CONFIDENTIAL

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DRAFT PRELIMINARY STATUS REPORT

Project Title: INJURY TO SALMON EGGS AND PRE-EMERGENT FRY IN PRINCE WILLIAM SOUND

Study ID Number: Fish/Shellfish Study Number 2

Lead Agency: State of Alaska, ADF&G; Commercial Fish Division

Cooperating Agency(ies): Federal: USFS
State: DNR

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EXECUTIVE SUMMARY

This study is part of an integrated group of Natural Resource Damage Assessment Studies (NRDA 1,2,3, and 4), that interrelate to quantify damage to pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon stocks from the Exxon Valdez oil spill. Each study will attempt to document injury to fish stocks at different stages in the life cycle. This project is designed to assess possible changes in pink and chum salmon egg to pre-emergent fry survival in intertidal and upstream areas relative to oil contamination from the Exxon Valdez spill.

Up to 75% of the pink and chum salmon in Prince William Sound spawn in intertidal areas which are highly susceptible to contamination from marine oil spills. Pink salmon alevins are more adversely affected by oil exposure in seawater than freshwater (Moles, Babcock, and Rice 1987).

Pre-emergent fry mortality immediately following the Exxon Valdez oil spill appeared to be minimal. No differences in fry numbers were detected between two sampling trips while significant differences were detected among stream zones and streams. A 5% difference in egg mortality between oiled and uncontaminated streams was observed.

This observed difference in egg mortality suggests that differences in overwinter survival may be detected with further post-spill pre-emergent fry and egg survey data.

OBJECTIVES

1. Estimate mortality of pre-emergent fry in oiled and non-oiled streams immediately following the Exxon Valdez oil spill of March 24, 1989.

2. Estimate overwinter survival (eggs to pre-emergent fry) of pink and chum salmon eggs in oiled and non-oiled areas.

3. Assess loss in production, if any, from changes in overwinter survival.

4. Use tissue samples from pre-emergent fry and mussels (*Mytilus sp.*) to document hydrocarbon contamination in streams where oil was not evident visually.

5. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

METHODS

Sample Sites

Thirty nine streams throughout Prince William Sound (PWS) were sampled during the first pre-emergent fry trip (Figure 1). Twenty- five of which were historically sampled for the following reasons:

1. They have a significant spawning population in both odd and even years.
2. They are representative of the spacial distribution of escapement in PWS.
3. They contribute significantly to the adult return forecasting model.
4. Environmental conditions allow sampling in most years.

The additional 12 streams were surveyed to assess their potential as egg and pre-emergent fry study streams. A second round of sampling occurred approximately two weeks after the oil spill and concentrated on the central and southwest areas of PWS. During the second event 14 streams were resampled (representing both oiled and non-oiled areas) and an additional 17 streams in oil impact areas were surveyed to evaluate their suitability as egg and pre-emergent fry study streams (Figure 1).

Egg deposition sampling was completed on 31 streams from 27 September to 15 October 1989 (Figure 2). The 31 streams are a subset of the 46 streams selected for the spring 1990 pre-emergent fry survey. New streams were selected using the following criteria:

1. There are sufficiently large adult salmon returns to indicate a high probability of success in egg/fry digging.
2. There is past history of egg/fry digging.
3. Streams which had low to no oil impact in the immediate vicinity of high oil impact streams. This will help account for possible variability due to differing climatic/stream conditions.

Egg dig streams had the following characteristics: ten were in areas where oil was visible at or in the vicinity of the stream mouth, seven were in areas where oil impact is suspected but the extent of contamination is unknown, and fourteen were in areas suspected of receiving little or no oil.

Hydrocarbon and Histopathological Sampling

Hydrocarbon and histopathological samples were collected to determine level of hydrocarbon impact at streams where oil was not visible. Tissue samples from pre-emergent pink salmon fry were collected from the intertidal channels of 14

