

Baseline Fish Health Monitoring Program

Bulkley River and Babine River

2003



**Prepared by
Brenda Donas, HEB-Northcoast
March 2004**

Executive Summary

Six stocks of salmon from the Babine and Bulkley Rivers were sampled for prevalence of bacterial and viral infections and for presence and level of parasite infestation. On the Babine River mainstem downstream of the Babine Lake outlet and at the Babine River fence, 60 each of sockeye, pink, chinook and coho were sampled. On the Bulkley River 60 each of coho and pink salmon were sampled from tributary streams.

Stocks were sampled for the presence of the causative agents for Bacterial Kidney Disease, Enteric Redmouth Disease, Furunculosis and other types of bacteria harmful to fish health. Stocks were also sampled for the presence of viral agents and especially for IHN (Infectious Hematopoietic Necrosis). Kidney and gut smears were taken to evaluate the presence and level of parasite infestation.

Samples were taken from every 15th fish for histological examination.

All fish sampled were examined externally for visible parasites and lesions, wounds and any other abnormalities.

A small number of sockeye(12) were sampled for heavy metals analysis and six pink salmon were also sampled for heavy metals.

Fish health sampling results have been categorized by stock, species and sample type. In general, there was an overall low prevalence of Bacterial Kidney Disease, Furunculosis, Enteric Redmouth Disease and other harmful bacteria and parasites. There was a low incidence of IHN in Babine River sockeye and that was the only viral agent identified.

All stocks of fish were sampled by the same crew with the exception of the 30 pink salmon sampled at the Moricetown Canyon which were sampled by the Wet'suwet'en Fisheries Program staff.

Acknowledgements

Andrea Osborn, Karen Mullen and Carl Westby from the Fish Health Diagnostics Lab at the Pacific Biological Station were all instrumental in the design of this study. Carl Westby, Karen Mullen, Marie Boichuk and Kerry Bate conducted the lab work and ensured that fish health samples were analyzed in a timely fashion. Jan Blackburn conducted the histopathology readings and reported the results which have simply been melded into this report. Dorothee Kieser and Karen Mullen provided assistance with data analysis and report editing.

Sue Farlinger, Brian Pearce and Bruce Shepherd provided the financial assistance for this program. Sue, Brian and Bruce provided encouragement and support for this program as a pilot study.

Cory Koenig, Ryan Frisk and Edi Young provided technical assistance through long days and extreme weather conditions. The program would not have been as successful without their dedication and diligence.

Thanks also to the Wet'suwet'en Fisheries Program staff who conducted the Bulkley River pink and coho salmon sampling at the Moricetown Canyon.

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1.0 Introduction

In the fall of 2002, representatives from the Wet'suwet'en Fisheries Program approached the Smithers area Community Advisor regarding support for a funding application for a project that would categorize anecdotal external fish health observations. During the beach seining and food fishery programs at the Moricetown Canyon, Wet'suwet'en Fisheries staff had been recording observations on the external condition of the fish being sampled in their programs. They thought that perhaps those observations of external symptoms could be compiled and used as a fish health indicator.

The proposal concept was passed on to F.O.C. Science staff who felt that categorizing external symptoms would not provide sufficient information. However, they encouraged a baseline fish health monitoring program.

The concept of a baseline fish health monitoring program was presented to Wet'suwet'en Fisheries with a request that they be the lead proponent for the program. The Wet'suwet'en felt that they did not have the resources for such a program but stated their interest in participating.

The Baseline Fish Health monitoring program was ultimately designed by F.O.C. Science staff as a pilot study. The study was conducted by Community Involvement Program staff.

The study involved a training session where sampling techniques and data collection were taught.

The study design called for the sampling of 60 returning adult fish per stock. Refer to Figure 1 for sampling locations. Some sampling was conducted where stocks could be sampled in pre-spawning condition and some stocks could only be sampled at the post-spawn stage. Samples included a swab of the posterior kidney for bacterial culture, a piece of posterior kidney for Bacterial Kidney Disease analysis using the ELISA test, spleen, anterior kidney and pyloric caeca for viral analysis, kidney smears for bacterial and parasite analysis and gut smears for parasite analysis. Selected fish also had tissues taken for histological analysis and heavy metal testing.

On the Babine River, 60 each of sockeye, pink, coho and chinook salmon were sampled. During the coho sampling, marked hatchery fish were included in the sample. Some of the sockeye and pink salmon were also sampled for the presence of metals.

All of the Babine pinks and chinook were sampled at the Babine River fence. Some of the coho were sampled at the Babine River fence and the majority were sampled from the spawning grounds at the post-spawning stage.

