Discovery Passage Plankton Monitoring and Juvenile Salmon Assessment 2007

Table of Contents

Introduction

Coho salmon (*Oncorhynchus kisutch*) are an important sport fish on the Pacific Coast, which provide limited commercial fishing opportunities as well. The Quinsam River Salmon Hatchery, along with other Fisheries and Oceans Canada (DFO) facilities, time the release of coho smolts using guidelines established in the early 1980's. These procedures are based on work that found survival rates for area coho were best when released near the third week of May at a size of 20-25g (Bilton et al 1984). However, over the past decade survival rates of both wild and hatchery released coho smolts have been decreasing, from highs in the 1980's of 10%, to more recent trends barely over 1%. It is believed that many factors are contributing to this decline including changes to the magnitude and timing of ocean productivity which may be related to global climate change trends.

Dr. R. Beamish of Fisheries and Oceans Canada (personal communications) suggests there is a relationship between the abundance levels of coho juveniles found in the Strait of Georgia in July and the resulting return of adults from that brood year. His catch sampling also indicates that growth, to a minimum size (nose-fork length), achieved by the summer solstice is critical to juvenile coho survival and migration out of Johnstone Strait. Dr. Ron Tanasichuk, Fisheries and Oceans Canada, has found similar results on the west coast of Vancouver Island (2002). Feed type and abundance in the spring marine environment plays a primary role in ensuring this essential growth.

Hatchery release programs in Alaska have historically used plankton abundance as a guide for timing releases of hatchery reared pink and chum salmon. The Quinsam Plankton Monitoring project focused on developing a program that would address the objectives of monitoring plankton productivity, evaluating nearshore coho diet components, assessing coho health parameters and establishing sampling protocols that would be effective and efficient in producing useful data. This program looked at the habitat in the near shore areas to determine through plankton surveys the productivity faced by out-migrating smolts. Additionally, the program assessed early growth information from April to June and diet components that can potentially be related back to the plankton data.

The information from this project will be shared with the staff at DFO's Quinsam River Salmon Hatchery allowing them the opportunity to adjust release schedules for coho smolts if conditions indicate a shift in plankton production. The success of this program will be measured by determining the survival of returning adult coho salmon to the hatchery through the retrieval of coded wire tag (CWT) data.

Methods and Materials

All plankton sampling was scheduled biweekly and was usually performed after dusk. Figure 1 shows sites for both plankton sampling and beach seines.

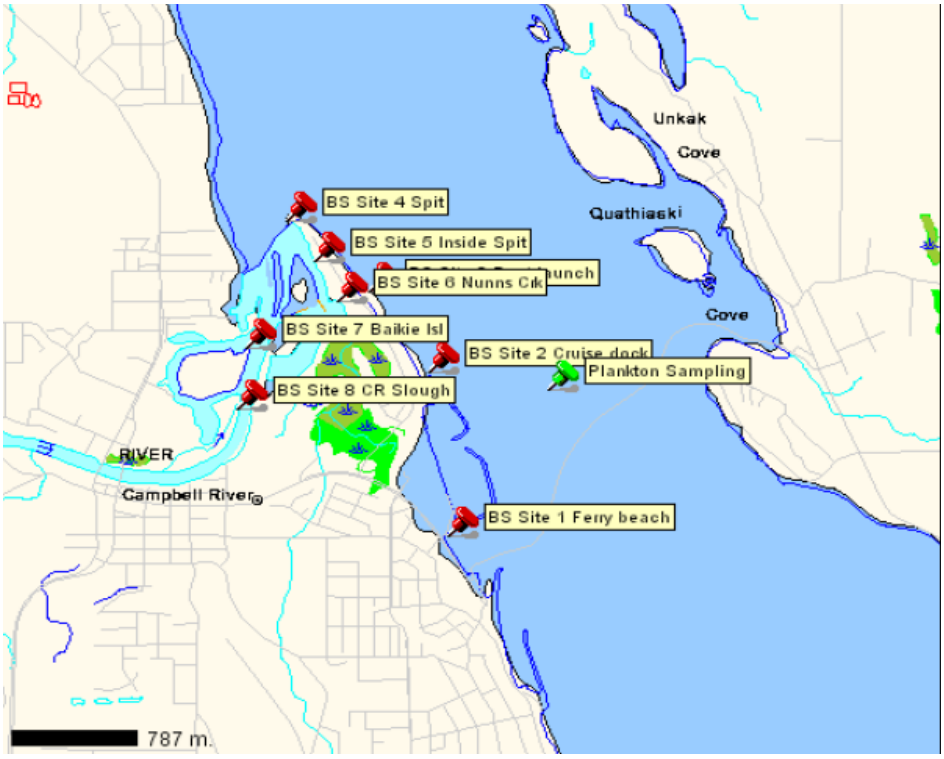

Figure 1 Beach seine (BS) and Plankton sampling sites- Discovery Passage, Campbell River 2007