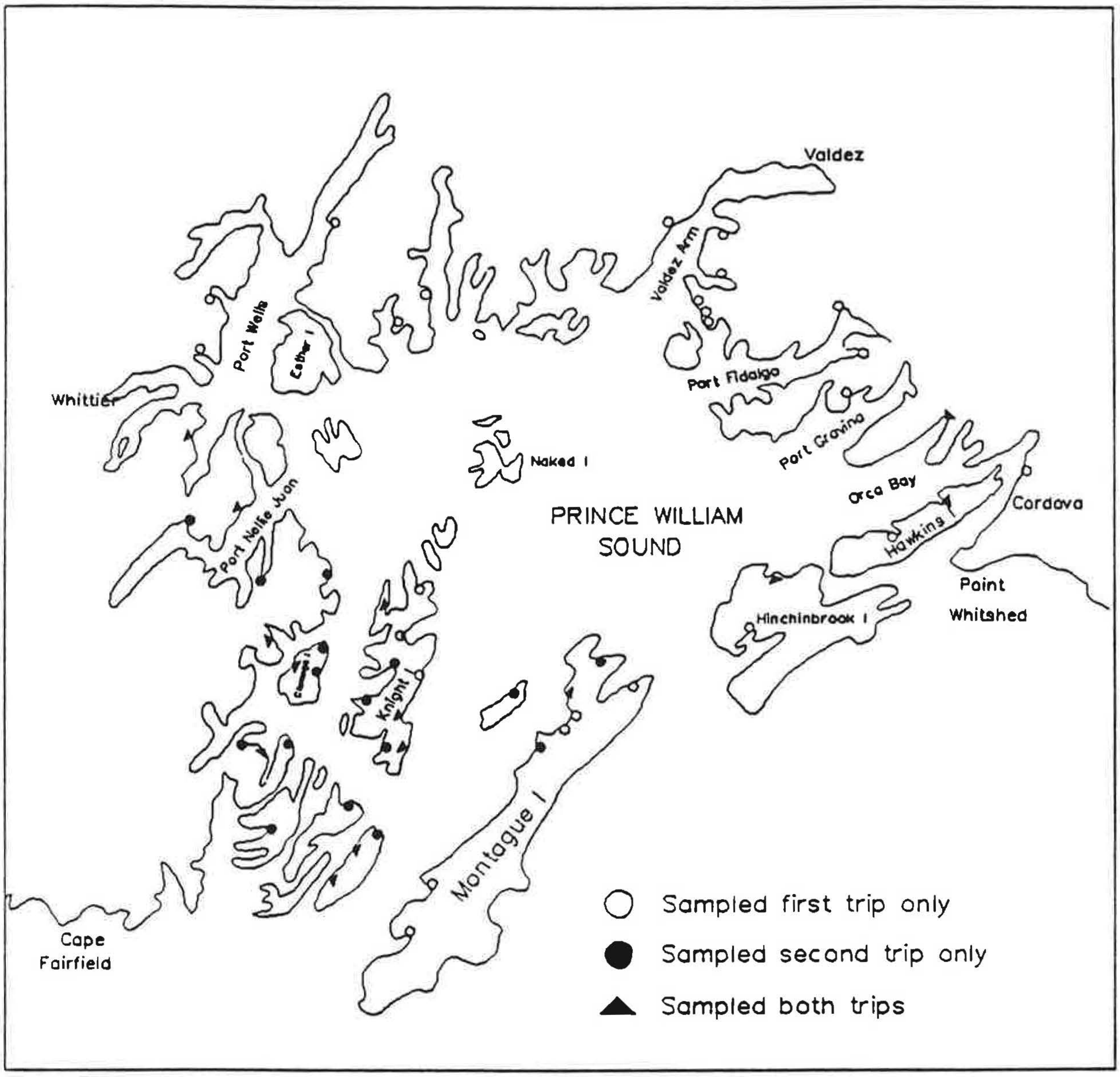

Figure 1. The locations of the 1989 Prince William Sound pre–emergent fry survey streams. Thirty nine streams were sampled on the first trip (26 March to 9 April). Thirty one streams were sampled on the second trip (4 April to 24 April). Fourteen streams were sampled on both trips.