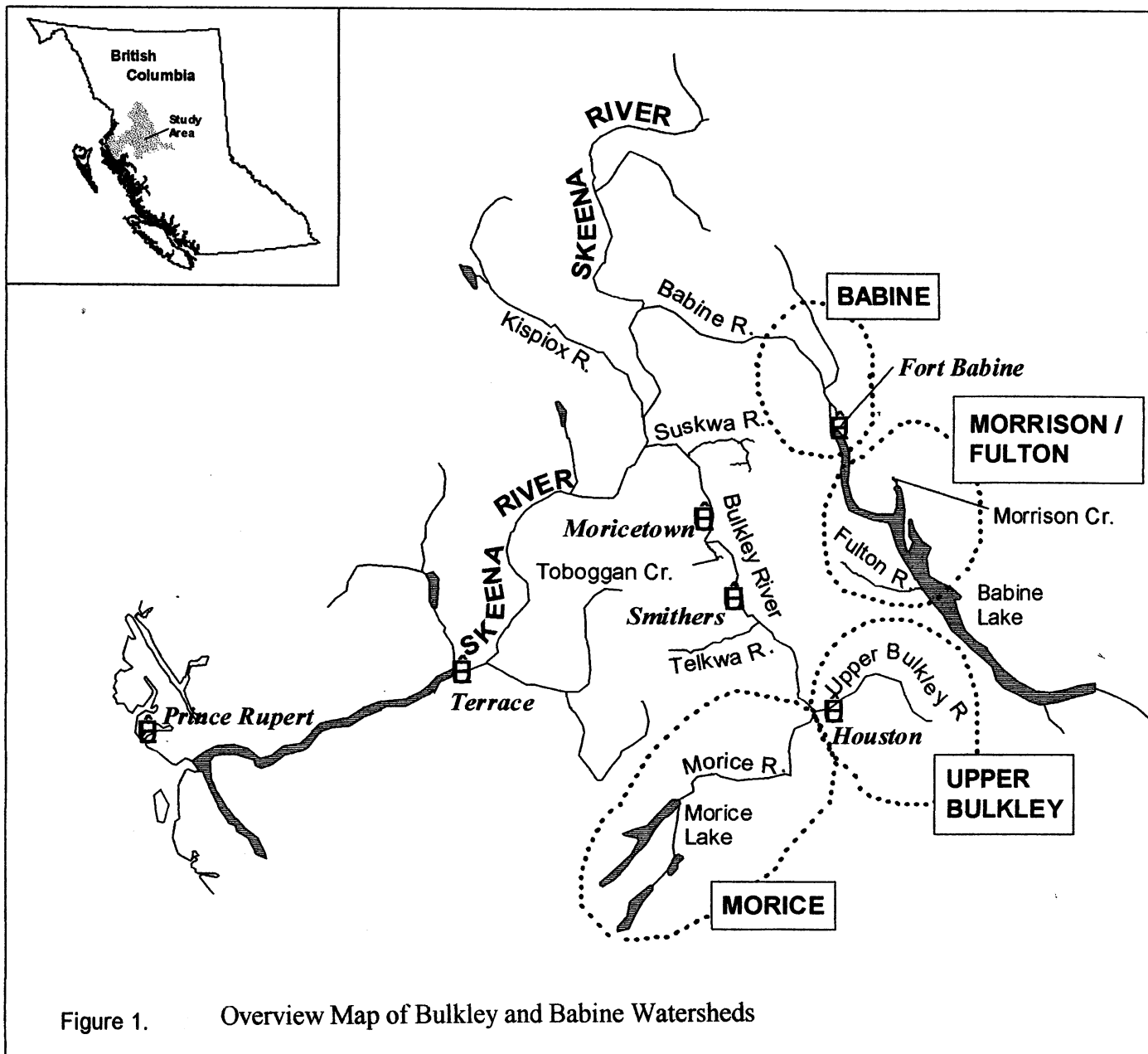


Figure 1. Overview Map of Bulkley and Babine Watersheds

Most of the Babine River sockeye were sampled at the fence and some were taken from the spawning grounds at the post-spawn stage. The sockeye and pink salmon that were sampled were not used in hatchery egg takes and although some of the carcasses were used as First Nations food fish, fish that were close to spawning condition were not valued as food. Most of the chinook that were sampled were used in hatchery egg takes.

On the Bulkley River, 61 coho and 60 pink salmon were sampled. The coho sample consisted of 26 Upper Bulkley River coho, 20 Toboggan Creek coho and 15 Mission Creek coho. The Upper Bulkley River coho were near spawning condition when sampled. The Toboggan Creek coho were in post-spawning condition as were the Mission Creek coho. A portion of the Mission Creek coho that were sampled were also used as broodstock. Where possible, coho being sacrificed for the coded wire tags were also used for fish health samples.

The 60 pink salmon samples consisted of 30 pinks sampled at the Moricetown Canyon (a combination of Bulkley River and Morice River pinks) and 30 pinks sampled at Mission Creek, a tributary of the lower Bulkley River. The pink salmon sampled at Mission Creek were removed from the spawning grounds in post-spawn condition.

2.0 Sampling Procedures

At the fences and in the smaller streams, fish were captured using dipnets. Samples collected from the Babine River spawning grounds were collected during the Coho Beach Seining Program.

Fish were killed with a blow to the head and then hung, head down, and bled by making a cut through the gills. Only that number of fish that could be sampled within one hour were killed i.e. fish had to be sampled within one hour of death. Each fish was assigned a number so that each sample could be identified as to stock, species and fish number.

While the fish were hanging and bleeding, an external examination was conducted. This involved an examination of the general condition of the skin, eyes and gills. Wounds, lesions and parasites were noted and some parasite samples were taken.

Once the fish had been bled for about 10 minutes, the fish was placed on a table. Using a scalpel (soaked in Iso-propyl alcohol and flamed), an incision was made along the fish's belly. The incision was made off to the side of the vent to avoid cutting into the intestine which could lead to contamination of the samples.

Instruments were soaked in alcohol, flamed and then cooled between each set of samples. Once the incision down the belly was made, an internal examination was conducted. This involved examining the general condition of the heart, liver, pyloric caeca, stomach, spleen, intestine and kidney.

These observations were recorded onto pre-formatted data sheets. (Refer to Appendix 1 for raw data and results tables). Once the internal examination was complete, the swim bladder was pulled away from the kidney in preparation for organ sampling.

Using a sterile scalpel, a small incision was made into the back (posterior) kidney. A sterile kidney swab was then removed from its packaging and inserted enough to cover the swab in kidney. The swab was returned to its sterile container and labelled as to stock, species and fish number.

Then a quarter size piece of posterior kidney was removed and put into a whirl pac for BKD ELISA testing. A small piece of posterior kidney was removed with forceps, rolled onto a clean paper towel to remove excess blood, and then the piece of kidney was smeared onto a labeled slide for future Gram stain analysis.

Using sterile instruments, a piece of the spleen, pyloric caeca and anterior kidney were removed and placed into a labeled whirl-pack bag. These samples were for viral analysis.

A gut smear was taken last by cutting out a small piece of gut, gently squeezing out the gut contents and then making a slide smear from the inside wall of the gut.

Some of the internal parasites that were observed were preserved in Davidson's solution for future analysis.

The above procedure was followed for each fish sampled. However, for each group of 60 fish sampled, some extra samples were taken for histological examination. Fish numbers 1, 15, 30, 45 and 60 had small pieces of heart, liver, spleen, pyloric caeca, posterior and anterior kidney and intestine removed. Tissues from individual fish were pooled and preserved in a vial of Davidson's solution for histological analysis.

In order to prevent degradation of the viral and other biological samples, the samples had to arrive at the lab within 24 hours of taking the samples. This was accomplished for each set of samples taken on every sampling date.