Environmental

Water Quality – temperature, salinity and dissolved oxygen.

An YSI 85 meter (Dynamic Aqua-Supply, Vancouver, BC) was used to measure dissolved oxygen (mg/L), salinity (parts per thousand, ppt), and temperature (º C) profiles from the surface every meter to a depth of 10m. A weight was attached to the probe end to create as vertical a profile as possible. The tidal waters of Discovery Passage have very fast and complex currents that made absolute vertical sampling very difficult, even during slack tides.

Plankton Sampling

Phytoplankton and Chlorophyll *a*

Discrete water samples were taken with the LaMotte water sampler (Dynamic Aqua-Supply, Vancouver, BC) at 1m, 5m and 10m depth. The sampler was sent down open to the sampling depth, then the messenger was sent down the line to trip the closing device and the 1 litre water sample was brought back to the surface.

From each 1'LT sample, chlorophyll *a* samples were taken by filtering the sample using a syringe and filter system (Appendix 1). The filter paper was stored in the dark and taken to the lab for processing chlorophyll *a* and phaeopigments (products of chlorophyll degradation found within algal cells).

From each 1'LT sample, approximately 125ml of sample was put into a 150ml sample container and 10-12 drops of Lugol's solution added to preserve the sample.

Zooplankton

Zooplankton was collected with a 0.5m diameter by 2.0m long 250μm conical plankton net mounted on a fixed metal frame with a removable, weighted cod-end sample container. Sampling was performed on most occasions after dusk to allow sampling of the upwardly migrating zooplankton. Three replicates were obtained on each trip. Total volume of seawater sampled was approximately 3.9m^3/haul.

The net was lowered to 20m depth and pulled up at a steady 1m per second rate to the surface. A small boom with block and hydraulic winch was set up on the side of the boat to allow consistent retrieving of the net. The net was kept as vertical as possible in the water and the boat was maneuvered to maintain the orientation. Plankton adhering to the net was rinsed off by immersing the net several times in seawater up to the opening. A top to bottom rinse concentrated the plankton into the cod-end. Figure 2 shows zooplankton sampling.
The three samples were collected in labeled 250ml containers and preserved with 20ml of 37% formalin. The cod-end was rinsed with filtered seawater. The volume of water was filled to the 250ml mark. If the sample took up more than 1/3 of the sample container, the sample was divided between additional containers.
The net and collection material was rinsed with freshwater after each use. Samples were transported to the lab at the BC Centre for Aquatic Health Sciences (BCCAHS) the next morning for processing.

Figure 2 Plankton net sampling, March 2007

Figure 3. Beach seine crew reloading net onto boat. Site 2

Beach seining

A number of sites in close proximity to the plankton sampling area were sampled by beach seine to assess the availability of coho juveniles for stomach analysis and health testing (Figure 3). Additionally, sites within the Campbell River estuary were sampled to determine the temporal and spatial distribution of coho juveniles, as an indicator of when and if there were still coho moving through the system. Sampling started May 9 th and ended on June 27, 2007. A 5.5m (18ft) Alumaweld boat powered by an 80 hp jet drive was used for beach seining.

The net was 13.5m long and 2.9m deep, consisting of 3 sections: two outer 4.5m wings of 1cm stretch mesh and the 4.6m centre bunt section of 0.6cm stretch mesh. Bridles were attached to the net gable end system with ropes marked off at 100m. The net was pulled off the boat by a crew on the beach, secured to a tow pole on the boat and set in a horseshoe shape to sample an area of 100 m^2. The 2 person boat crew pulled the line to the beach and the net was pulled slowly and evenly to shore by both crews.