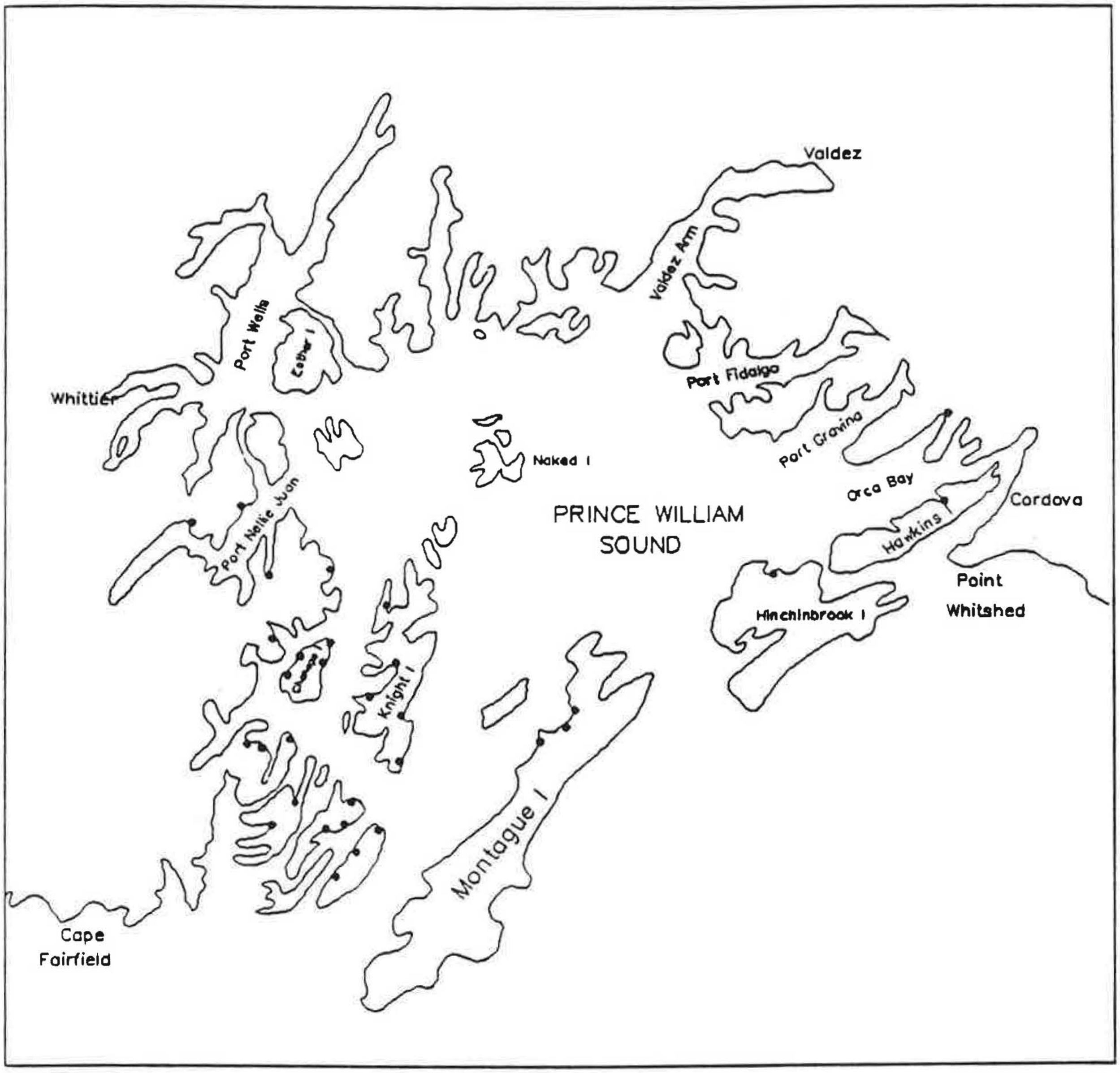

Figure 2. The locations of the 1989 Prince William Sound egg deposition survey streams.

streams for hydrocarbon analysis and 22 streams for histopathology examination (Appendices B.1 and B.2). All samples were collected during the second pre-emergent fry sampling trip. Mussel samples were also collected from all streams examined in NRDA Study 1. Fry samples were collected from the stream bed approximately 2.5 m above mean low water. Samples were collected when the tide stage was below the sampling level to avoid contamination from any surface film of oil on the salt water. Fry were dislodged with a clam rake and collected in a stainless steel strainer or flushed from the gravel using normal fry/egg dig sampling methods. Captured fry were placed in glass jars containing Bouin's solution, topped with teflon lined lids, and frozen. Glass jars and lids were pre-rinsed three times with dimethylchoride, dried, and kept in locked storage prior to use.

Sample Design

Four zones, 3 intertidal and one above tidal inundation were sampled when possible for each sample stream. The zones were established with a hand level and stadia rod during the fry dig. Crews conducting stream surveys for NRDA Study 1 identified and marked the zones on most egg dig streams before sampling began. A surveyors level, tripod, and stadia rod were used to survey the zones on streams not marked prior to the egg dig. The zones were 1.8-2.4 m, 2.4-3.0 m, 3.0-3.7 m above mean low water, and upstream of tidal influence.

Separate linear transects 30.5 m long were established for each zone on the egg and pre-emergent fry digs; however, transects were sometimes shorter due to steep stream gradients. Transects were historically selected in riffle areas where significant spawning was consistently observed. On streams with no history of fry/egg digs or ground surveys transect locations were subjectively selected in areas that appeared to be good spawning habitat. Transects ran diagonally across the river, the downstream end at one bank and the upstream end against the opposite bank. Fry dig transects started downstream against the right bank and moved upstream to the left bank. Streams resampled on the second fry dig trip used the opposite transect orientation to avoid resampling. Egg dig transects started downstream against the left bank and moved upstream to the right bank. Opposite orientations of egg and fry dig transects should reduce sampling overlap and the influence of fall egg digging on perceived spring fry abundance. A map was drawn of each egg dig stream indicating the tide zones and transect locations in relation to the major landmarks. Each egg dig transect was marked with surveyors flagging to help assure that egg and fry dig transects will be in the same immediate area. A better estimate of egg to fry survival within the sample zone should result.

Ten circular digs, each 0.3 m^2, were systematically dug along each transect during the 1989 pre-emergent fry survey. The number of digs was increased to fourteen for the egg survey. The increased sample size was a compromise between reducing variance and practicality. Fewer digs were completed on narrow stream channels to avoid excessive sampling of the stream. Streams that split into two or more channels within a zone were sampled by allocating digs among the channels based on the spawner distribution observed during NRDA Study 1; or by an equal allocation where spawner distribution was unknown. Pirtle and McCurdy (1977) describe the methods used for both egg and pre-emergent fry digs.

Data on the numbers and condition (live or dead) of fry and eggs by species were collected for each dig on the pre-emergent fry and egg deposition surveys and recorded in "Rite in the Rain" books. Additional information such as date, time, zone, and a subjective estimate of the overall percent absorption of fry egg sacs in each sample was also collected. Pink fry were differentiated from chum fry by their smaller size and lack of parr marks. Pink eggs were separated from chum and coho (*O. kisutch*) salmon eggs by their smaller size. Chum eggs were separated from coho eggs by their greater development and different coloration. Eggs were considered dead if opaque or discolored with concentrations of lipids. Live eggs showed a consistent color with no opaqueness.