Laboratory Methods:

Samples submitted to the Fish Pathology Program were analysed according to the following methods:

- 1) Enzyme-linked Immunosorbant Assay (ELISA):
Kidney tissue was analysed based on the method described by R.J. Pascho and D. Mulcahy (Enzyme-linked Immunosorbent Assay for a soluble antigen of *Renibacterium salmoninarum*, the causative agent of salmonid bacterial kidney disease, Can. J. Fish. Aquat. Sci., Vol. 44, 1987)
- 2) Other bacterial detection methods: culture of kidney tissue, Gram stain of kidney tissue. Methods used were based on the Fish Health Protection Regulations, Manual of Compliance (Department of Fisheries and Oceans, 1984. Fish.Mar.Serv.Misc.Spec.Publ. 31 (revised): 43 p.). It is to be noted that in addition to examining Gram stains for *Renibacterium salmoninarum*, smears were also checked for microsporean and myxosporean parasites and for other bacteria.
- 3) Viral assays: Detection methods were based on the Fish Health Protection Regulations, Manual of Compliance (Department of Fisheries and Oceans, 1984. Fish.Mar.Serv.Misc.Spec.Publ. 31 (revised): 43 p.). Cell cultures used: EPC and CHSE -214.
- 4) Histology: The Davidson-fixed material received by the laboratory was embedded in paraffin. Sections (5 μ) were stained with hematoxylin-eosin and examined.

3.0 Results

Since this is a pilot baseline study, the results of the fish health analysis simply provide a snapshot of fish health in the areas surveyed. The results will therefore be reported without any statistical comparison between stocks and species or marked and unmarked fish. The results have been categorized and reported according to prevalence of bacterial and viral agents and by presence/absence of parasites. All sample results are tabulated in Appendix 1.

The following abbreviations apply to all of the following summary tables :

A.s.	<i>Aeromonas salmonicida</i>
Y.r.	<i>Yersinia ruckeri</i>
H	High
M	Medium
L	Low
Li	Light
Ra	Rare
Sphaer	Sphaerospora
Spor	Sporoblasts

IHN	Infectious Hematopoietic Necrosis
K.r.	<i>Kryzeomonas luteola</i>
Myx	<i>Myxidium</i>

3.1 Babine River Sockeye

The sockeye sample consisted of 25 females, 30 males and 2 jacks. Three of the sockeye sampled were not identified as to sex. All fish were in near to post-spawning condition. Sampling commenced July 23rd and was completed on November 4th, 2003.

External examination showed a very low prevalence of gill or other parasites. However, external examination showed that of the 60 sockeye sampled, 6 had internal parasites (roundworms). The number of parasites was not quantified but by visual examination, the range of infestation was from very few (isolated) parasites to quite dense areas of parasite infestation usually around the pyloric caeca, stomach and intestine.

Using ELISA testing, no *Renibacterium salmoninarum* (BKD agent) was detected in Babine River sockeye. The bacterial culture results from the kidney swabs showed that one of the 60 sockeye sampled was positive for *Aeromonas salmonicida*, the causative agent of Furunculosis. The kidney smears showed a very low prevalence of parasite sporoblasts and Sphaerospora spores. All gut smears were negative. Ten of the 60 sockeye sampled were positive for IHN. All of those fish were sampled late in the run and were either actively spawning or in post-spawn condition. Results are summarized in Table 1 below.

Table 1 : Babine Sockeye Results

Total Number sampled	60
No. of Males	30
No. of Females	25
No. Jacks	2
Other	3

Bacterial agents found

Bacterial agents found (#positive/# tested)		Viral Agents		Parasites
ELISA	Culture	Kidney Smear	Gut Smear	Spec-tiss-infestation
0/60	1/60 A.s.	6/60 Sphaer - R 1/60 Sphaer- Li 5/60 Sporo-R 1/60-Sporo-Li	0/60	10/60-IHN 2/5-Myxozoan 2/5- Kidney tubule parasite(un- identified) 1/5-Cestodes

3.2 Babine River Pink

The pink samples consisted of 35 females and 25 males. All fish were in near to post-spawning condition. Sampling commenced on September 2nd and was completed by September 9th, 2003.

External examination showed a very low prevalence of gill or other visible parasites. Internal examination showed that of the 60 pink salmon sampled, 41 had internal parasites. The prevalence of parasites was not quantified and as for the sockeye, the internal parasite infestation ranged from isolated parasites to higher densities of parasites normally situated around the pyloric caeca, stomach and intestine. There was a very low prevalence of what appeared to be roundworms on the spleen and livers.

Babine Pink results are summarized in Table 2 below.

Table 2 : Babine Pink Results

Total Number sampled	60
No. of Males	25
No. of Females	35
No. Jacks	0
Other	0

Bacterial agents found

(#positive/# tested)

<u>ELISA</u>	<u>Culture</u>	<u>Kidney Smear</u>	<u>Gut Smear</u>	<u>Viral Agents Detected</u>	<u>Parasites Spec-tiss-infestation</u>
0/59	0/60	9/60 Spor-Ra 18/60-Spor-Li 10/60-Spor-M 6/60-Spor-H 2/60-Sphaer 1/60-Loma-Ra 5/60-Loma-Li 6/60-Loma-M 3/60-Loma-H	0/60	0/60	4/5-unidentified kidney tubule parasite 4/5-Cestodes

3.3 Babine River Chinook

Sixty Babine River chinook were sampled of which 23 were females and 37 were males. All fish were in spawning condition. Sampling commenced on September 17th and was completed on September 23rd, 2003.

External examination showed a very low incidence of gill or other visible parasites. Internal examination showed that of the 60 chinook sampled, 13 had internal parasites. These were all very low infestation levels ie. usually isolated parasites around the liver or pyloric caeca, stomach and intestine.

BKD ELISA testing showed that of the 60 fish tested, 1 male was a high positive and fifty-nine chinook were negative. The bacterial cultures showed that of the 60 samples taken, 2 fish were light positives for *Aeromonas salmonicida*, the causative agent of Furunculosis and one fish was a light positive for the causative agent of Enteric Redmouth Disease, *Yersinia ruckeri*. All chinook were negative for viral infections.

Babine River chinook results are summarized in Table 3 below.

Table 3 : Babine River Chinook Results

Total Number sampled	60
No. of Males	37
No. of Females	23
No. Jacks	0
Other	0

Bacterial agents found

(#positive/# tested)

<u>ELISA</u>	<u>Culture</u>	<u>Kidney Smear</u>	<u>Gut Smear</u>	<u>Viral Agents Detected</u>	<u>Parasites Spec-tiss-infestation</u>
1/60-H	2/60-A.s-Li 1/60-Y.r.-Li	3/60-Sphaer-Ra 1/60-Myx-Ra	0/60	0/60	0/5

3.4 Babine River Coho

Sixty Babine River coho were sampled of which 26 were females, 30 were males, 1 was a jack and 3 fish were not identified as to sex. All coho were in near to post-spawn condition. Sampling commenced on October 28th and was completed on November 18th, 2003.

