Discovery Passage Plankton Monitoring and Juvenile Salmon Assessment 2008

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Summary

Spring 2008 was the second year of the Discovery Passage Plankton Monitoring Program. As a result of the first years results (available on www.cahs-bc.ca website), refinements and improvements were made to the sampling protocols and analysis in 2008. A Bongo net was purchased this year to bring the sampling protocol in line with zooplankton sampling techniques used worldwide.

In 2008, productivity was delayed in the spring as compared to 2007. This occurrence was seen up and down the coast of the coast of BC. The water temperatures in Discovery Passage remained below 10 C until late May. The phytoplankton blooms occurred later and there were fewer blooms compared to 2007.The zooplankton bloom occurred very soon after the initial phytoplankton bloom but did not bloom again with similar densities. Phytoplankton levels were correlated to chlorophyll a measurements suggesting that chlorophyll a might be a useful surrogate for predicting phytoplankton blooms.

Quinsam Hatchery coho smolt releases coincided with the increase in plankton productivity seen in the marine environment. The beach seining resulted in fewer coho (both enhanced and wild) captured than in 2007. Both the wild and hatchery reared coho were smaller and more variable in weights than coho sampled in 2007. Stomach analysis showed that the coho salmon were feeding on large amounts of smaller salmonid species (i.e. pink and chum salmon). It is beyond the scope of this project to conjecture as to why this is but does raise some interesting questions about how food availability affects the feeding behaviour and growth of juvenile coho, and how the prey species (the smaller salmonids) are affected by later productivity.

The results from the coho jack returns in 2008 indicate that the hatchery reared coho smolts released during a zooplankton bloom in 2007 had 2 times higher survival levels than those released outside the bloom period. Based on these preliminary findings, the information available to the hatchery on productivity in the marine environment could be invaluable for the decision making process in determining optimal release periods for the hatchery reared coho. Fall 2009 jack returns data will be used to provide an indication as to the survival of this year's hatchery reared coho and to see whether the pattern observed in 2008 is repeated.

Introduction

Coho salmon (*Oncorhynchus kisutch*) are an important sport fish on the Pacific Coast, and also provide limited commercial fishing opportunities. The Quinsam River Salmon Hatchery, along with other Fisheries and Oceans Canada (DFO) facilities, time the release enhanced of coho smolts according to guidelines established in the early 1980's. These procedures are based on observations that 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). At the Quinsam Hatchery, (Campbell River, BC), and other salmon hatcheries that border the Strait of Georgia, recent smolt to adult survival for coho have been approximately 1%, down from the 8-10% in the 1980's, when these guidelines were first established. It is believed that many factors are contributing to this decline including changes in the magnitude and timing of ocean productivity which may be related to modification in global climate. In the past decade, the temporal patterns of the plankton blooms in the Strait of Georgia have changed, perhaps as a result of changes in climate and /or in ocean currents. As a result of these variations, it is suspected that there is a mismatch between release of smolts and the plankton blooms they rely on as a primary food source.

Dr. R. Beamish of Fisheries and Oceans Canada (personal communications) suggests there is a strong correlation between the abundance of coho juveniles found in the Strait of Georgia in early summer and the corresponding return of this population as adults. His research suggests that survival of the juvenile coho salmon is tied to the fish reaching a critical size (nose-fork length), by the summer solstice. Dr. Ron Tanasichuk of DFO, in his studies on the west coast of Vancouver Island (2002) suggests that feed type, particularly zooplankton, and its abundance during Spring plays a primary role in ensuring this early growth in the marine environment.

Hatchery release programs in Alaska have historically used plankton abundance as a guide for timing releases of hatchery reared pink and chum salmon. The Discovery Passage Plankton Monitoring project has focused on developing a program to monitor plankton productivity, and examine the diets of coho captured in the near-shore marine environment. The key of this program is to develop and assess a monitoring program that could assist Quinsam Hatchery in optimizing coho survival by timing releases with favourable marine food availability (marine productivity). This project has now completed its second year where phytoplankton and zooplankton surveys were conducted, to assess productivity faced by out-migrating smolts. The program sampled salmon to collect early growth and diet information for juvenile coho salmon during the spring. Fish specific information was related back to the plankton data to establish what the juveniles are eating when they exited the estuary and enter the near-shore marine environment.

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

Methods and Materials

All plankton sampling was scheduled biweekly and was during daylight hours. Figure 1 shows sites for both plankton sampling and beach seines.

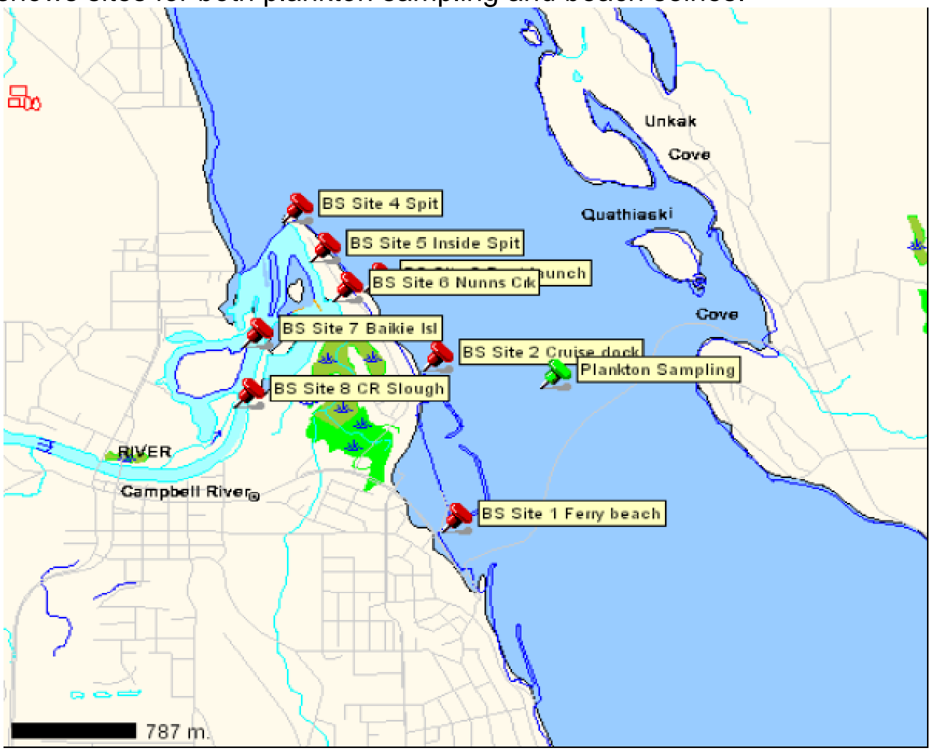

Figure 1 Beach seine (BS) and Plankton sampling sites- Discovery Passage, Campbell River 2007

Environmental

Water Quality – temperature, salinity and dissolved oxygen.

A YSI 85 meter (Dynamic Aqua-Supply, Vancouver, BC) was used to measure dissolved oxygen (mg/L), salinity (parts per thousand, ppt), and temperature (° 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 *α*

Discrete water samples were taken with the LaMotte water sampler (Dynamic Aqua-Supply, Vancouver, BC) at 5m 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 *α* samples were taken by filtering the sample using a syringe and filter system (Appendix 2). The filter paper was stored in the dark and taken to

the lab for processing chlorophyll α and phaeopigments (products of chlorophyll degradation found within algal cells).
From the 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.
In addition to the discrete sampling, a 50um conical phytoplankton net was used to collect a vertical tow from 5m to surface. The net was rinsed into the cod-end and then poured into a 125 ml container and 10-12 drops of Lugol's Iodine solution was added.