Data Analysis

Pre-emergent Fry Digs

Data from the two passes of pre-emergent fry digs were examined for presence or absence of dead fry by stream and stream zone. If dead pink salmon fry were detected for the majority of the stream/stream zone groupings, a blocked fixed effects 2-way analysis of variance would be used to determine whether mortality increased with oiling. If few of the groupings contained dead pink salmon fry, the data would be examined for differences in number of fry between passes using a similar analysis.

$$Y = \mu_{...} + P_i + Z_j + (PZ)_{ij} + S_k + \epsilon_{ijk}$$

Data were blocked by stream (S_k) while dig pass (P_i; 2 levels; first pass prior to oil impact, second pass 2 weeks later) and stream zone (Z_j; 4 levels) were treatments. A dig pass/stream zone interaction was also considered. The equality of variances assumption was tested using the F_{max}-test (Sokal and Rohlf, 1969) while normality was visually assessed. Appropriate transformations were considered.

Egg Mortality

Pink salmon egg mortality was estimated for each stream using;

$$M_{egg} = \frac{DPE}{DPE + LPE + DPF + LPF},$$

where DPE is number of dead pink eggs, LPE is number of live pink eggs, DPF is number of dead pink fry, and LPF is the number of live pink fry.

Differences in egg mortality possibly due to oiling were tested using a mixed effects two-factor experiment with repeated measures on one factor (Neter, Wasserman, and Kutner, 1985).

$$Y_{ijk} = \mu_{...} + O_i + Z_j + (OZ)_{ij} + S_{k(i)} + \epsilon_{(ijk)}$$

The two treatments were extent of oiling, (O_i, 2 levels; oiled and control), and height in the intertidal zone (Z_j, 4 levels; 2.1, 2.7, and 3.4 m above mean low water and upstream) both fixed effects. The data were also blocked by stream ($S_{k(i)}$), a random effect which was nested within extent of oiling. The interaction of extent of oiling and height in the intertidal zone was also examined.

Adequate data on level of oiling for each of the streams sampled is not available at this time; consequently, no quantitative stratification could be made for extent of oiling. As a result, streams were subjectively classified as definitely oiled and most likely not oiled (control). Care was taken to insure that control and oiled streams were selected in close geographic proximity. Streams which could not be classified to one of the two groups with any level of subjective confidence were excluded from the analysis. Streams selected for controls were 485, 498, 623, 656, 666, 692, 695, 699, and 744. Streams classified as oiled were 506, 618, 628, 637, 663, 677, 678, 681, and 682. The equality of variances assumption was tested using the F_{max}-test (Sokal and Rohlf, 1969) while normality was visually assessed. Appropriate transformations were considered.

RESULTS

Pre-emergent Fry Digs

Few dead pink salmon fry were found during either of the 1989 pre-emergent fry digs. Only 9 of the fifty two transects examined (fourteen streams with 3 or 4 stream zones, each dug twice) averaged more than 5 dead fry. Moles, Babcock, and Rice (1987) found that 5- and 60-day alevins had no mortality when exposed to 1.5 mg/liter aromatic hydrocarbons for 30 days in a simulated tide cycle and 100% mortality in less than 30 days when exposed to 2.4 mg/liter. Hydrocarbon concentrations exceeding 1 ppm were measured during the Amoco Cadiz spill, but generally the concentrations were 0.5 ppm or lower (Calder and Boehm 1981 as cited in Moles, Babcock, and Rice 1987). Minimal direct pre-emergent fry mortality from the Exxon Valdez spill probably resulted from sublethal concentrations of hydrocarbons; but this does not preclude sublethal effects that could make fry more susceptible to predation or disease. Pink salmon tissue samples collected for histopathological testing may help show any sublethal effects of oil contamination. Because of the low numbers of dead fry, the data were examined for differences in number of pink salmon fry between passes.

The F_{max}-test indicated homoscedastic variances ($p<.01$) for the untransformed data and visual examination of factor level distributions indicated near normality. No difference in numbers of pink salmon fry was detected between the 2 passes ($p=0.87$) while significant differences were detected between stream zones ($p=0.0002$) and streams ($p=0.0001$) (Appendix A). The pass/stream zone interaction was not significant ($p=0.42$). The lack of significant differences