External examination showed a higher prevalence of gill parasites than previously observed when sampling other Babine River stocks. Of the 60 fish examined, 12 had visible gill parasites. Internal examination showed that of the fish sampled, 26 had internal parasites. Parasite infestations were very light and usually parasites were found around the pyloric caeca, stomach and intestine with some isolated parasites around the liver.

The BKD results by ELISA showed that of the 60 fish sampled, 3 coho were high positive for BKD and 57 fish were negative. The bacterial cultures showed that 2 fish were moderate positives for *Aeromonas salmonicida*, the causative agent of Furunculosis and 1 coho was a light positive for *Aeromonas salmonicida*. The other 57 coho were negative for bacterial agents. Babine River Coho results are summarized in Table 4 below.

Table 4 : Babine River Coho Results

Total Number sampled	60
No. of Males	30
No. of Females	26
No. Jacks	1
Other	3

Bacterial agents found

Bacterial agents found (#positive/# tested)		Viral Agents		Parasites	
ELISA	Culture	Kidney Smear	Gut Smear	Detected	Spec-tiss-infestation
3/60 - H	1/60 Li A.s. 2/60 -M A.s.	8/60-Spor-Ra 13/60-Spor-Li 17/60 Spor-M 3/60-Spor-H 8/60-Loma-Ra 6/60-Loma-Li 1/60-Loma-M 3/60-Myx-Ra	1/60-Myx	0/60	5/5-Myxozoan

3.5 Bulkley River Pink Salmon

Bulkley River pink salmon were sampled at two locations, Moricetown Canyon and Mission Creek, a tributary to the lower Bulkley River. The 30 pink salmon sampled at Moricetown Canyon would have been a mixture of stocks bound for the Bulkley River upstream of the Moricetown Canyon and stocks bound for the Morice River. The Moricetown pink salmon sampling was conducted by the Wet'suwet'en Fisheries program staff.

Moricetown Canyon Pink Salmon

The Moricetown sample consisted of 22 female and 8 male pink salmon. Sampling commenced on August 6'th and was completed on August 13'th, 2003. All pinks were near or in spawning condition.

External examination showed few to no external parasites. Internal examination showed that of the 30 pinks sampled, 22 contained parasites which were usually found around the pyloric caeca, stomach and intestine.

All 30 fish were negative for BKD by ELISA. Bacterial cultures showed one fish positive for *Kryzeomonas luteola* and the remaining 29 samples were negative. All fish were negative for viral agents.

Moricetown Pink results are listed in Table 5 below.

Table 5 : Moricetown Pink(Bulkley/Morice) Results

Total Number sampled	30
No. of Males	8
No. of Females	22
No. Jacks	0
Other	0

Bacterial agents found

(#positive/# tested)				Viral Agents	Parasites
<u>ELISA</u>	<u>Culture</u>	<u>Kidney Smear</u>	<u>Gut Smear</u>	<u>Detected</u>	<u>Spec-tiss-infestation</u>
0/30	1/30-K.I.	1/30-Spor-Ra 1/30-Spor-Li	0/30	0/30	2/3-Myxozoan 1/3-Trematodes and Cestodes

Mission Creek Pink Salmon

The Mission Creek sample consisted of 15 females and 15 males. All 30 pink salmon were sampled on September 1st, 2003 and all fish were in spawning condition.

External examination showed that of the 30 fish sampled, two fish each had one visible gill parasite. Internal examination showed that 11 fish contained internal parasites with very light to moderate infestation. Internal parasites were found mostly around the digestive system organs.

All 30 fish were negative for BKD by ELISA. Bacterial cultures showed that 1 fish was a light positive for *Aeromonas salmonicida*, the causative agent of Furunculosis. The remaining 29 fish were negative. All 30 fish were negative for viral agents.

Mission Creek Pink salmon results are shown below in Table 6.

Table 6 : Mission Creek Pink Results

Total Number sampled	30
No. of Males	15
No. of Females	15
No. Jacks	0
Other	0

Bacterial agents found

(#positive/# tested)

<u>ELISA</u>	<u>Culture</u>	<u>Kidney Smear</u>	<u>Gut Smear</u>	<u>Viral Agents Detected</u>	<u>Parasites Spec-tiss-infestation</u>
0/30	1/30- A.s-L	5/29-Spor-Ra 14/29-Spor-Li 3/29-Spor-M 1/30-not read	0/29 1/30-not read	0/30	

3.6 Bulkley River Coho

The 61 fish sample of Bulkley River coho consisted of 26 Upper Bulkley River coho, 20 Toboggan Creek coho and 15 Mission Creek coho. There were also three coho sampled at the Moricetown Canyon but due to that small sample size, those results have not been included in this report.

Upper Bulkley River Coho

There were 26 Upper Bulkley coho sampled of which 10 were females and 16 were males. Sampling commenced on Sept 8th and was completed on September 29th, 2003. These coho were in silver-bright to pre-spawning condition. Of the 26 coho sampled, the hatchery coho sampled consisted of 4 Adipose/Right maxillary clipped and 1 adipose clipped coho.

External examination showed no external parasites. Internal examination showed that 11 of the 26 coho sampled had parasites present. However, parasite infestations were very light with parasites usually being found around the digestive system organs.

BKD ELISA showed that 23 of the 26 fish were negative for BKD while 1 fish had a light infection and 2 fish were classified as high. The bacterial cultures showed that 24 coho were negative for bacterial agents and one was light for *Aeromonas salmonicida*, the causative agent of Furunculosis. All fish sampled were negative for viral agents.

Upper Bulkley River Coho results are shown in Table 7 below.

Table 7 : Upper Bulkley River Coho Results

Total Number sampled	26
No. of Males	16
No. of Females	10
No. Jacks	0
Other	0

Bacterial agents found

(#positive/# tested)				Viral Agents	Parasites
<u>ELISA</u>	<u>Culture</u>	<u>Kidney Smear</u>	<u>Gut Smear</u>	<u>Detected</u>	<u>Spec-tiss-infestation</u>
3/26 -H	1/25- A.s.-Li	4/26-Spor-Ra 12/26-Spor-Li 1/26-Spor-M 1/26-Loma-Ra 1/26-Loma-Li 1/26- Henneguya	0/26	0/26	1 / 2-Myxozoan 1 / 2 – unidentified parasite in the kidney tubules

Mission Creek Coho

There were 15 Mission Creek coho sampled and some of those coho were used in hatchery egg takes. Of the 15 coho sampled, 7 were adipose clipped and there were 6 females, 8 males and 1 jack. Sampling occurred on October 22nd, 2003 and all coho were in spawning condition.