Zooplankton

Zooplankton sampling was performed late morning of the sampling day, and three replicates were obtained on each trip. 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.
The net was lowered to 20m depth (which at the sampling site was near bottom) 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.

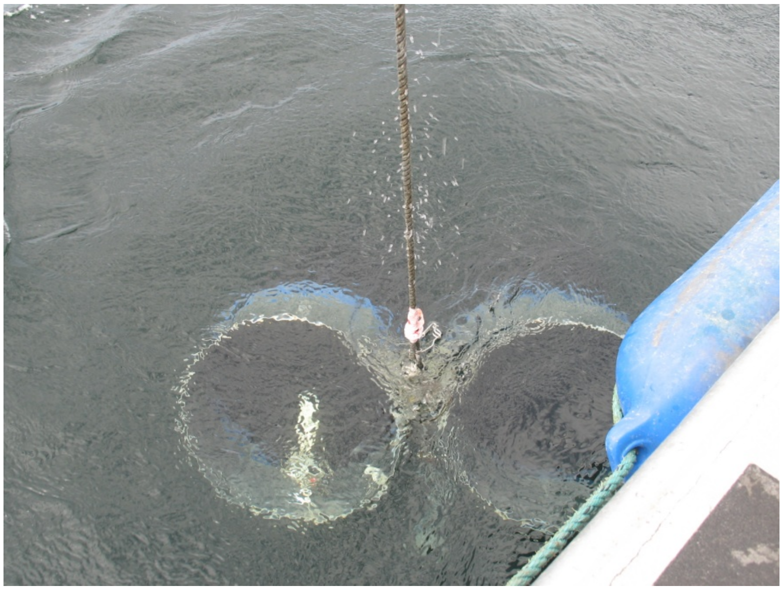

Figure 2 Plankton net sampling, March 2008

Figure 3. Beach seine crew seining and resulting small salmonids. Site 2

Beach seining

A number of sites in close proximity to the plankton sampling area were sampled by beach seine to catch 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 and ended on June 27, 2008. 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. All 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 groups (groups were divided into diatoms, dinoflagellates, flagellates and zooplankton) and 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.
A 1ml subsample was removed from the 125 ml vertical tow sample and placed on a Sedgwick-Rafter counting cell. All plankton was counted according to the same group divisions as the discrete sample and reported similarly.

Chlorophyll α

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 Valerie Forsland of the Ocean Chemistry Division at Institute of Ocean Sciences in Victoria, BC 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.

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 24, 2008 and July 27, 2008, 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 Apr 30 and June 25, 2008. Table 1 provides a summary of the sampling dates.

Table 1 Sampling dates for plankton and beach seining for juvenile salmon, in 2008

Date	February	March	April	May	June
Plankton sampling	24,27	4,13,18,20,25,2731	3,7,11,14,18, 23,25, 28	1,5,8,12,15,20,22,27, 29	3,5,9,12,1,20,24,27
Beach seine			30	15,22,28	4,18,25

All of the plankton sampling occurred in the late morning of each day. All tide phases were covered over the sampling period.

Environmental data collected is summarized in Figure 5. There was a trend towards increasing water temperatures. Salinity over the sampling period was steady around 30ppt. The dissolved oxygen levels did not change during the sampling period and stayed below 10 mg/L. In the spring of 2007, the salinities were lower, indicating more freshwater influence. In 2008 the temperatures rose above 10 C much later than in 2007.

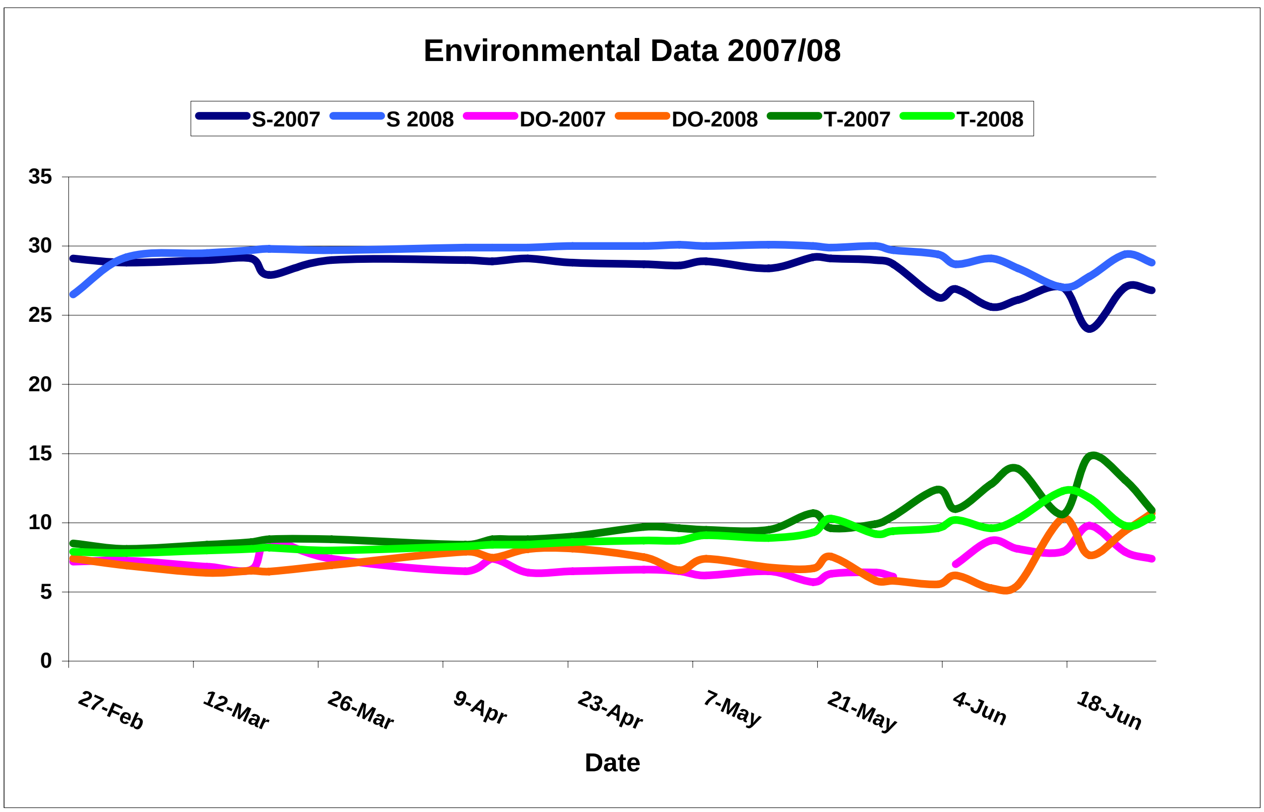

Figure 5 Salinity (S), dissolved oxygen (DO) and temperature measurements at 5m.

The first main phytoplankton bloom observed occurred in early May, 2008 (see Fig. 6) with cell counts remaining high until late May. The composition of this bloom was primarily diatoms with the majority of the diatoms being identified as *Coscinodiscus* which bloomed as the dominant species and stayed in the samples for the entire month of May into June. Comparison of the phytoplankton blooms of 2007 and 2008 shows a later bloom in 2008 (Figure 7). In 2007 there were two more phytoplankton blooms but 2008 saw one major bloom with no reoccurrence over the spring.