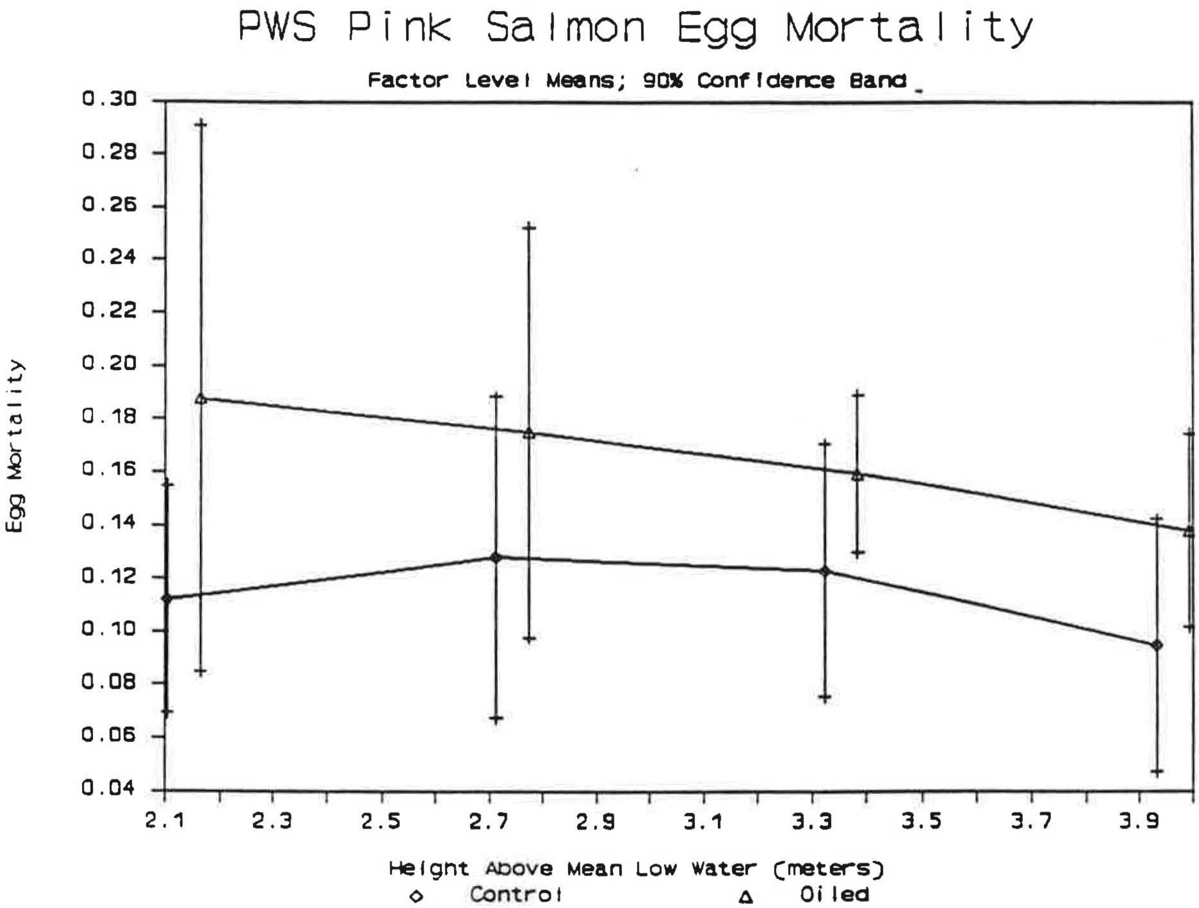

Figure 3. Pink salmon egg mortality by tide zone for oiled and non-oiled (control) streams. Data from the 1989 Prince William Sound egg deposition survey.

between passes indicates that the pre-emergent sampling can produce repeatable results.

Egg Mortality

The F_{max}-test indicated homoscedastic variances ($p<.01$) for the untransformed data although visual examination of factor level distributions indicated possible non-normality. The data were transformed using the square root and arcsin transformations and the analysis was performed on both untransformed and transformed data. While the transformations helped to normalize the data, no differences in results were obtained; consequently, all further analysis were performed using untransformed data.

A five percent increase in egg mortality was detected for the oiled streams ($p=0.0437$; overall average of 11.5% for controls vs. 16.5% for oiled) while no significant differences were found between stream zones ($p=0.724$) (Figure 3) or for the interaction of extent of oiling and stream zone ($p=0.880$). The blocking variable, stream, showed no significant effect ($p=0.379$). Pairwise contrasts between oiled and control for each stream zone were made. A possible difference was detected for the 1.8 to 2.4 m zone ($p=0.078$) while no differences were found for the 2.4 to 3.0 m, 3.0 to 3.7 m, and upstream zones ($p=0.309$, 0.429, 0.350, respectively). The inclusion of hydrocarbon data into the analysis will allow for better partitioning of the variability due to oiling.

A cursory examination of the experiments power indicated the ability to detect a 15% difference in egg mortality at $\alpha=0.05$, 95% of the time or a 10% difference 80% of the time.

The relatively small difference in egg mortality between oiled streams and controls was not unexpected. Eggs have lower rates of hydrocarbon uptake than alevins or fry; consequently, the damage to egg embryos from long-term exposure to hydrocarbons may not be manifest until after hatching (Korn and Rice 1981).

STATUS OF INJURY ASSESSMENT

The first objective was to estimate the mortality of pre-emergent fry in oiled and control streams immediately following the Exxon Valdez oil spill. The direct mortality from short-term exposure to oil appeared to be minimal.

Objective two was to estimate overwinter survival (eggs to pre-emergent fry) of pink and chum salmon in oiled and non-oiled areas. Egg dig data were examined for evidence of mortality differences between oiled and non-oiled areas, and a 5% difference in mortality was observed. Eggs have low rates of hydrocarbon uptake and may not show visible injury after short-term exposure (Korn and Rice 1981). No conclusion can be reached yet regarding overwinter survival differences; however, the observed difference in egg mortality indicates more post-spill data should be collected. Items needed before final conclusions can be reached include hydrocarbon sample and coastal habitat study results indicating the extent of oiling, historical summary and analysis of egg and pre-emergent fry pre-spill data bases, and further egg and pre-emergent fry surveys for a post-spill data base.