External examination showed that 4 of the 15 coho sampled had gill parasites but in very low numbers i.e. one or two visible parasites per fish. Internal examination showed 3 coho to contain parasites and none of those fish had a heavy infestation of parasites.

The BKD ELISA results showed that 14 of the coho were negative and 1 was a high positive. The bacterial cultures showed 1 fish was positive for *Aeromonas salmonicida*. All fish were negative for viral agents. The results for the Mission Creek coho are summarized in Table 8 below.

Table 8 : Mission Creek Coho Results

Total Number sampled	15
No. of Males	8
No. of Females	6
No. Jacks	1
Other	0

Bacterial agents found

(#positive/# tested)

<u>ELISA</u>	<u>Culture</u>	<u>Kidney Smear</u>	<u>Gut Smear</u>	<u>Viral Agents Detected</u>	<u>Parasites Spec-tiss-infestation</u>
1/15-H	1/15-A.s. 1/15-Spor-Ra 1/15-Loma-Ra	4/15-Spor-Ra 3/15-Spor-Li 6/15-Spor-M 3/15-Spor-H 1/15-Myx-Ra 3/15-Myx-Li 1/15-Myx-M	0/13 2/15-NA	0/15	1/1-unidentified kidney tubule parasite

Toboggan Creek Coho

The Toboggan Creek coho sample consisted of 9 females, 9 males and 2 jacks and 9 of the 20 coho sampled were adipose clipped hatchery coho. Sampling occurred on October 15th, 2003 and all coho were in post-spawn condition.

External examination showed no gill or other visible external parasites. Internal examination showed 7 of the 20 fish sampled as having internal parasites. However, parasite infestation was very light with parasites mostly being concentrated around the digestive system organs.

The BKD ELISA results showed that 19 fish were negative and 1 coho was a moderate positive for BKD. All bacterial cultures were negative and all virus results were negative. Results for Toboggan Creek coho are summarized in Table 9 below.

Table 9 :Toboggan Creek Coho Results

Total Number sampled	20
No. of Males	9
No. of Females	9
No. Jacks	2
Other	0

Bacterial agents found

(#positive/# tested)

ELISA	Culture	Kidney Smear	Gut Smear	Viral Agents Detected	Parasites Spec-tiss-infestation
1/20-M	0/20	1/20-Spor-Ra 7/20-Spor-Li 5/20-Spor-M 1/20-Spor-H 7/20-Loma-Ra 2/20-Loma-Li 1/20-Myx 1/20-Henneguya 1/20-Philonema larva	1/19-Henneguya 1/20-NA	0/20	2/2-unidentified kidney tubule parasite

3.7 Histopathology Results (Prepared and Reported by Janice Blackburn – PBS)

Following are the results of the histopathological examination of the tissue samples submitted to the Pacific Biological Station (PBS) by Brenda Donas in October 2003. These samples were collected from several locations and from several species of wild salmon adults as shown in Table 10.

Macro parasites were also collected and included with the samples. Nematode larvae (roundworms) were found in a pink (fish #30) in the Bulkley River collection, in sockeye (fish #1 and #15) collected from the Babine River and in a coho (fish #15) sampled from Mission Creek. Larval nematodes (probably *Anisakis sp.*) are relatively non-pathogenic to salmon and are commonly found in wild fish. The salmon is an intermediate host, mammals are the definitive host. (Kent, M.L and Poppe, T.T. 1998).

A single copepod (a relative of sea lice) was collected from the gills of a coho (fish #10) from Mission Creek. It has been identified as *Salmincola californiensis* (personal communication: T.E. McDonald). In very high numbers it can seriously reduce respiratory function. (Roberts, R.J. 2001).

A skin lesion was submitted for examination from a coho (fish #1) collected from the Upper Bulkley River. Histologically, an acute inflammatory lesion and developing granuloma was observed. The aetiological agent (cause) was not evident. In addition, tissue surrounding a wound from a sport caught coho (H03-297) was also submitted for histopathological examination. Opportunistic bacteria with associated necrosis and inflammation of the surrounding tissues was observed, but an aetiological agent was not apparent.

Table 10 lists the histopathology observed in tissue samples from each salmon submitted.

Table 10 Histopathological observations ^a in tissues sampled from several species of adult wild salmon collected from several locations in October 2003.

Location	Species	Fish #	Heart	Kidney (posterior)	Kidney (anterior)	Liver	Pyloric caeca Stomach	Spleen	PBS I.D. # ^b
Upper Bulkley River	Coho <i>Onchorhynchus kisutch</i>	1	-ve	Unidentified myxozoan in the lumina of renal tubules	-ve	Small focal patches of inflammatory cells	-ve	-ve	2003-233 H03-289
		15	-ve	CKX	-	-ve	Developing granulomas in the submucosa	-ve	
Bulkley River (Moricetown)	Pink <i>Onchorhynchus gorbuscha</i>	1	-ve	CKX	-ve	Early cirrhosis, small patch of fatty degeneration	-ve	-ve	2003-208 H03-291
		15	-ve	-ve	-	Focal patches of hepatocyte necrosis	Trematode and cestodes in lumen	-ve	
		30	-ve	CKX	-	-ve	-ve	-ve	
Babine River	Coho <i>Onchorhynchus kisutch</i>	1	-ve	CKX	-	Necrosis and hepatitis	-ve	-ve	2003-265 H03-290
		15	-ve	CKX	-ve	cirrhosis	-ve	-ve	
		Sockeye <i>Onchorhynchus nerka</i>	1	-ve	Unidentified myxozoan in the lumina of renal tubules	-ve	hepatitis	-	-ve

Table 10 (continued) Histopathological observations ^a in tissues sampled from several species of adult wild salmon collected from several locations in October 2003.									
Location	Species	Fish #	Heart	Kidney (posterior)	Kidney (anterior)	Liver	Pyloric caeca Stomach	Spleen	PBS I.D. # ^b
Babine River	Chinook <i>Onchorhynchus tshawytscha</i>	1	-ve	-	-	-ve	-ve	-	2003-239 H03-296
		15	-	-ve	-	-ve	-ve	-ve	
		30	-ve	-ve	-ve	-ve	-ve	-ve	
		45	-ve	-ve	-ve	-ve	-ve	-	
		60	-ve	-ve	-	cirrhosis	-ve	-ve	
Mission Creek	Coho <i>Onchorhynchus kisutch</i>	1	-ve	CKX	-	hepatitis	-ve	-ve	2003-249 H03-292
Tobbogan Creek	Coho <i>Onchorhynchus kisutch</i>	1	-ve	CKX	-ve	cirrhosis	-ve	-ve	2003-259 H03-293
		15	-ve	CKX	-	-ve	-ve	-ve	

a - : not submitted for examination
 -ve : negative (tissues appear unremarkable)
 CKX unknown coho salmon kidney parasite

b Pacific Biological Station identification number (diagnostics and histology)

HISTOPATHOLOGICAL OBSERVATIONS

Heart: The ventricle was submitted from some sampled fish, histologically there were no abnormalities noted in any cardiac tissues.