Three sites in the marine near-shore (Fig 1, site 1-3) were sampled, all catch was enumerated and juvenile salmon species and origin (hatchery, wild or indeterminate) identified. Beach seines were repeated at the same site or in the immediate vicinity if few fish, or insufficient numbers of coho, were captured on the first set. Nearly all hatchery-origin coho had their adipose fin clipped. A small percentage of hatchery origin fish, with or without adipose clipping, had CWTs applied.) If catches were very large, a subsample of at least 100 was taken. The remaining

number was estimated by counting the number of dip net releases into the ocean. This estimate was added to the subsample number to approximate the total catch number.) Sites were sampled at a number of different tide phases. Samples were placed in labeled Ziploc bags, stored in a cooler, and transported to the lab.

Figure 4 Beach seine sampling within the Campbell River estuary. Site 8

Laboratory analysis

Zooplankton

The preserved plankton net samples were poured into 250ml graduated cylinders and allowed to settle for 1-2 hours. The total biomass of plankton was estimated using the settled volume, reported as the height in millimeters measured from the cylinder bottom. The volume was then recorded as millimeters of plankton per cubic meter of seawater filtered through the net.

A subsample was prepared by splitting the original sample using a Folsom Plankton Splitter (Aquatic Research Instruments, Idaho). This allowed the sample to be divided evenly into workable subunits for identification and counting. Accurate identification and counting of zooplankton utilized a good quality binocular microscope or an inverted microscope on lowest power. The split sample was concentrated and placed in a plankton counter. The top five occurring zooplankton groups (were identified (usually to Order) and counted; the resulting split number was used to calculate the number of each group in the total sample (recorded as#/m^3).

Phytoplankton

A 1ml subsample was removed from the 125ml phytoplankton sample. Using a Sedgwick-Rafter cell, all phytoplankton species were counted and recorded on a standardized sample analysis form (Appendix 2). The percentage of diatoms, dinoflagellates, flagellates, etc. was determined and the counts applied to the sample and reported in #cells/ml.

Chlorophyll a

The chlorophyll filter samples were all stored in the freezer (at -18 ºC) until the completion of sampling. Once collected, all samples were sent to Department of Fisheries and Oceans Institute of Ocean Science in Victoria for analysis.

Fish sampling

All coho were visually inspected to assess physical appearance (i.e. normal vs. abnormal). Each coho was weighed and the nose to fork length measured. Fish from the final 3 of 6 of coho sampling events were scanned using a CWT tag detector to ensure the origin designation was correct. Scanning was put in place when it was realized that not all hatchery fish were adipose clipped.

Coho stomach analysis –

Stomachs were excised and placed in 125 ml of 10% formalin. Separate pools of wild and hatchery coho stomachs were prepared for each seine date. All samples were sent to Al Hirst of Jensyd Bio Tech Ltd (Nanaimo,BC) for enumeration and identification of contents. The report included values for % fullness and % digestion as well as identification and count of each species or group of zooplankton.

Samples were also collected to determine fish health status. The kidney was removed from each fish and put into individual bags for testing for *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD), using enzyme-linked immunosorbent assay (ELISA) (Appendix 3). Gills were excised and fixed in 10% formalin for future examination of infection by ***Loma salmonae***, a gill intracellular microsporidian parasite.

Results

Between February 21, 2007 and July 21, 2007, 34 plankton sampling trips were completed in Discovery Passage near Campbell River. Sampling was done weekly until mid-March and then biweekly thereafter. Beach seining to sample juvenile salmonids in the nearshore marine habitat, as well as the Campbell River estuary, was done 7 times between May 9 and June 27. Table 1 provides a summary of the sampling dates.

Table 1 Plankton sampling and beach seining for juvenile salmon sampling dates in 2007

Date	February	March	April	May	June	July
Plankton sampling	21	2,9,14, 20, 28	2,5,9,12,16,19,23,30	3,7,10,13,17,21,24,28,31	4,7,11,14,18,21,25,28	3,9,16

Beach seine				9,23,30	8,13,22,27	

The majority (86%) of the plankton sampling (29 trips) occurred at night, between 8 and 10 p.m. The first 5 trips (14%) were conducted during daylight (between 11 a.m. and 6 p.m.) to enable the researchers to assess equipment and methods. There was no bias towards tides during plankton sampling: 44% of sampling occurred during slack tide, 32% during ebb and 23% during flood.