Assessment of the loss in production, if any, from changes in overwinter survival was the third objective. No adult production loss due to changes in overwinter survival can be assessed until the 1991 catch and escapement are evaluated. Loss in adult production cannot be estimated without the measures of production generated by NRDA Study 1 (wild stock escapement) and NRDA Study 3 (allocation of the catch between wild and hatchery stocks using coded wire tag results). A loss in wild stock production could occur due to poor adult spawner escapement and subsequent egg deposition, low overwinter survival, poor early marine survival, or low marine survival. Stock injury due to oil at one life stage may not cause an overall loss in adult production; consequently, without estimates of survival and extent of oil exposure at all life stages, no case could be made for loss in adult production due to injury at one life stage. NRDA Studies 1,2,3, and 4 will provide the framework to assess whether a possible loss in production is attributable to lower overwinter survival due to oil injury or some other factor.

The fourth objective of the study is to document hydrocarbon contamination in streams where oil was not visually evident. Pre-emergent fry samples were collected from 14 streams during the study for hydrocarbon analysis. Mussel samples were collected from all streams examined by NRDA Study 1. The samples are being analyzed, and no results are available.

The final objective of the study is to identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is documented. We are working in cooperation with the FRED Division to examine the possibility of enhancing wild stocks where injury is documented.

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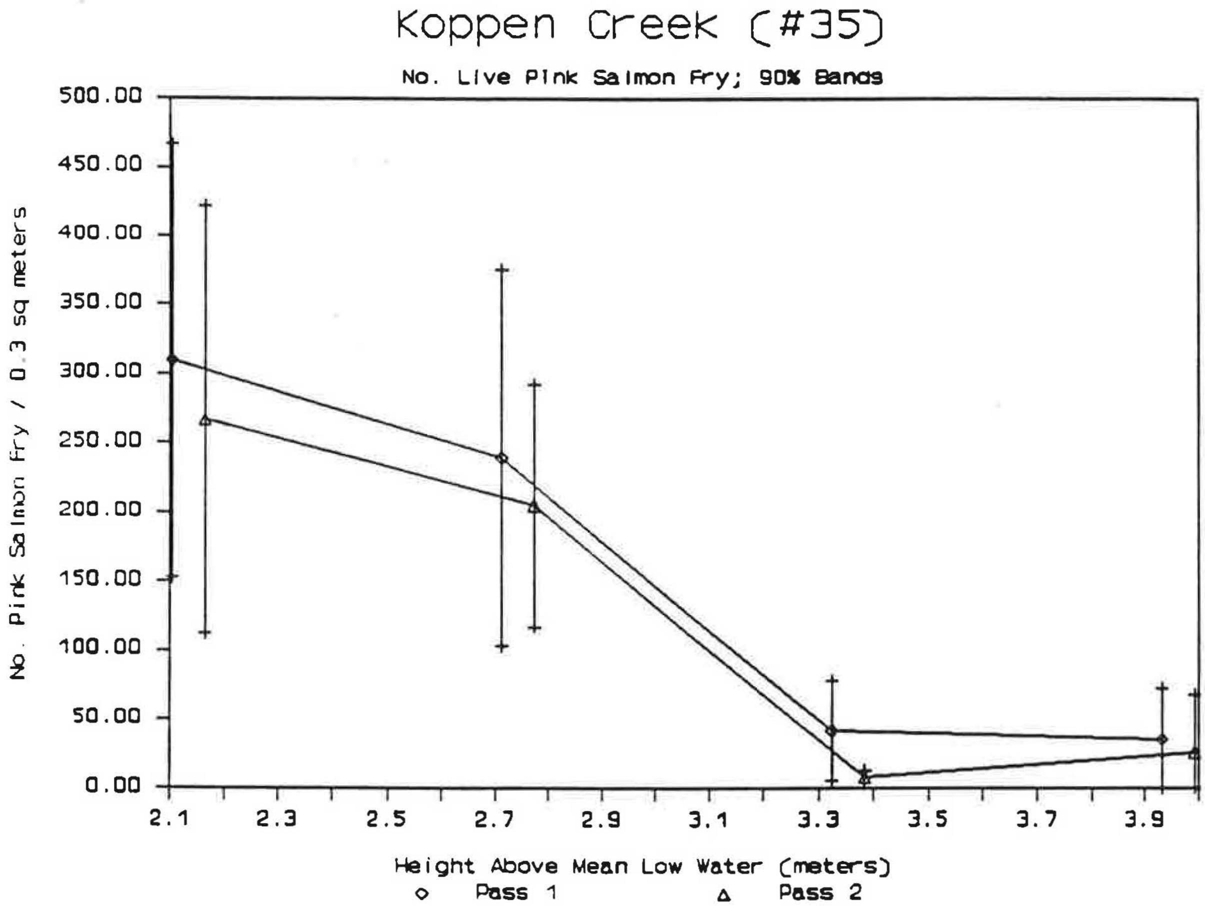
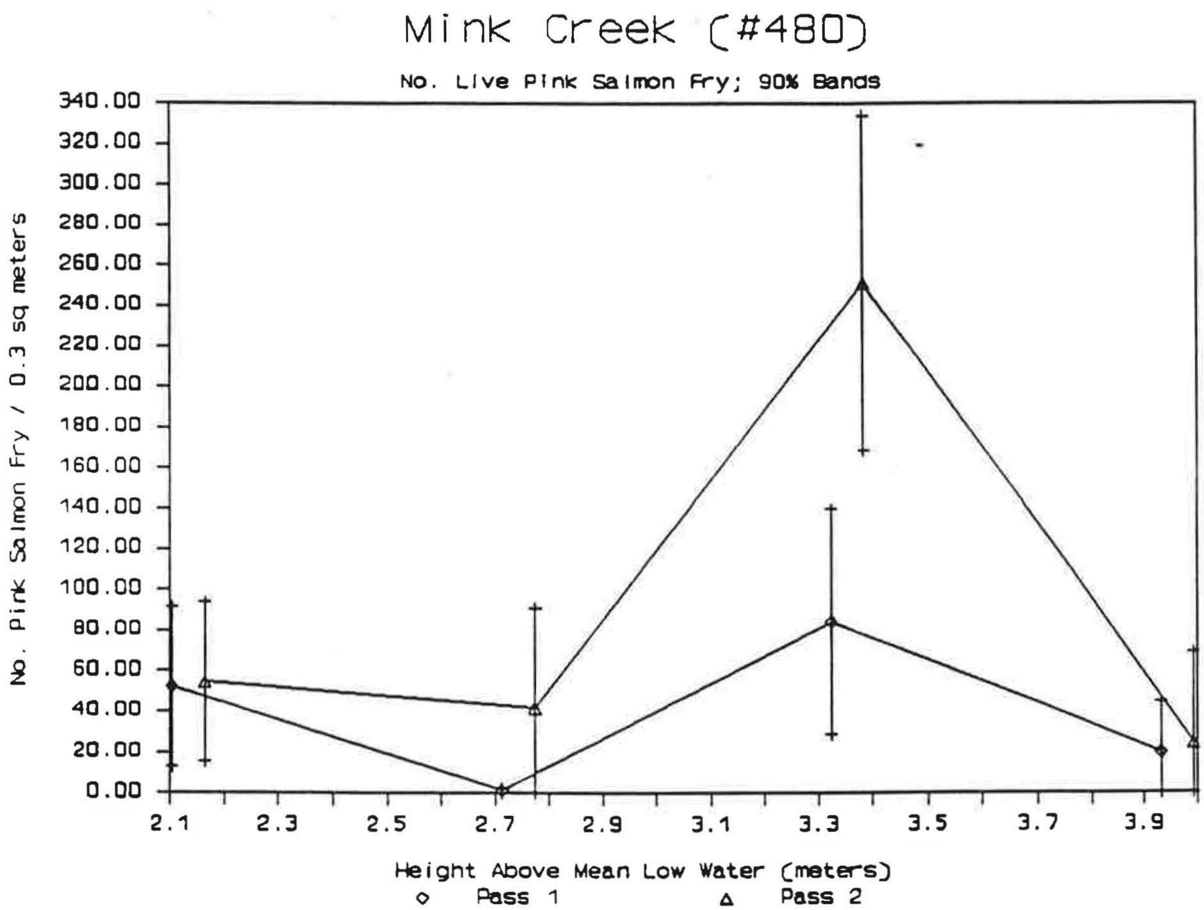
APPENDICES