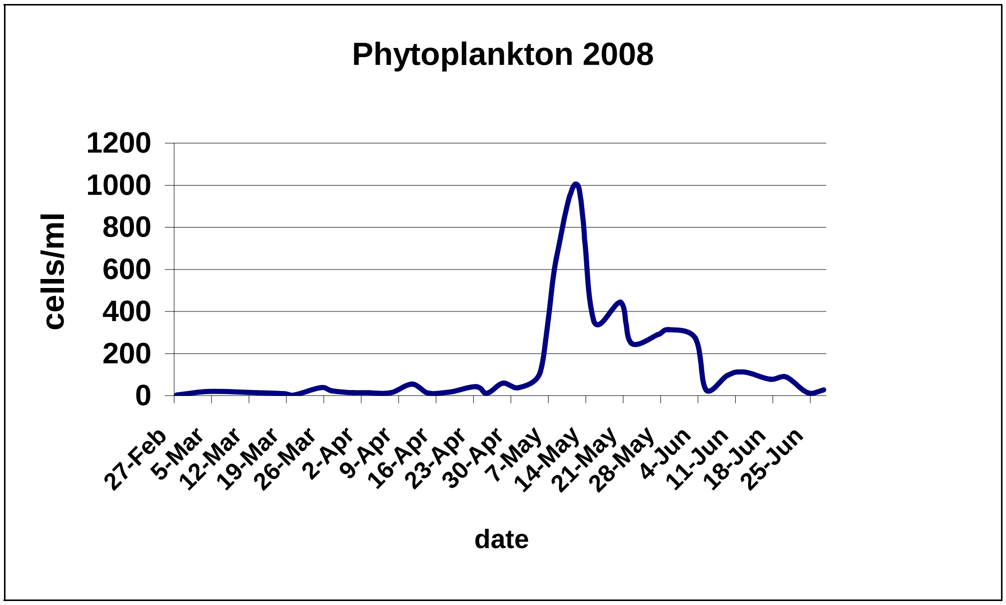

Figure 6 Discrete phytoplankton levels measured at 5m

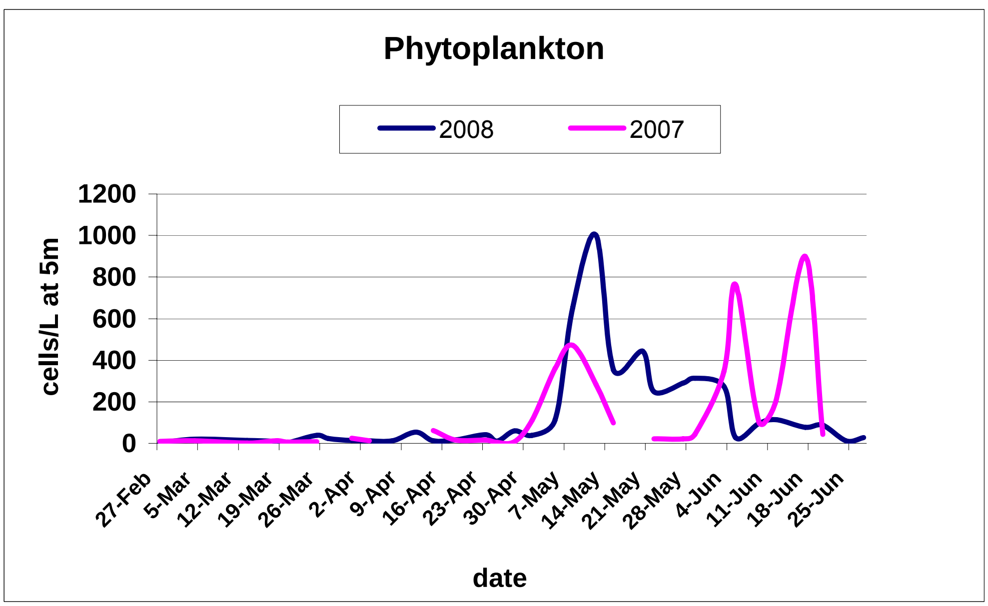

Figure 7 Comparison of phytoplankton levels (measured at 5m) for 2007 and 2008

Phytoplankton densities (measured at 5m) were compared with results from chlorophyll *a* and phaeopigments (Figure 8). Cholorophyll/phaeopigment analysis indicates an additional spike mid-April which does not show up in our discrete samples. The correlation between phytoplankton concentration and chlorophyll *a* is good (r=0.65 p=0.0002).

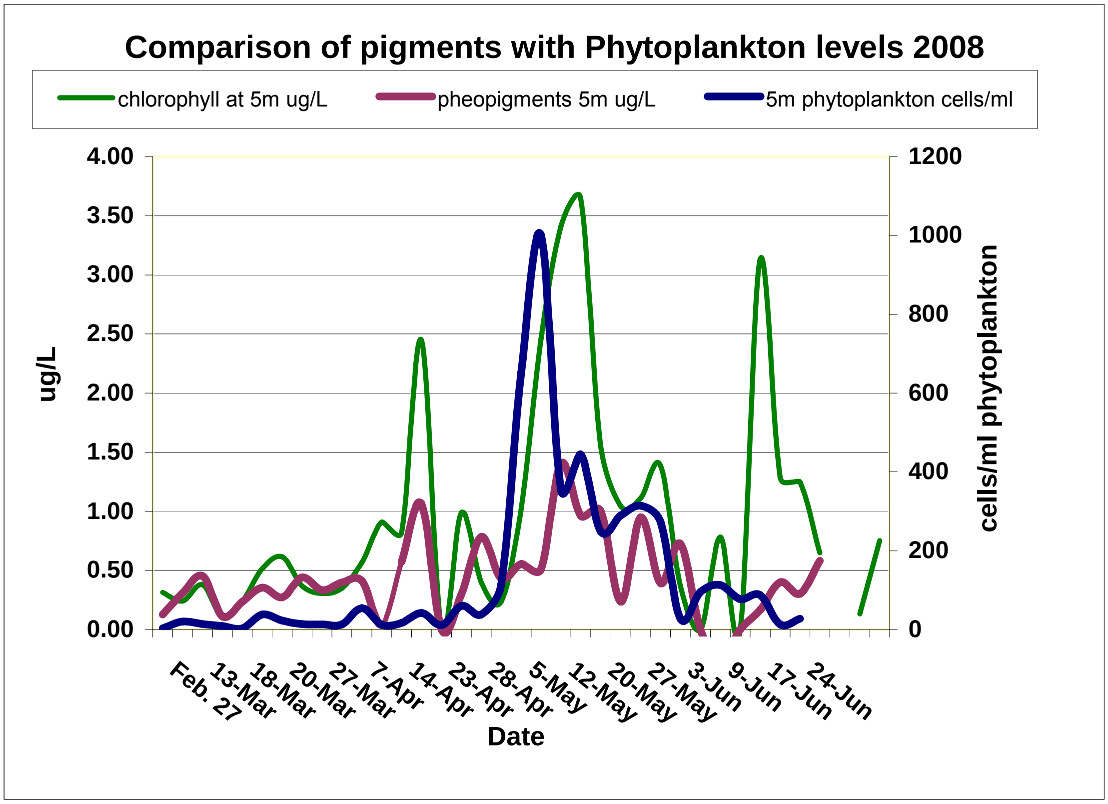

Figure 8 Comparison of discrete plankton sample counts (cells/ml) with chlorophyll a /phaeopigment analysis (µ/l) from the same sample.