Environmental data collected is summarized in Figure 5. There was a trend towards increasing water temperatures and decreasing salinities over the sampling period (February to July) whereas the dissolved oxygen levels did not change during this period. There was no significant difference in water temperature (p=0.91) and dissolved oxygen (p=0.72) measurement at the three water depths (1m, 5m and 10m) and with the exception of two sampling dates, one in April and one in May, there was no significant differences in salinity (p=0.12). The evaluation of the phytoplankton samples revealed little difference between the phytoplankton concentrations (cells/ml) at the 1m, 5m, or 10m vertical grab samples (Figure 6). This indicates that the water column in the sampling region is well mixed, not stratified. Diatoms were the dominant phytoplankton group in all samples.

Figure 5 Salinity (S), dissolved oxygen (DO) and temperature measurements at 1, 5 and 10m.

Figure 6 Discrete phytoplankton samples from 1, 5 and 10m indicating well mixed waters

Two main phytoplankton blooms were captured in the discrete samples, one in mid-May and another in mid-June, with the chlorophyll/phaeopigment analysis indicating an additional spike mid-April. Major *Noctiluca scintillans* blooms in the region were captured in the zooplankton tows; however, these same blooms were not observed in any of the discrete phytoplankton samples. Phytoplankton densities (measured at 1m) were compared with results from chlorophyll *a* and phaeopigments (Figure 7). The correlation between phytoplankton concentration and chlorophyll *a* is only low to moderate (r=0.53 p=0.002) whereas correlation between chlorophyll *a* and phaeopigment is moderate to high (r=0.77 p=0.001). There was poor correlation between phytoplankton concentration and its degradation product, phaeopigment (r=0.20 p=0.30).

Figure 8 shows the relationship of phytoplankton and zooplankton density for each sample date. The zooplankton densities appeared to increase in conjunction with phytoplankton levels as measured by chlorophyll *a.* Figure 9 shows a breakdown of the top five zooplankton groups found at each sampling date. Cirripedians (barnacle larvae) as well as calanoids and cycloids (both copepods) were the dominant zooplankton in the tows throughout the sampling period.

Figure 7 Comparison of discrete plankton sample counts (cells/ml) and chlorophyll a /phaeopigment analysis (μ/l)

Figure 8 Comparison by date of plankton and zooplankton densities 2007

Figure 9 Relative densities of zooplankton species over the 2007 sampling season

During the initial and final beach seines on May 9 and June 27 respectively, no coho salmon were captured in the near shore salt water; however, the in-between trips - May 23, 30, June 8, 13, and 22nd- saw many coho salmon caught. The highest densities of coho juveniles were observed during the May 30 sampling. Chum and pink were predominant in the early sets. Chinook, comprising mainly of hatchery sea-pen and river releases, were also caught. Figure 10 and 11 show the average weight and length of the sampled hatchery and wild coho. Hatchery fish were larger than their wild counterparts and this was expected as they are fed a constant diet and expend less energy searching for food. The last beach seine in which coho were captured (June 22 included a very large wild coho. This individual increased the average weight for that group.

The stomach contents from sampled coho juveniles are summarized in Table 2. Only 9% of the stomachs were empty indicating that the majority of the fish, wild and hatchery, were actively feeding. There appears to be a difference in target prey items between the juvenile hatchery and wild coho salmon with the hatchery salmon targeting primarily cyclopoids, amphipods and polychaetes. Cyclopoids become dominant in the water samples around the middle of April and increase in numbers through to the middle of June. Conversely, the wild coho appeared to consume large numbers of amphipods and euphasiids neither of which was found to be dominant in the zooplankton tows. Small fish, most likely juvenile salmonids (pink or chum salmon), were also observed as a significant food item for hatchery and wild coho. Pink and chum juveniles were more prevalent in wild coho stomachs during the earlier seines. The digestion of the stomach contents was generally quite advanced so there is the possibility that larger animals made up a larger portion of the sample because they were digested last. Ninety-five (95) kidney samples were analyzed using ELISA. Based on DFO cut-off levels, 93% were BKD negative with the remaining 7% low positive for BKD. All fish tested were below levels set by DFO for release (less than 0.4 optical densities).