Koppen Creek (#35)
No. Live Pink Salmon Fry; 90% Bands
No. Pink Salmon Fry / 0.3 sq meters
500.00
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
3.7
3.9
Height Above Mean Low Water (meters)
Pass 1
Pass 2



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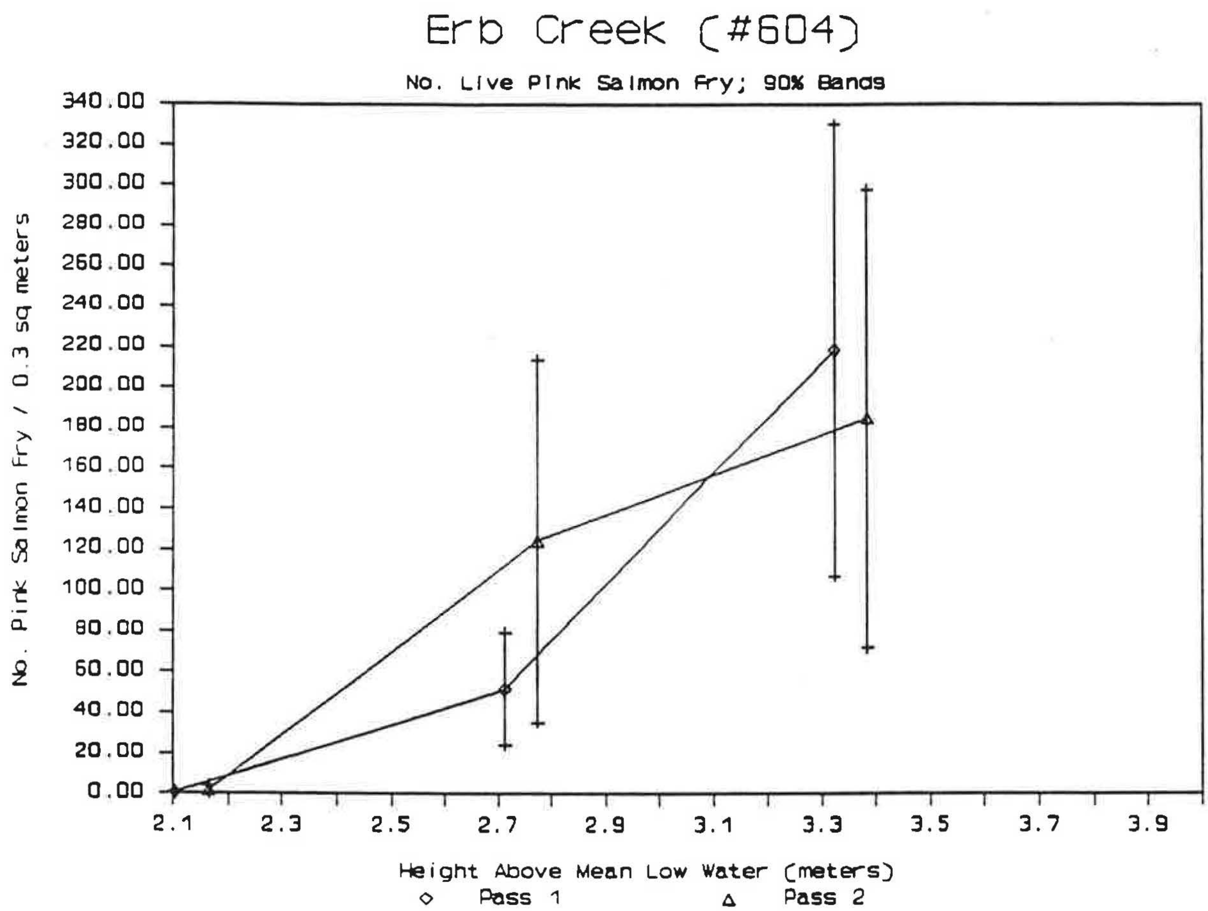