Kidney (posterior): Myxozoa are parasitic eukaryotic organisms that are classified by the structure of the spores. The unidentified myxozoan that were observed in renal tubule lumina of coho of the Upper Bulkley River, and a Babine River sockeye did not contain mature spores, which precluded classification. These organisms may be part of the life cycle of the unknown coho salmon kidney parasite (CKX) that was observed in all species of fish (and in all locations) collected except in the sample of Chinook collected from the Babine River. CKX is a pre-sporogonic stage of a myxozoan that is found within the epithelial cells of renal tubules. It was first noted in coho salmon (hence it's name) but has also been observed in other salmon species. CKX has not yet been given it's scientific name, currently, research is being conducted to determine it's effects, life cycle and relationships to other myxosporean parasites. CKX may cause extensive damage to infected kidneys.

Kidney (anterior): The anterior kidneys sampled did not exhibit any abnormalities.

Liver: 1) Several degenerative (necrosis, inflammation) changes were noted in the livers of the fish collected from every location. Some of these changes may be due to the life stage of the salmon collected. 2) Cirrhosis is a diffuse increase in the fibrous tissue of the liver, usually associated with chronic damage of the cellular components of the organ. The damage can result from a wide range of stimuli from longstanding biliary obstruction, heavy metal or pesticide poisoning, to chronic parasitism. The cirrhotic livers in wild fish are seen with little evidence for cause. (Roberts, R.J. 2001). 3) In hepatitis, numerous lymphocytes and leucocytes (white blood cells) are observed around the blood vessels in the liver. Degenerative changes are recognized in the neighbouring hepatocytes (liver cells) and may eventually progress to an increase of connective tissues in the affected area (Hibiya, T. 1982).

Pyloric caeca / Stomach: A single trematode (fluke) was observed in the lumen of a single pink collected from the Bulkley River (Morice town), it was surrounded by bacteria. Most adult flukes of fish infect the alimentary tract and seldom cause significant tissue damage. (Kent, M.L and Poppe, T.T. 1998)

Cestodes (tapeworms) are parasitic helminths usually found in the lumen of the gut. The cestodes observed were the larval stage of *Scolex pleuronectis* (pers. comm. T.E. McDonald). They were found in fish from all locations sampled except in the coho from Upper Bulkley River and the coho from the Babine River. Many species cause varying degrees of pathology (Roberts, R.J. 2001). Pathology was not associated with these cestodes.

Spleen: All the spleens sampled were unremarkable except that from a sockeye collected from the Babine River. It showed an increase in fat and connective tissues which may be a degenerative condition.

3.8 Heavy Metals Testing on Babine Sockeye and Babine Pink

Babine Sockeye were initially tested for heavy metals levels for a comparison with heavy metals levels in Lower Fraser River sockeye. This heavy metals testing was additional to this Baseline Fish Health study and was for the interest of Fish Pathologists at the Pacific Biological Station.

Initially, six Babine River sockeye were tested for heavy metals by taking liver samples. Analysis of those six sockeye showed that levels of some heavy metals were much higher than levels found in Lower Fraser River sockeye and were higher than the standard safe levels as listed at the bottom of Table 11. The heavy metals results for the initial six Babine sockeye are shown below in Table 11.

Table 11 : Heavy Metals Results for Babine Sockeye Sampled on July 23, 2003

Heavy Metals Results in PPM - Livers

Ca	Cd	Cu	Fe	Mg	Mn	Pb	Se	Zn
Calcium	Cadmium	Copper	Iron	Magnesium	Maganese	Lead	Selenium	Zinc
44	0.2	510.9	156	174	2.7	<2	21.22	48
43	1.4	910.2	49	194	3	<2	43.15	68
65	1.2	260.3	43	240	3	<2	15.23	39
49	0.4	380.4	108	187	2.4	<2	22.89	69
58	0.9	810.1	224	190	2.2	<2	24.7	90
45	0.6	680.6	155	185	2.3	<2	26.87	49

Safe Levels

40-80ppm	< 2 ppm	10-75 ppm	100-450 ppm	175-275ppm	1.2-3.2ppm	<2 ppm	1 - 3 ppm	30-60 ppm
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The levels of copper, selenium and cadmium were all above standard safe levels. This prompted further sampling and an additional six Babine sockeye were sampled on August 26th, 2003. Samples of liver, kidney, gill and eggs were taken for heavy metals analysis. These results are summarized in Tables 12a through 12d below.

Table 12a : Babine River Sockeye Heavy Metals Results from Liver Samples:

Aug 26, 2003

Ca	Cd	Cu	Fe	Magnesium	Maganese	Pb	Selenium	Zn
Calcium	Cadmium	Copper	Iron	Mg	Mn	Lead	Se	Zinc
54	2	873	101	208	2.5	<2	27.61	52
65	1.7	598	130	170	2.7	<2	13.28	47
88	0.9	66.8	172	260	2.6	<2	5.54	30
89	0.6	92.9	71	260	2.4	<2	5.25	32
62	0.5	703	172	204	2.2	<2	25.45	85
63	0.7	794	186	190	2.1	<2	34.07	91

40-80ppm	< 2 ppm	10-75 ppm	100-450 ppm	175-275ppm	1.2-3.2ppm	<2 ppm	1 - 3 ppm	30-60 ppm
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Table 12b : Babine River Sockeye Heavy Metals Results from Kidney Samples

Ca Calcium	Cd Cadmium	Cu Copper	Fe Iron	Magnesium Mg	Maganese Mn	Pb Lead	Zn Zinc
181	2.5	6	73	200	1.1	<2	76
96	1.2	2.8	72	154	0.7	<2	62
154	3.6	3.7	144	194	0.9	<2	51
144	1.7	2.9	92	183	0.9	<2	54
123	0.6	3.6	120	183	1	<2	63
170	0.9	7.1	76	211	2	<2	72
40- 80ppm	<.2 ppm	10-75 ppm	100-450 ppm	175- 275ppm	1.2- 3.2ppm	<2 ppm	30-60 ppm