Table 2 Dominant stomach contents for wild and hatchery origin coho juveniles

	Wild						Hatchery				
	23-May	30-May	8-Jun	13-Jun	22-Jun		23-May	30-May	8-Jun	13-Jun	22-Jun
Amphipod	53%	28%	39%	73%	26%		33%	14%	9%	64%	3%
Polychaete	7%	14%	0%	0%	55%		0%	0%	22%	16%	17%
Decapod	7%	0%	11%	1%	2%		3%	20%	13%	16%	0%
Insect	0%	3%	0%	22%	3%		3%	0%	3%	4%	0%
cyclopoid	0%	0%	0%	2%	12%		40%	63%	46%	0%	39%
euphasiid	0%	52%	17%	0%	0%		0%	1%	0%	0%	0%
cumacea	0%	1%	0%	1%	0%		0%	0%	3%	0%	0%
cirripedia	0%	0%	0%	0%	3%		3%	0%	1%	0%	38%
Fish	33%	3%	33%	1%	0%		17%	2%	4%	0%	2%

Figure 10 Comparative weights of wild and hatchery origin coho sampled

Figure 11 Comparative lengths of wild and hatchery origin coho sampled

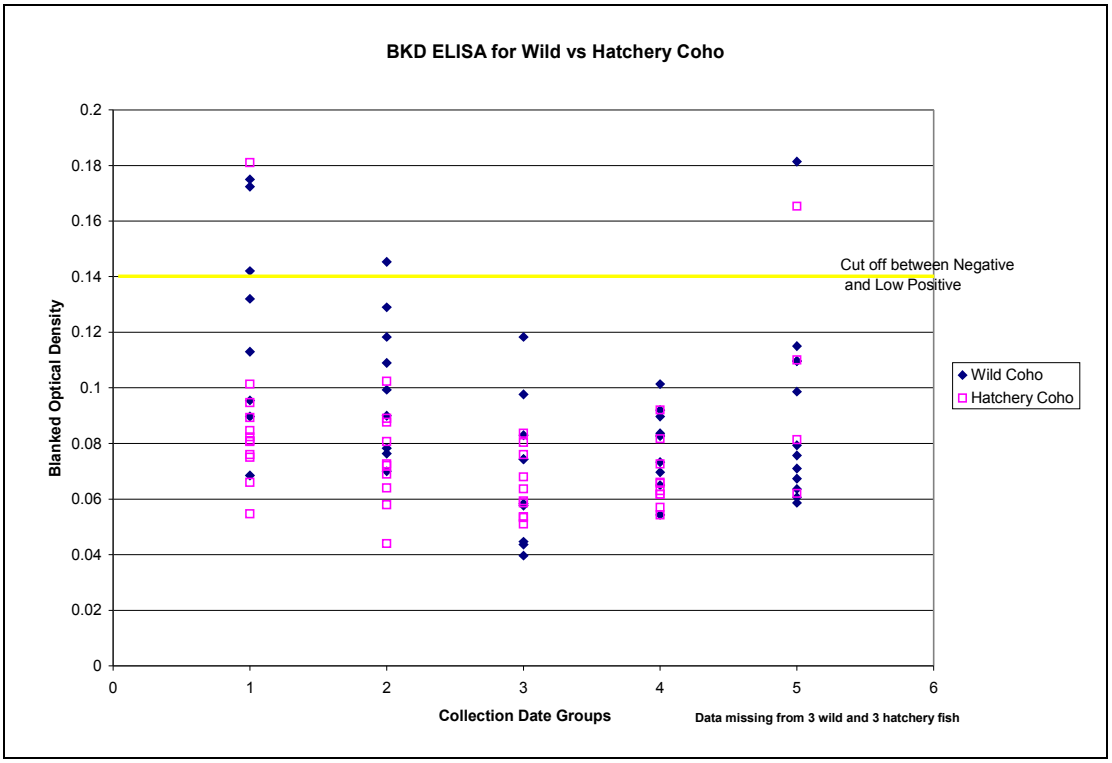

Figure 15 Bacterial Kidney Disease Screening results (ELISA test)

Discussion

This project is a cooperative pilot initiative between the BC Centre for Aquatic Health Sciences and Fisheries and Oceans Canada (DFO) to establish a plankton monitoring program for the local area, specifically Discovery Passage (the water body immediately adjacent to the Campbell River) and the near shore ocean habitats encountered by out-migrating juveniles from Quinsam River Salmon Hatchery. This area is also a major migration route of many of the lower eastern Vancouver Island and BC interior salmonid stocks. Data on ocean conditions from February to July 2007, including phytoplankton and zooplankton densities, environmental conditions, juvenile salmon diet, timing and distribution have been summarized.