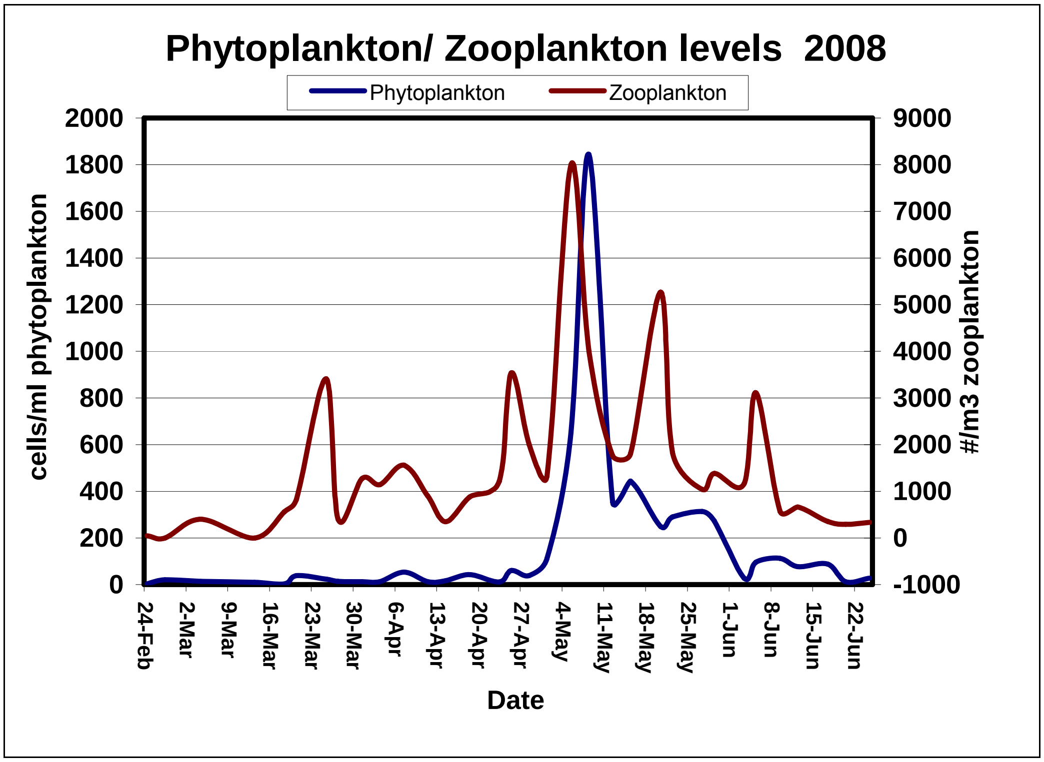

Figure 9 Phytoplankton vs zooplankton trends. Black arrows indicate coho release from Quinsam Hatchery

Figure 9 shows the relationship of phytoplankton and zooplankton density for each sample date. The zooplankton densities appeared to increase in association with phytoplankton levels as measured by counts at 5m. Chlorophyll levels in Fig. 8 would indicate a smaller increase in phytoplankton numbers in mid to late April which may lead to the increase in zooplankton in early May.

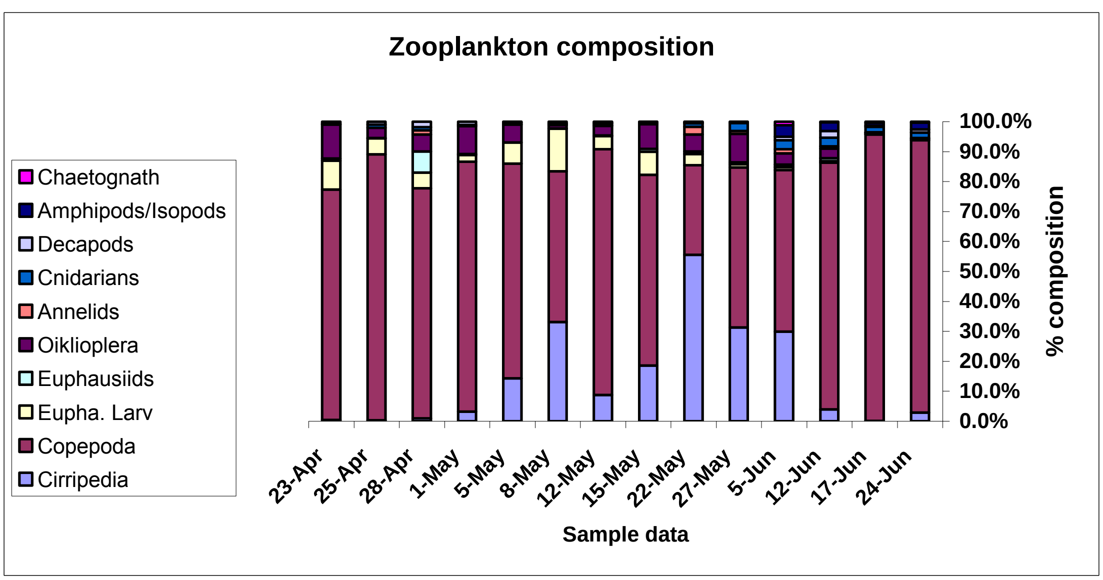

Figure 10. Zooplankton breakdown for each sample date.

Figure 10 shows a breakdown of zooplankton groups found at each sampling date. The graph covers the time period shortly before coho releases from Quinsam Hatchery until the end of the sampling period. Cirripedians and copepods were the dominant zooplankton in the tows throughout the sampling period.

During the initial and final beach seines on May 16 and June 25 respectively, no wild coho salmon were captured in the near shore salt water; however, during the in-between trips – May 16, 22, 28, June 5, 12, and 19 sampling, we were able to capture both wild and hatchery coho although the N values for each sampling were very low for each group. Only on the June 5th sample were we able to capture the targeted 10 wild and 10 hatchery coho. Chum and pink were predominant in the early sets. Chinook, comprising mainly of hatchery sea-pen and river releases, were also caught. Figure 11 and 12 show the average weight and length of the sampled hatchery and wild coho. When sample sizes were large enough to warrant comparison we found that there was no difference between the length and the weight of the enhanced and wild coho. There was consistently more body fat in the hatchery coho which may be explained by the fact that unlike wild fish, hatchery reared fish are fed on a regular basis allowing them to build up fat reserves.

