upper Nass River populations appearing to be closely related to Skeena River populations. The EcoSection names (e.g. Nass basin for the Kispiox, Cranberry Uplands for the Kitwanga etc.) provide additional support for this possibility.

The results generated from the Bayesian and AMOVA analyses are similar to those produced for regional groups of Atlantic salmon (*Salmo salar*) populations in eastern Canada although the differences were slightly greater in magnitude for Atlantic salmon (2.54% and 2.02%, respectively), potentially reflecting the larger geographic range considered in that study (Dionne et al.. 2008). In addition, they incorporated some *a priori* spatial information that may have influenced results.

# 7. DISCUSSION AND CONCLUSIONS

The purpose of this paper was to assemble steelhead habitat, life history and genetics information into a consistent framework of Conservation Units suitable for the management and protection of Skeena steelhead genetic variation. The approach was to first accept the current Freshwater Adaptive Zones (established by the Federal government), then determine if subdivision of those zones for steelhead was appropriate based on the analysis of each of the above categories of information.

Obtaining representative samples for life history and genetics analysis represented a significant challenge. Also, size at age was a composite age structure averaged over a number of different brood years since we did not have complete data from a single brood year. To reconstruct individual brood years would require determining the age structure of the catch, which of course would only be possible if the catch was properly sampled and individual stocks could be properly identified.

The fact that portions of adult samples could be enroute to another watershed, or that juveniles may have either been displaced or actively migrated from their natal watershed, added to the complexity and sources of error.

In conducting the work it became apparent that each of the datasets had its own strengths and weaknesses. The habitat classifications that are broadly used for land use planning, have a widespread understanding, and are consistent over the entire province. However, the relationship of vegetation patterns or other physical characteristics of watersheds to fish conservation values is uncertain. Similarly, while there were dramatic differences in life history among some populations, the extent to which these were the result of environmental differences versus genetic factors remains unclear. Finally, measurement of genetic differences did not necessarily relate to the adaptive value of a given set of traits and therefore provided only a limited picture of the genetic variation in a given analysis unit. Therefore, while no single approach offers a definitive methodology for identifying critical adaptive variation, it seems clear that the combination of methods provides a useable framework of 11 Conservation Units based on the EcoSection classification.

As for the definition of Conservation Units themselves, the final result is a combination of units unambiguously derived from the various analyses (i.e. habitat, life history and molecular genetics) are all consistent, and those for which a number of possibilities still remain. Additional sampling may be the only method for resolving some of these possibilities, but one need give serious consideration to the premise that the marginal improvement in fine tuning the Conservation units would not be worth the cost. This would be particularly true if, as seems to be the case, that there is a relatively high level of auto correlation among the easily measurable indicators and the objectives of the wild salmon policy. The exceptions to this suggestion would be the Upper Skeena CU (for which we have no life history or genetic information and the Upper Bulkley (including Buck Creek), which might assist in clarifying the Bulkley Conservation Unit. The other areas of uncertainty relate to the impact of the various fisheries, particularly on the small unproductive systems and the role of these systems (including recruitment of juveniles to the mainstem Skeena River) in the broader life history strategies of Steelhead in the Skeena. We know with considerable certainty through boat and shore-based electrofishing that steelhead juveniles rear at habitat capacity densities in the mainstem river (Ron Ptlomey pers. com.)

Table 19 Final steelhead Conservation Units suggested for the Skeena River. The various
colors represent the Lower(green), Middle(blue) and Upper Skeena(yellow) FAZ Units

	Habitat			Life history	Genetics
CONSERVATION UNIT	EcoSection	Hydro Zone	BEC	L Hist	BAPS
Coastal Winter Runs	Kitimat Range	17	7 cwh-mh-cma	n/a	
Coastal Summers	Nass Mountains	18	3 cwh-mh-cma	?	Zymoetz, Lakelse, Kitsumkalum
Middle Skeena	Cranberry Uplands	18	3 ich-essf-bafa	М	
Kispiox	Nass basin	12	2 ich-essf-bafa		Kispiox, Bulkley, Morice, Toboggan, Kitwanga
Bulkley	Bulkley basin	19	) sbs-essf-bafa	S	
Morice	Bulkley Ranges	19	) sbs-essf-bafa	S	
Suskwa	Southern Skeena Mnts	11	L ich-sbs-essf-bafa	L	Suskwa,, Kitseguclq
Babine	Babine Uplands	10	) ich-sbs-essf-bafa	L	Babine
Upper Skeena	N Skeena Mnts	11	L sbs-essf-bafa	?	
Upper Skeena hdwtrs	East Skeena mnts	10	) essf-sbs-bafa	?	Kluatantan, Upper Skeena, Lower Sustut
Upper Sustut	North Omineca mnts	10	) essf-swb-bafa	L	Upper Sustut

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