The project involved developing a routine sampling program that gathered data, summarized it and identified trends in bloom cycles. The phytoplankton data showed two main phytoplankton blooms, one in mid-May and the other in mid-June. Chlorophyll *a* and phaeopigment levels showed an additional spike in mid-April. Phytoplankton densities were compared with the chlorophyll *a* and phaeopigment levels and were not found to correlate highly with one another. However, increases in chlorophyll *a* levels were followed by increases in zooplankton density indicating a possible relationship between phytoplankton and zooplankton levels. Zooplankton densities increased fairly steadily from mid-April, peaked around May 21st, and rapidly decreased until June 14th. The phytoplankton levels dropped off dramatically during this zooplankton peak. The second phytoplankton bloom in the 3rd week of June was followed by a quick spike in zooplankton productivity. During the sampling period, two large *Noctiluca scintillans* blooms were seen in the zooplankton tows but not captured in the phytoplankton samples. The data indicates that zooplankton densities increase in conjunction with increasing

phytoplankton density. It is possible that any lag in increase of zooplankton numbers would have been missed due to the frequency of sampling (2 times per week).

A chlorophyll meter will be purchased ((e.g. 6025 Chlorophyll sensor) which will permit for real-time measurement of chlorophyll *a* and provide the flexibility to modify the monitoring frequency. Our evidence suggests that discrete sampling at specific depths may not be the most appropriate method to assess phytoplankton. Plans are to replace discrete sampling with vertical phytoplankton net tows next year. For example, samples from 1m, 5m and 10m showed no appreciable difference in density or community; furthermore, these tows missed two large *Noctiluca scintillans* blooms which were seen in the zooplankton samples and were visually apparent by the orange-coloured water in the area. *Noctiluca scintillans* is a zooplankton food source that, as a dinoflagellate, has no chloroplast. Thus, even though this species contributes to zooplankton nutrition, its impact as a food source can not be assessed by measuring chlorophyll levels.

Cyclopoids, a dominant species observed in the zooplankton samples, were a significant food type in the hatchery reared coho diet. Amphipods and euphasids were important in both wild and hatchery diets, but neither were dominant in the zooplankton samples. This could be a result of the zooplankton sampling method and/or the sampling gear. Alternatively, it could indicate that the coho actively hunt for these prey items even when they do not make up a large part of the zooplankton fauna. Another factor to consider is the difference between the beach seining and plankton sampling environments. In an attempt to address these concerns, the next phase of the project will incorporate oblique tows using a Bongo net which will permit zooplankton sampling closer to shore and will provide a larger capture diameter. This change will attempt to reduce the capture-avoidance behaviour of larger zooplankton. Sampling times will change so that sampling occurs during day-light hours. Although many zooplankton species are known to have vertical migration patterns (moving up the water column at night), it is believed that the effects will be minimized by sampling the entire depth of the water column. The new area sample depth will be 14 to 16m at low tide.

Results from the pilot project which examined the marine conditions in the spring of 2007 indicate that this year's mid-May release date for the coho was optimal. This will be verified through the assessment of jack returns in fall 2007 (jack coho survival is a reliable indicator of brood year strength) as well as the adult returns of Quinsam's coho release groups. The adult return data will be available in late fall 2008 and will be assessed relative to historical returns as well as returns to other regions.

Changes to be made for the next phase of the Quinsam Plankton Project are summarized in Appendix 4.

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Appendices

Appendix 1

Chlorophyll sampling protocol

- Fill the 60mL syringe from the bucket.
- Compress plunger until 50mL of water remains and there are no bubbles in the syringe.
- Screw the Swinnex filter holder marked "1m" onto the syringe.
- SLOWLY squeeze the water out through the filter (~15 seconds per 50mL), discarding the water.
- Remove the filter holder from the syringe and check for colour. Circle 50mL on the data sheet for the 1m depth.
- If no colour is apparent on the filter repeat steps above (circling 50mL more on the data sheet each time) until some colour is apparent or 250mL has been filtered. Mark total amount filtered on 1m chlorophyll sample bottle (30mL).
- Unscrew Swinnex filter holder, and, with forceps, carefully fold filter and place in Chlorophyll sample bottle. (Note: this step can be done indoors later if weather is wet or windy). Try to keep filter paper in the dark i.e. store in black bag in freezer

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Appendix 2

Plankton identification form

Appendix 3

Bacterial Kidney Disease (BKD) Sampling – Elisa Protocol

Sample Preparation

Kidney Samples were diluted 8 times with PBST then crushed until an even mixture was obtained. The samples were transferred to a 2mL micro-tube, boiled for 15 minutes at 100°C and finally centrifuged at 1100 rpm for 5 minutes.