Table 12c : Babine River Sockeye Heavy Metals Results from Gill Samples

Ca Calcium	Cd Cadmium	Cu Copper	Fe Iron	Magnesium Mg	Maganese Mn	Pb Lead	Zn Zinc
4600	<0.2	2.3	161	1370	2.4	<2	30
9200	<0.2	1.5	76	750	1.9	<2	25
7500	<0.2	1.5	92	800	1.8	<2	22
6800	<0.2	1.4	111	700	2.4	<2	23
11920	<0.2	2.1	176	810	4.1	<2	48
8460	<0.2	2.1	216	560	3.3	<2	39
40- 80ppm	<.2 ppm	10-75 ppm	100-450 ppm	175- 275ppm	1.2- 3.2ppm	<2 ppm	30-60 ppm

Table 12d : Babine River Sockeye Heavy Metals Results from Egg Samples

Ca Calcium	Cd Cadmium	Cu Copper	Fe Iron	Magnesium Mg	Maganese Mn	Pb Lead	Zn Zinc
492	<0.2	49.8	25	670	1	<2	41
519	<0.2	44.6	20	700	1.1	<2	30
40- 80ppm	<.2 ppm	10-75 ppm	100-450 ppm	175- 275ppm	1.2- 3.2ppm	<2 ppm	30-60 ppm

Results from the liver and kidney sampling conducted on August 26th, 2003 showed levels of cadmium and copper above the standard safe levels. It is interesting to note that copper was found in the eggs of the two female sockeye sampled.

To determine if other Babine salmon stocks would test positive for heavy metals, samples were taken from six pink salmon. The liver, kidney, gill and egg sample results are shown in Tables 13a through 13d below.

Table 13a : Babine River Pink Salmon Metals Results – Liver

<u>Se</u> <u>Selenium</u>	<u>Cu</u> <u>Copper</u>	<u>Zn</u> <u>Zinc</u>	<u>Fe</u> <u>Iron</u>	<u>Mn</u> <u>Maganese</u>	<u>Pb</u> <u>Lead</u>	<u>Cd</u> <u>Cadmium</u>	<u>Ca</u> <u>Calcium</u>	<u>Mg</u> <u>Magnesium</u>
1.32	78.3	131	302	2.2	< 2	0.7	90	221
1.5	48.7	90	240	1.7	< 2	0.6	62	197
1.59	96.4	60	234	1.7	< 2	0.4	66	202
1.96	177.2	108	239	1.7	< 2	0.7	61	213
1.79	89.1	91	142	1.5	<2	0.8	72	177
1.46	52.9	88	179	<0.3	<2	0.4	57	196
1 - 3 ppm	10-75ppm	30-60 ppm	100-450 ppm	1.2-3.2ppm	<2 ppm	<.2 ppm	40-80ppm	175-275ppm

Table 13b : Babine River Pink Salmon Metals Results – Kidney

<u>Se</u> <u>Selenium</u>	<u>Cu</u> <u>Copper</u>	<u>Zn</u> <u>Zinc</u>	<u>Fe</u> <u>Iron</u>	<u>Mn</u> <u>Maganese</u>	<u>Pb</u> <u>Lead</u>	<u>Cd</u> <u>Cadmium</u>	<u>Ca</u> <u>Calcium</u>	<u>Mg</u> <u>Magnesium</u>
NA	2.7	29	50	0.9	< 2	0.8	65	180
NA	2.4	34	43	0.8	< 2	0.8	81	168
NA	2.8	40	106	0.9	< 2	0.6	75	164
NA	2.9	26	64	0.6	< 2	0.6	84	149
	2	27	87	0.8	<2	0.8	71	152
	2.4	31	89	1	<2	0.7	101	157
1 - 3 ppm	10-75ppm	30-60 ppm	100-450 ppm	1.2-3.2ppm	<2 ppm	<.2 ppm	40-80ppm	175-275ppm

Table 13c : Babine River Pink Salmon Metals Results – Gill

<u>Se</u> <u>Selenium</u>	<u>Cu</u> <u>Copper</u>	<u>Zn</u> <u>Zinc</u>	<u>Fe</u> <u>Iron</u>	<u>Mn</u> <u>Maganese</u>	<u>Pb</u> <u>Lead</u>	<u>Cd</u> <u>Cadmium</u>	<u>Ca</u> <u>Calcium</u>	<u>Mg</u> <u>Magnesium</u>
NA	1.9	24	79	2.4	< 2	<0.2	9900	640
NA	1.6	26	85	2.5	< 2	<0.2	10070	680
NA	2	24	78	2.7	< 2	<0.2	9200	730
NA	<0.2	24	48	2.3	< 2	<0.2	8800	800
	1.8	20	60	3.3	<2	<0.2	12390	85
	1.4	19	69	2.1	<2	<0.2	11400	640
1 - 3 ppm	10-75ppm	30-60 ppm	100-450 ppm	1.2-3.2ppm	<2 ppm	<.2 ppm	40-80ppm	175-275ppm

Table 13d : Babine River Pink Salmon Metals Results – Egg

<u>Se</u> <u>Selenium</u>	<u>Cu</u> <u>Copper</u>	<u>Zn</u> <u>Zinc</u>	<u>Fe</u> <u>Iron</u>	<u>Mn</u> <u>Maganese</u>	<u>Pb</u> <u>Lead</u>	<u>Cd</u> <u>Cadmium</u>	<u>Ca</u> <u>Calcium</u>	<u>Mg</u> <u>Magnesium</u>
NA	7.1	24	25	1.4	< 2	<0.2	693	69
	6.2	20	24	1.9	<2	<0.2	455	520
	6	21	24	1.2	<2	<0.2	589	610
1 - 3 ppm	10-75ppm	30-60 ppm	100-450 ppm	1.2-3.2ppm	<2 ppm	<.2 ppm	40-80ppm	175-275ppm

The liver results for cadmium, copper and selenium for Babine sockeye and Babine pinks are included in Tables 14a and 14b below. The mean levels for Cadmium, Copper and Selenium in Sockeye livers are well above the standard safe levels. The mean levels for Pink livers are all within standard safe levels.