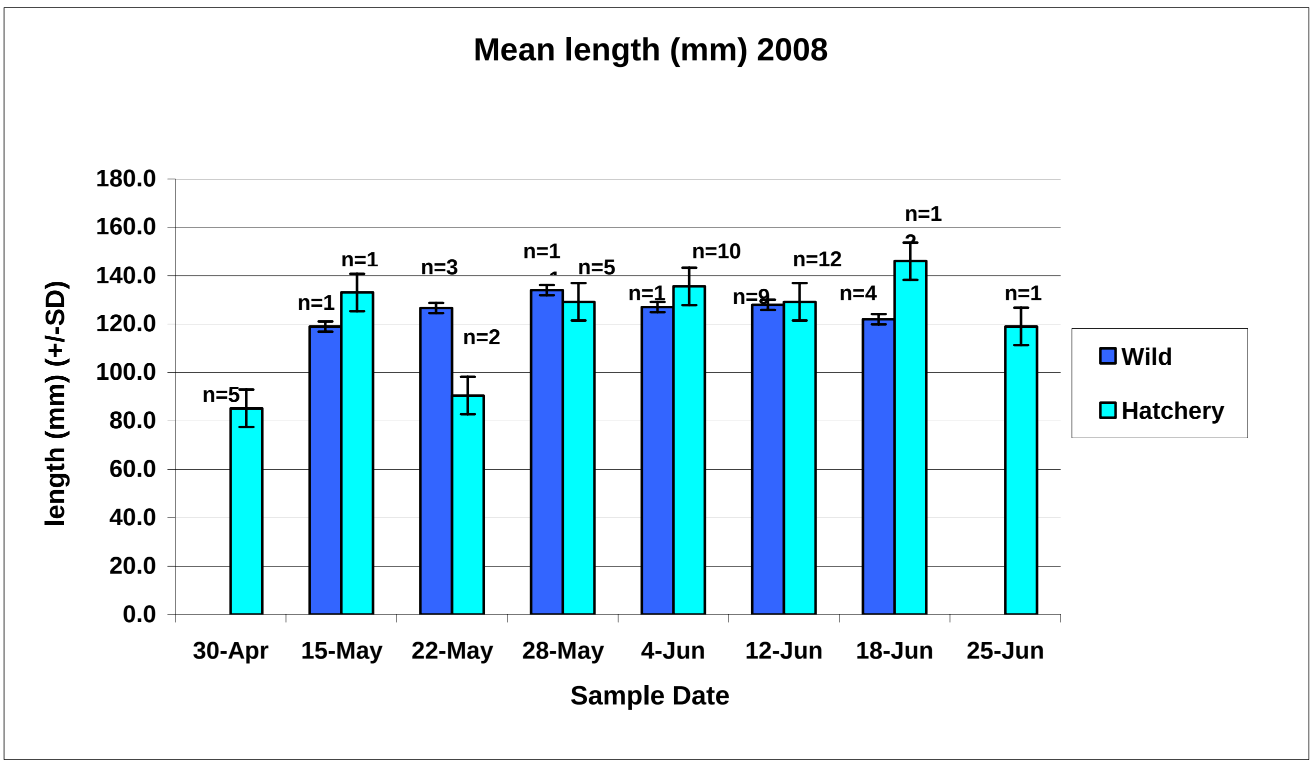

Figure 11 Average length of sampled hatchery and wild coho (n=number of fish sampled)

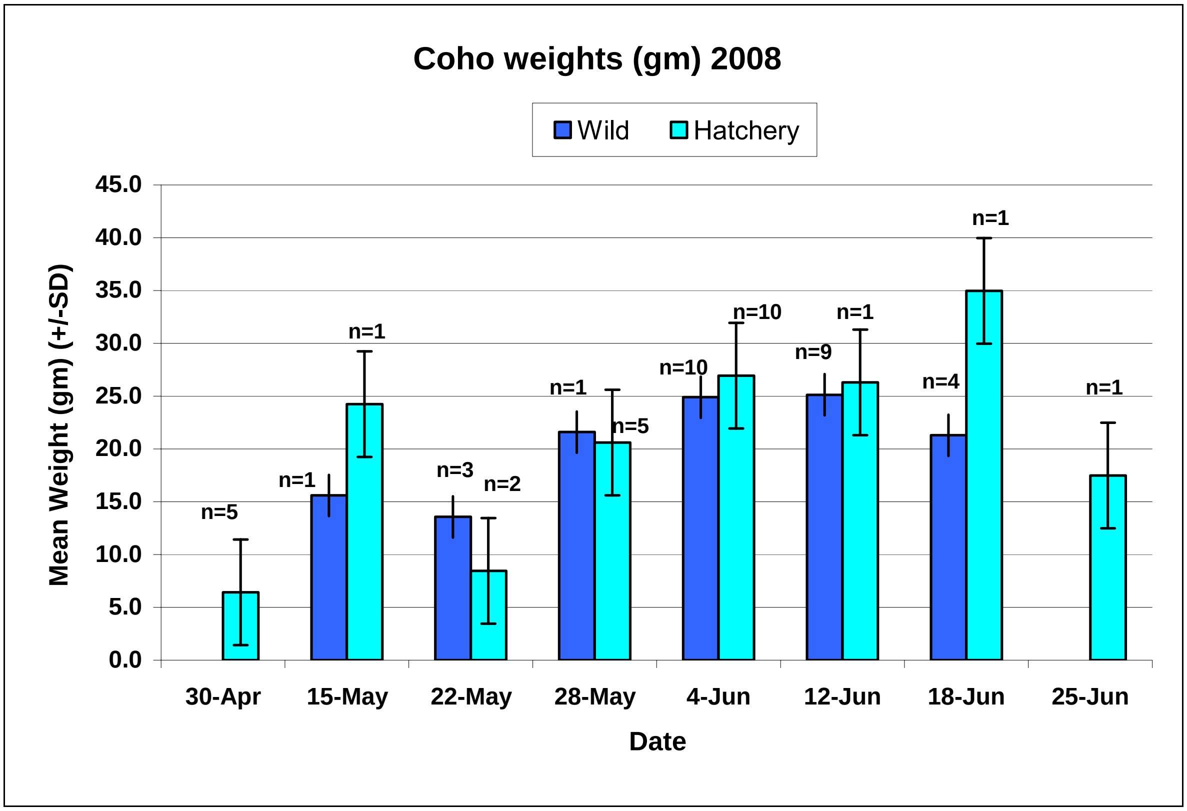

Figure 12 Average weight of sampled hatchery and wild coho. (n=number of fish sampled)

The stomach contents from sampled coho juveniles are summarized in Figure 13. The diets of both groups were dominated by amphipods, polychaetes, isopods, and fish. There appears to be a difference in target prey items between the juvenile hatchery and wild coho salmon with the hatchery salmon targeting a wider variety of zooplankton.

Figure 14 summarizes the zooplankton composition of the near shore environment near the date of the beach seines. The composition indicates a much higher number of smaller species, i.e. copepod, cirripedia, and much lower concentrations of the larger zooplankton found in the stomach contents of the fish. It should be noted that the digestion of the stomach contents was generally quite advanced. The dominant zooplankton seen in the stomach samples represent the larger prey items in the zooplankton sample. There is the possibility that any smaller prey would be digested quickly leaving the remains of larger animals in the stomach contents. Only 9% of the stomachs were empty (see Table 2) indicating that the majority of the fish, wild and hatchery, were actively feeding. Small fish, most often juvenile salmonids (pink or chum salmon), were also observed as a major food item for hatchery and wild coho.