ELISA Assay

The protocol for *Renibacterium salmoninarum* was obtained from the Fish Health Section Blue Book 2005 Edition. The ELISA plates were coated with the capture antibody at a concentration of 1:1500 using Kirkegaard & Perry Laboratiories prepared Affinity purified antibody, goat anti-*Renibacterium salmoninarium* (Lot # 030852). The capture antibody was left overnight and washed with PBST in the morning. Samples were added to the ELISA plate according to the Pacific Biological Station Plate Layout. Blank, substrate, conjugate, negative and positive controls were included on the ELISA plate. Blank Controls were plated using PBST in the place of sample, substrate controls had no primary

or secondary antibody in those wells, and conjugate control wells contained only secondary antibody. Negative controls were obtained from the Pacific Biological Station (PBS) and were pooled coho samples that had previously tested negative according to the PBS cutoff values. *Renibacterium salmoninarum* positive control (Lot # 040381) prepared by Kirkegaard & Perry Laboratories was plated at four different concentrations 1:2000, 1:6000, 1:8000, and 1:12000. The samples and controls were incubated at room temperature for 3 hours and then washed with PBST. Kirkegaarde & Perry Laboratories Affinity purified antibody, goat anti-*Renibacterium salmoninarum* secondary antibody (Lot # 040520) was mixed with 5% milk diluent (Lot # 042093) and plated at a concentration of 1:2000 in all wells, except the substrate control, and incubated at room temperature for 2 hours. The plate was then washed with PBST and a 50:50 mixture of ABTS Peroxidase Substrate A (Lot # 050361) and B (Lot # 050097) was added to the plate before incubating for 20 min at 37°C. 5% Stop solution was added to all wells and the plates were read at 405 nm using a PowerWave XS plate reader.

Cut off values for Coho ELISAs obtained from PBS (blanked data)

Negative - <0.14 (eggs are kept and fry are released)
Low Positive - 0.14-0.4 (eggs are kept and fry are released)
Moderate Positive – 0.4-0.6 (Out plant eyed eggs)
High Positive - >0.6 (Eggs are destroyed)

Appendix 4

Modifications to 2008 program:

1. Look at day vs. night sampling. Analyze changes in zooplankton occurrence between the two different sampling times and sampling techniques – vertical vs. oblique, slack vs. mid-tide.

2. Do assessment of catch by vertical haul 20m (as per 2007) and Bongo net tows to determine differences in samples obtained. Each net could be of a different size mesh (for example, 200µm and 300µm) to give a result that may be a more complete picture of what is in the water. The oblique angle of the tow gives a more complete sample of zooplankton. This would result in more technologist time for analysis.

3. Initiate a phytoplankton net sample – either oblique or vertical 50μm mesh to 5m depth to give better illustration of phytoplankton community Discrete depth sampling yielded very low densities of phytoplankton.

4. Add to the zooplankton analysis by determining density of the top five zooplankton groups found in stomach samples from 2007.

5. Continue seining for collection and analysis of stomach contents. Keep stomachs separate and tied to specific fish health, size info.

6. Expand sample to include Chinook stomachs, and, if indicated, chum and pink to integrate link between zooplankton and coho/Chinook growth -2007 data indicate that chum and pink are important components of the coho diet; therefore, samples should be collected to analyze the productivity of this food source).

7. Determine an Index of Relative Importance for stomach contents to ensure the volume, and not just the number, of prey species is quantified.

8. Continue phytoplankton sampling using discrete sampler at 5m only and continue taking chlorophyll samples.

9. Purchase Chlorophyll meter to get real time data that can be correlated to phytoplankton and zooplankton sampling.

10. Continue to take kidney samples for ELISA and take gill samples for *Loma* analysis.

11. Provide zooplankton densities to Quinsam hatchery within 48 hours of sample collection.