Table 14a: Babine Sockeye Metal Results Summary : Livers

Metal	Minimum Level(ppm)	Maximum Level(ppm)	Mean Level(ppm)	Safe Level ppm
Cadmium	0.2	2	0.928	<0.2
Copper	66.8	910.2	556.7	10-75
Selenium	2.25	43.15	22.11	1 - 3

Table 14b: Babine Pink Metal Results Summary : Livers

Metal	Minimum Level(ppm)	Maximum Level(ppm)	Mean Level(ppm)	Safe Level ppm
Cadmium	0.4	0.8	0.6	<0.2
Copper	48.7	177.2	90.4	10-75
Selenium	1.32	1.96	1.60	1 - 3

The stated safe levels of heavy metals in the organs cannot be related to safe levels for human consumption. Tissue sampling may be necessary to determine if residual levels of heavy metals warrant consumption warnings.

4.0 Discussion

This Baseline Fish Health monitoring program represents a snapshot of fish health for the Bulkley and Babine stocks sampled in this program. This snapshot does not provide a complete baseline of fish health as a one year sampling program does not consider year to year variation in fish health. However, as a pilot study, the feasibility of a monitoring program for fish health has been proven.

A total of 360 salmon adults were sampled for pathogens and parasites that are considered to impact salmon survival. The prevalence of BKD, Enteric Redmouth Disease, Furunculosis, IHN and nuisance parasites was low for all stocks and species. However where present, these disease agents can have negative impacts on individual fish, and if present in a hatchery can cause significant losses in the reared salmon populations even once they leave the hatchery (e.g. *Renibacterium salmoninarum* infections can influence seawater survival).

Background Information on the Pathogens

Bacterial Kidney Disease:

This is a bacterial infection that can affect the kidney, liver, spleen and heart of the fish. Commonly, the centre of infection is the kidney and as the infection develops, kidney function is impaired. The kidney cleans the blood and also produces red blood cells. The kidney is a crucial organ when changes in osmoregulation occur during the salmon smolting process. As a result of impaired kidney function, the abdominal cavity can become filled with fluid causing the belly of the fish to become distended.

Capillaries in the skin can burst giving the appearance of small bruises. Hemorrhaging may also occur at the base of the fins.

The causative agent of Bacterial Kidney Disease is *Renibacterium salmoninarum*. This bacteria can be transmitted vertically through the egg from the female to the progeny. Fry can already show clinical signs of the disease. Smolts can carry the infection to the salt water where survival can be compromised (Kent 1992). In a hatchery situation, it is important to screen the female broodstock for BKD where it is known to occur in the source population. This is usually done with the ELISA test.

Enteric Redmouth Disease:

This bacterial infection is characterized by inflammation of the jaws of salmonids. Often the stomach and intestine can become fluid-filled. Fish become lethargic and unable to feed. In hatchery rearing situations, fish to fish contact promotes spread of the disease.

Furunculosis:

This water born bacterium can enter the fish through a wound or through the digestive tract. In older fish, it may be characterized by “furuncles”, which are excavations containing blood tinged fluid, occurring in the flank muscle. These “furuncles” are not seen in all cases (Hicks 1989). The bacteria produce a toxin that destroys the ability of the fishes immune system to fight the disease. Internal bleeding results from capillary breakdown and blood and serum leak out into the surrounding tissues. The gill capillaries can also break down and the fish can bleed to death from the gills. In hatchery rearing situations, significant mortality can occur. Smolts infected with *Aeromonas salmonicida* (causative agent of Furunculosis) can carry the bacteria into the salt water (Kent 1992).

Infectious Hematopoietic Necrosis:

IHN is a viral disease that attacks the blood forming tissues within the kidney. This virus is strongly influenced by water temperature and is quite prolific at water temperatures near 9 to 10 degrees Celsius. It can be especially devastating to alevins of host species. It is easily transmitted by waterborne exposure and may also be vertically transmitted (Kent 1992). In BC, sockeye and kokanee are often carriers of this virus.

Myxosporidia :

Internal parasites such as Myxosporidia are not usually life threatening but pose an irritant to the fish and may leave the fish immunocompromised, and thus more vulnerable

to other infections. Parasites such as *Henneguya* can be found in the body or musculature of salmon rendering the fish less aesthetically desirable for human consumption.

Myxidium is a non life threatening internal parasite that can be found in the renal tubules of the kidney.

Loma:

Systemic infections of *Loma* sp. have caused high mortality in chinook, but infections in coho in fresh water are usually mild and cause little disease. Infections can persist after fish are transferred to sea water. Gills are the primary site of infection, but parasites and associated lesions can occur in the heart, spleen, kidney and pseudobranchs (Kent 1992).

Of the 360 fish sampled, 10 salmon or 3% of the salmon sampled, were positive for *Renibacterium salmoninarum*. The positive fish consisted of 8 coho salmon from the Bulkley and Babine watersheds, 1 Babine chinook and 1 fish was a Babine pink salmon. None of the salmon examined had obvious external symptoms of the disease.

A light infection of Enteric Redmouth disease was found in 1 of the 360 salmon examined. That fish had no obvious external symptoms of the disease.

Furunculosis was found in 9 salmon and infection levels were light to moderate. None of the salmon examined had obvious external symptoms of the disease.

Most of the salmon examined had some internal parasites in various life stages in the kidneys. None of the parasites were life threatening. Very few fish (2) had internal parasites present in the gut.

Babine River sockeye were the only stock and species examined that were diagnosed with IHN. There were 10 sockeye diagnosed with IHN and all were in post spawning condition and these fish were all examined late in the sockeye run. This is a very common finding in post-spawned sockeye.

Levels of heavy metals in the kidneys, livers, gills and eggs of the Babine sockeye were often above the stated safe limits for optimal fish health. However, it is unclear how the stated laboratory safe limits relate to safe limits for human consumption. Further research is required to determine what types of testing should occur to determine metals levels in tissue and how those levels relate to safe human consumption levels.

5.0 Recommendations

There are two main recommendations resulting from this program :

1. Repeat the Baseline Fish Health Monitoring Program in 2004 so that year to year variation in fish health can be documented and included in the baseline. Knowing the occurrence and amount of significant pathogens year to year gives a view of the health of the various stocks over time. A solid sampling history can result in educated management decisions being made with the health of the stocks as an obvious priority. Ongoing sampling will help to pinpoint fish health problems such as increases in significant pathogens. Detection of new pathogens (especially viruses) is also important.
2. Conduct further sampling on Babine River sockeye for heavy metals and determine the implications of heavy metals levels as they relate to safety levels for human consumption.

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