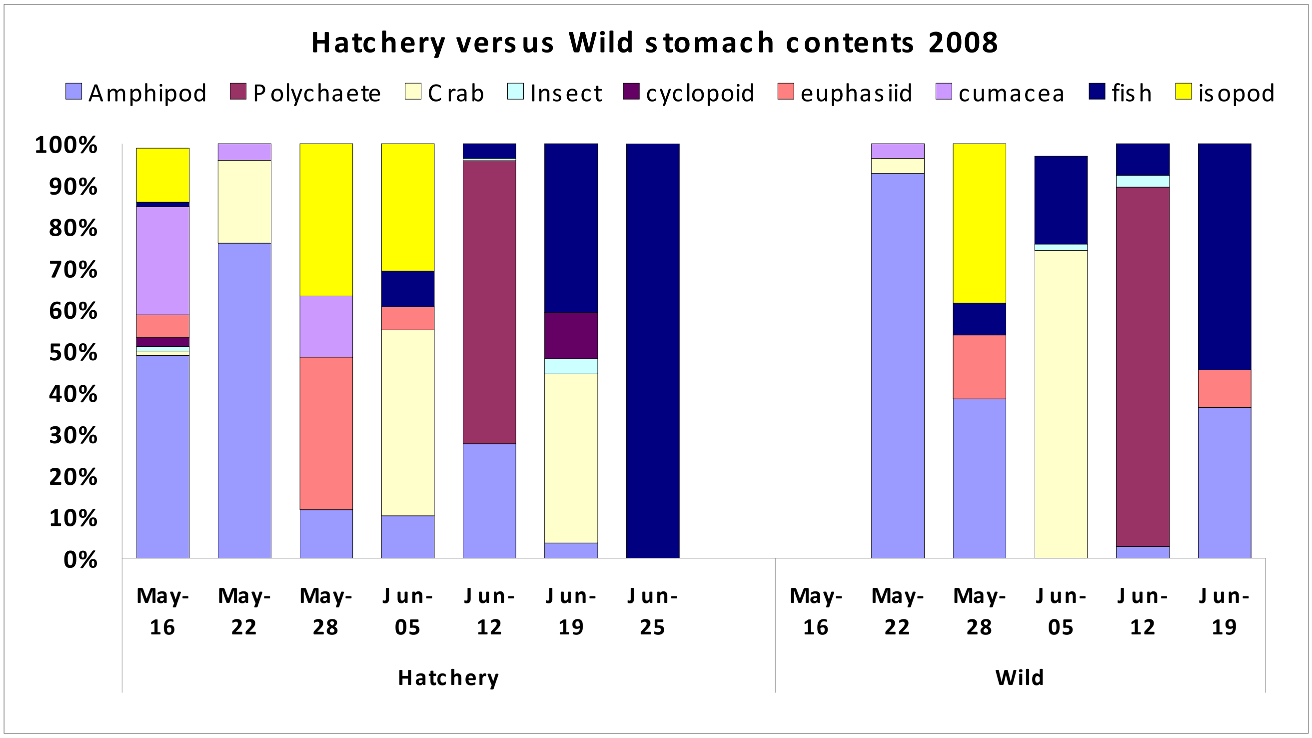

Figure 13 Hatchery and wild coho stomach compositions. % composition based on total numbers of organisms in stomach.

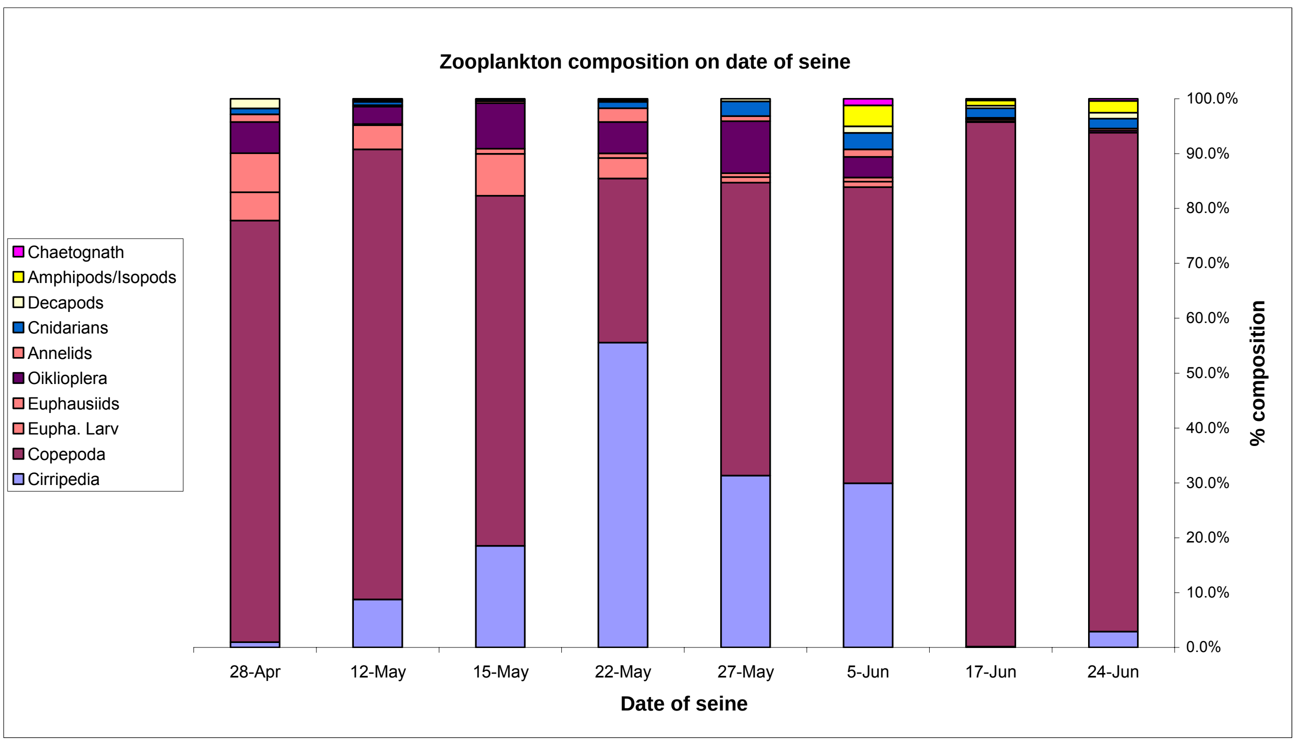

Figure 14 Illustrates the zooplankton composition on or near the date nearest to the beach seine.

Table 2 Degree of stomach fullness

% fullness of stomach	0% full		1-25% full		26 – 50 % full		51% - 75% full		76 – 100% full	
	wild	hatchery	wild	hatchery	wild	hatchery	wild	hatchery	wild	hatchery
% fish in each category	0%	0%	15%	17%	11%	21%	7%	13%	59%	42%

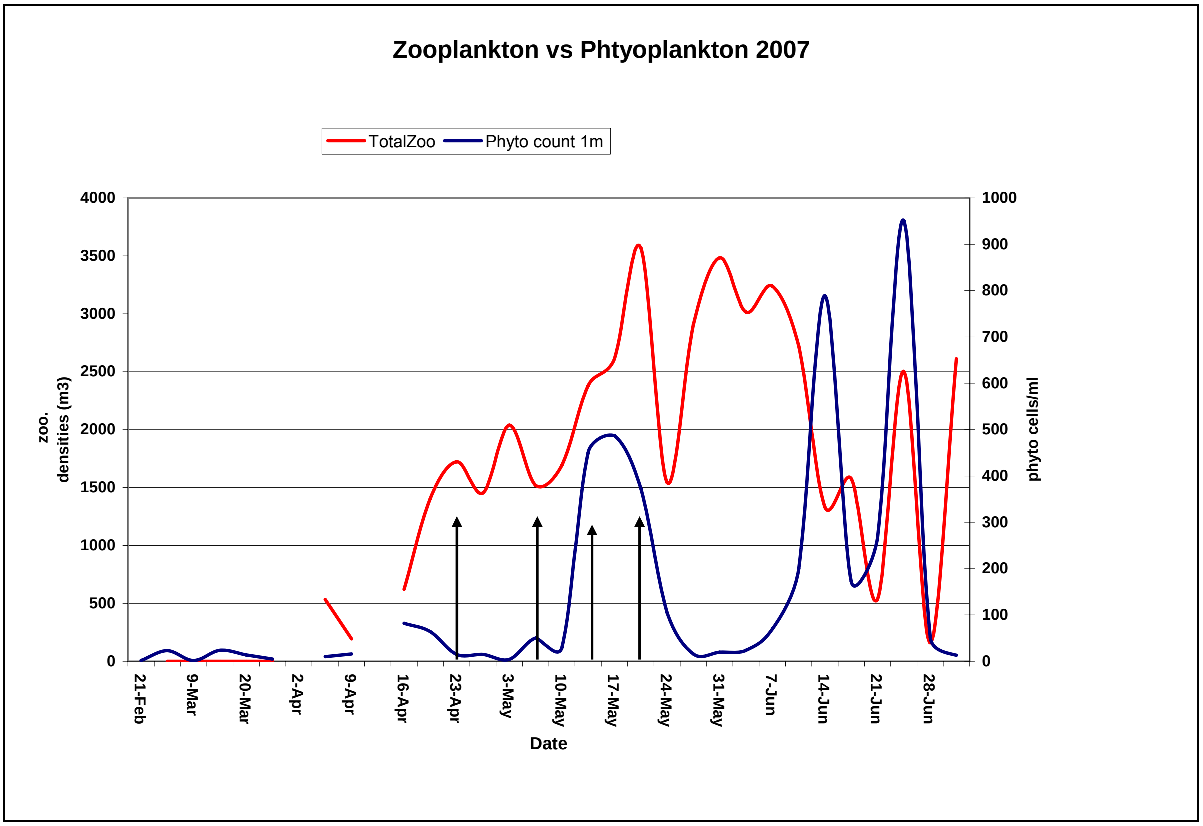

Figure 15. Zooplankton vs. Phytoplankton 2007. Black arrows indicate coho release dates from Quinsam Hatchery

Figure 15 shows the zooplankton bloom in 2007 with the coho release dates from Quinsam Hatchery. There were 4 release dates beginning Apr. 25 continuing through May 23. The second and third release dates occurred during the bloom of zooplankton. The fourth release date happened towards the end of the primary bloom.

Table 3 shows preliminary results from early returning coho salmon (Jack) in fall of 2008. This is Coded Wire Tag data from returns to the hatchery only. There is no catch added in and the numbers are real numbers, no extrapolation. It shows the different release groups and how many tags were recovered compared to the tags released as smolts in the spring.

It shows that the tag recoveries from Jacks that were released in mid-May were two times higher than Jacks released in late April and late May; suggesting better survival.

2006 Brood Coho (Returned 2007) **Release group**	**Jacks** **% Survival**	**#tags recovered**	**Tags Released**
April 25 Release: 26.7 gm avg wt	0.09%	21	23,353
May 9 Release: 25.1 gm avg wt	0.24%	56	23,445
May 16 Release: 26.2 gm avg wt	0.24%	56	23,204
May 23 Release: 24.6 gm avg wt	0.12%	25	20,738

Table 3 Jack returns from 2007. CWT data to hatchery only.

Ninety-five (95) kidney samples were analyzed using ELISA (Figure 16). 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).

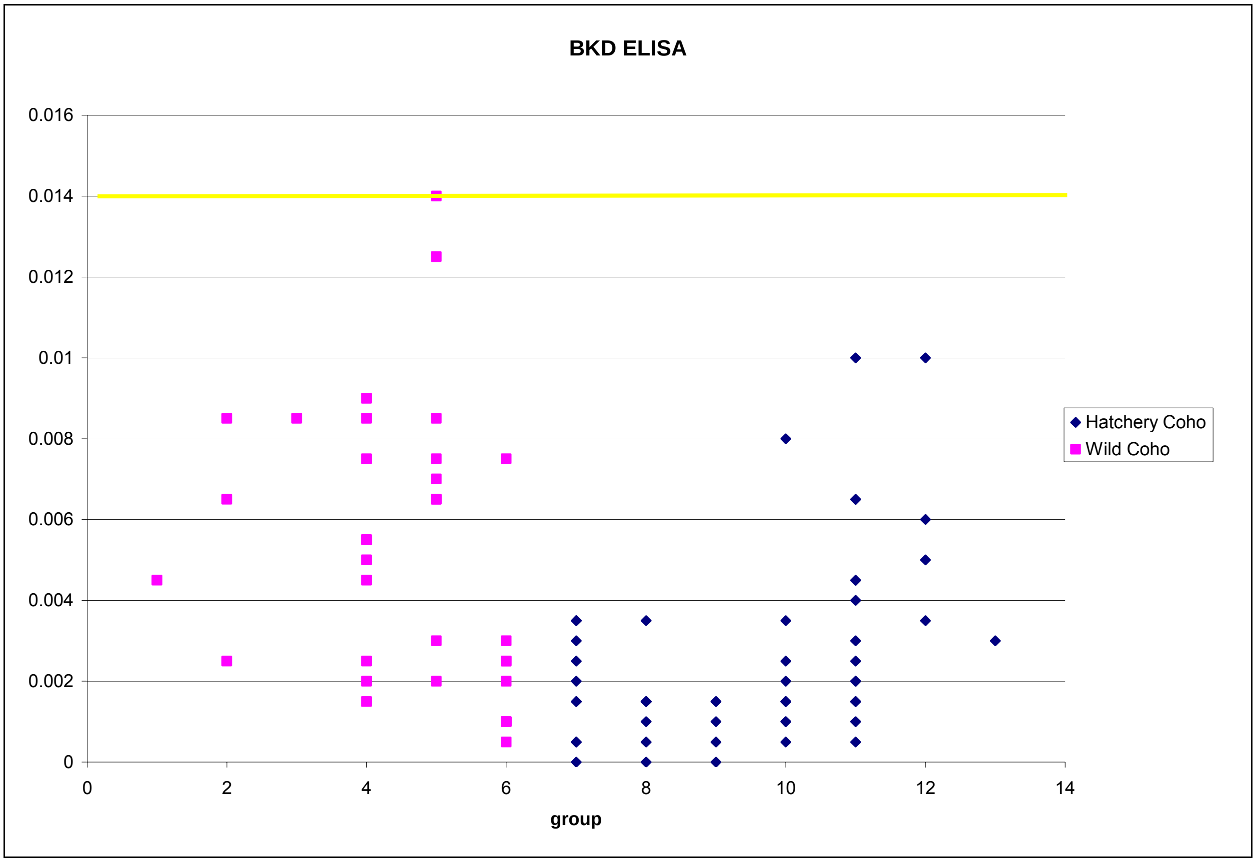

Figure 16 Bacterial Kidney Disease Screening results (ELISA test). Yellow line indicates DFO release levels of 0.4 optical densities.

Discussion

This project is a cooperative pilot initiative between the BC Centre for Aquatic Health Sciences (BC CAHS) 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 2008, 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 one main phytoplankton bloom in mid-May 2008, which was much later than the first bloom in 2007 which occurred mid-April. Anecdotal information (from Nicky Haigh, Harmful Plankton Watch, VIU, Nanaimo, and Valerie Forsland, IOS, Victoria, BC) indicated that phytoplankton blooms were late up and down the coast this spring (2008). Water temperatures in 2008 were cooler than 2007 and did not rise until later in the spring. Phytoplankton densities were compared with the chlorophyll *a* levels and were found to correlate with one another. The data also showed that an increase in phytoplankton levels was followed by an increase in zooplankton density indicating a possible relationship between phytoplankton and zooplankton density. Zooplankton densities increased in late March and the primary constituents of the samples were small copepods. The zooplankton levels were at their highest in early May right around the time the first coho were released from Quinsam Hatchery, suggesting that there was a larger variety of zooplankton in the water at this time so the juvenile coho had a good food supply. During all three releases of coho, the fish encountered high levels of prey although it would appear that the first two releases encountered the best food availability. In comparison, in 2007, there were a 2 additional phytoplankton and zooplankton blooms throughout the spring. Both 2007 and 2008 data showed that zooplankton levels increased in conjunction with increasing phytoplankton density.

Chlorophyll and phytoplankton abundance

Chlorophyll a levels were positively correlated with phytoplankton density ($r=0.65$ $p=0.0002$) and would therefore be a good indicator of phytoplankton titres. Our evidence suggests that discrete sampling at specific depths may not be the most appropriate method to assess phytoplankton due to boat and crew availability. Laboratory analysis of phytoplankton samples is also time consuming, making quick turnaround of results difficult, especially if multiple samples are being submitted from various areas. The vertical net tow proved useful in identifying what the make up of the phytoplankton was but the time commitment for analysis was extreme, especially during periods of blooms. In both 2007 and 2008, chlorophyll a levels indicated a small phytoplankton bloom preceding the larger main bloom in spring. The discrete sampling and phytoplankton net tows did not indicate a bloom in either year. Being able to identify this increase in phytoplankton would allow an increase in frequency of sampling phytoplankton. Identifying when the major phytoplankton bloom occurs may give more flexibility in adapting the monitoring schedule for zooplankton. The hatchery would then have more lead time to adjust any changes in the release schedule of the coho smolts. In 2009, we would like to purchase a bench top chlorophyll meter (e.g. 6025 Chlorophyll sensor) to facilitate real-time measurement of chlorophyll *a* and provide the flexibility to modify the monitoring frequency and allow samples to be analyzed from additional hatchery facilities. We would work closely with Valerie Forsland (Ocean Chemistry Division, IOS, Victoria, BC) and instigate a more stringent sampling regime including 10% duplicates to maintain data quality and integrity.

Zooplankton versus gut content abundance

According to coho smolt gut content analysis, cyclopoids were a dominant species observed in the zooplankton samples, but not a significant food type in the hatchery reared coho diet. Conversely, amphipods, euphasiids and other fish were important in both wild and hatchery smolt diets, but neither were dominant in the zooplankton samples. This finding could be a result of sampling for zooplankton during daylight hours, as many zooplankton species are known to have vertical migration patterns (moving up the water column at night). This migration behavior was mitigated by sampling the entire depth of the water column near shore (from 20m to surface) and the change to Bongo nets (which is standard sampling gear for zooplankton).

The hatchery coho appear to have a more varied diet than the wild coho perhaps indicating a learning curve as to the best prey type when the hatchery juveniles get to the marine near shore environment. The last two seines produced hatchery coho with primarily small fish in their stomachs. The corresponding zooplankton titres at this time were not at the highest levels so perhaps this influenced which prey items were used by these juveniles in the nearshore environment.

Results from this spring sampling would indicate that this year's release dates for the coho was optimal.

Coded Wire Tag Returns to Quinsam Hatchery

Jack returns in fall of 2007 indicated that the juveniles that were released into a zooplankton bloom, (early May, a period of high productivity), had better returns than the fish released earlier, (late April), and later, (end of May). 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.

The jack returns in the fall of 2008 will give a good indication of what the survival will be for the three release dates in spring of 2008. Adults from this release brood year will be assessed in the fall of 2009.

Fish Health

The ELISA results for Bacterial Kidney Disease indicate that the hatchery coho had low levels of BKD but were well below the DFO cutoff for release. There would appear to be low to negative levels of BKD in these stocks would indicate that returning brood also have low levels of BKD. This disease is endemic in our wild fish but Quinsam Hatchery would appear to have healthy returning stocks. This could be further evaluated through broodstock screening at the hatchery during spawning.

Proposed changes in 2009

Proposed changes and additions for the 2009 portion of the project are outlined in Appendix 1. Having established that there is a relationship between chlorophyll a and phytoplankton levels, the project would look at adding chlorophyll a testing capacity in Campbell River with the purchase of a bench top Chlorophyll a analyzer. This would allow for real time measurements and faster turnaround of results for Quinsam Hatchery and for other facilities on the coast of BC. The plankton sampling would continue to compare to chlorophyll and track zooplankton production. There will also be the addition of secchi disk readings (a black and white disk lowered into the water to record visibility). The secchi disk is an easy indication of phytoplankton densities in the water and can be related to phytoplankton densities and chlorophyll a levels.

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Appendices

Appendix 1

Modifications to 2009 program:

1. Streamline zooplankton analysis to determining density of small (<2.5mm) copepods, large copepods, amphipods, annelids, Euphasiids (larvae and adult) and decapods. This brings all reporting into alignment with stomach sample analysis.

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

3. Continue phytoplankton sampling using discrete sampler at 5m only and continue taking chlorophyll samples. Revise monitoring protocol to include 10% duplicates.

4. Purchase Chlorophyll meter to get real time data that can be correlated to chlorophyll a, phytoplankton, and zooplankton sampling. This will increase capacity on Vancouver Island for this analysis.

5. Add secchi disk readings to environmental data sampling. This is an easy tool to employ to monitor changes in the water.

6. Add plankton and chlorophyll sampling off of a shore based location; the Pier. This would help to establish if shore based monitoring can be substituted for boat sampling. In this way we can incorporate volunteers into the sampling program.

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

8. Provide chlorophyll a levels and zooplankton densities to Quinsam hatchery within 48 hours of sample collection.

Appendix 2

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

Appendix 3

Plankton identification form

Plankton Sample Analysis

Company: | **Analysis Date:**

	Site			Site		
	Date			Date		
	1m	5m	10m	1m	5m	10m
Diatoms						
Achnanthes sp.						
Asterionella japonica						
Bacteriastrum sp.						
Biddulphia sp.						
Cerautalina sp.						
Chaetoceros sp.						
Corethron hystrix						
Coscinodiscus sp.						
Cylindrotheca closterium						
Dactyliosolen sp.						
Detonula pumila						
Ditylum brightwellii						
Eucampia zoodiacus						
Grammatophora sp.						
Haslea wawrikae						
Lauderia annulata						
Leptocylindrus sp.						
Licmophora sp.						
Melosira sp.						
Navicula sp.						
Pleurosigma / Gyrosigma sp.						
Pseudonitzschia sp.						
Rhizosolenia sp						
Skeletonema costatum						
Stephanopyxis sp.						
Thalassionema sp.						
Thalassiosira sp.						
misc. centric						
misc. pennate						
Dinoflagellates						
Alexandrium sp.						
Amphidinium sp.						
Ceratium furca						
Ceratium fusus						
Ceratium sp.						
Cochlodinium cf. polykrikoides						
Cochlodinium sp.						
Dinophysioid sp.						
Diplopsalis sp.						
Gonyaulacoid sp.						
Gymnodinioid sp.						
Gymnodinium sanguineum						
Gyrodinium spirale - type						
Heterocapsa triquetra						
Noctiluca scintillans						
Oxyphysis oxytoxoides						
Polykrikos sp.						
Prorocentrum gracile						
Prorocentrum sp.						
Protoceratium reticulatum						
Protoperidinioid sp.						
Pyrocystis lunula						
Scrippsiella trochoidea						

	Site			Site		
	Date			Date		
	1m	5m	10m	1m	5m	10m
Raphidophytes						
Heterosigma akashiwo						
Chattonella cf. marina						
Other Flagellates						
Cryptomonads						
Dictyocha fibula						
Dictyocha speculum						
Dictyocha non-skeletal						
Ebria tripartita						
Euglenoid						
Phaeocystis pouchetii						
Unid. nanoflagellates						
Zooplankton						
Amoebae						
Appendicularian						
Barnacle nauplius						
Ciliate						
Copepod						
Fungus						
Nauplius						
Pluteus						
Radiolarians						
Rotifer						
Tintinnid						
Zoea						
other						
Dominant Species/Group						
count (cells/mL)						
% constituent						
Diatoms						
Dinoflagellates						
Raphidophytes						
Other Flagellates						
Zooplankton						
Biomass						
Comments						

Appendix 4

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 Laboratories prepared Affinity purified antibody, goat anti- *Renibacterium salmoninarum* (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 Kirkegaarde & 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)