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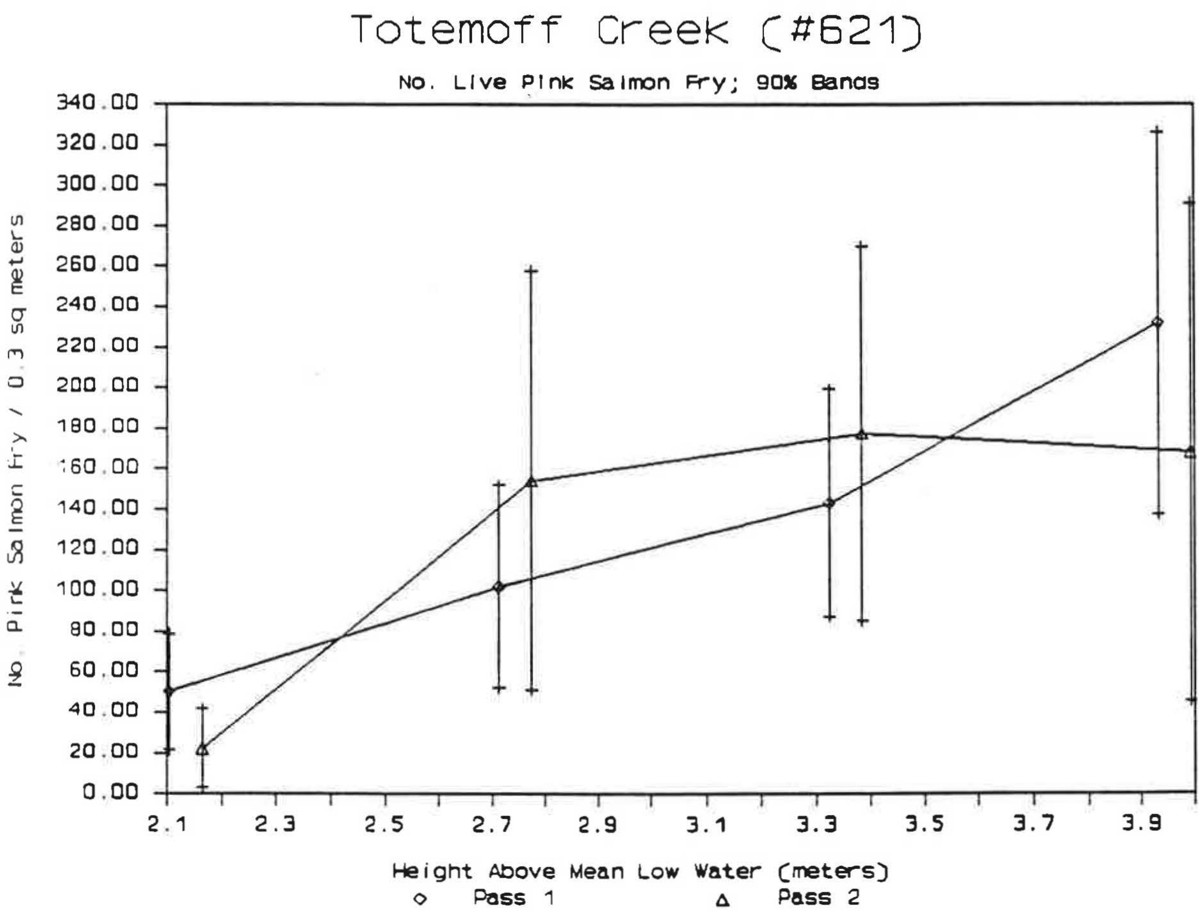

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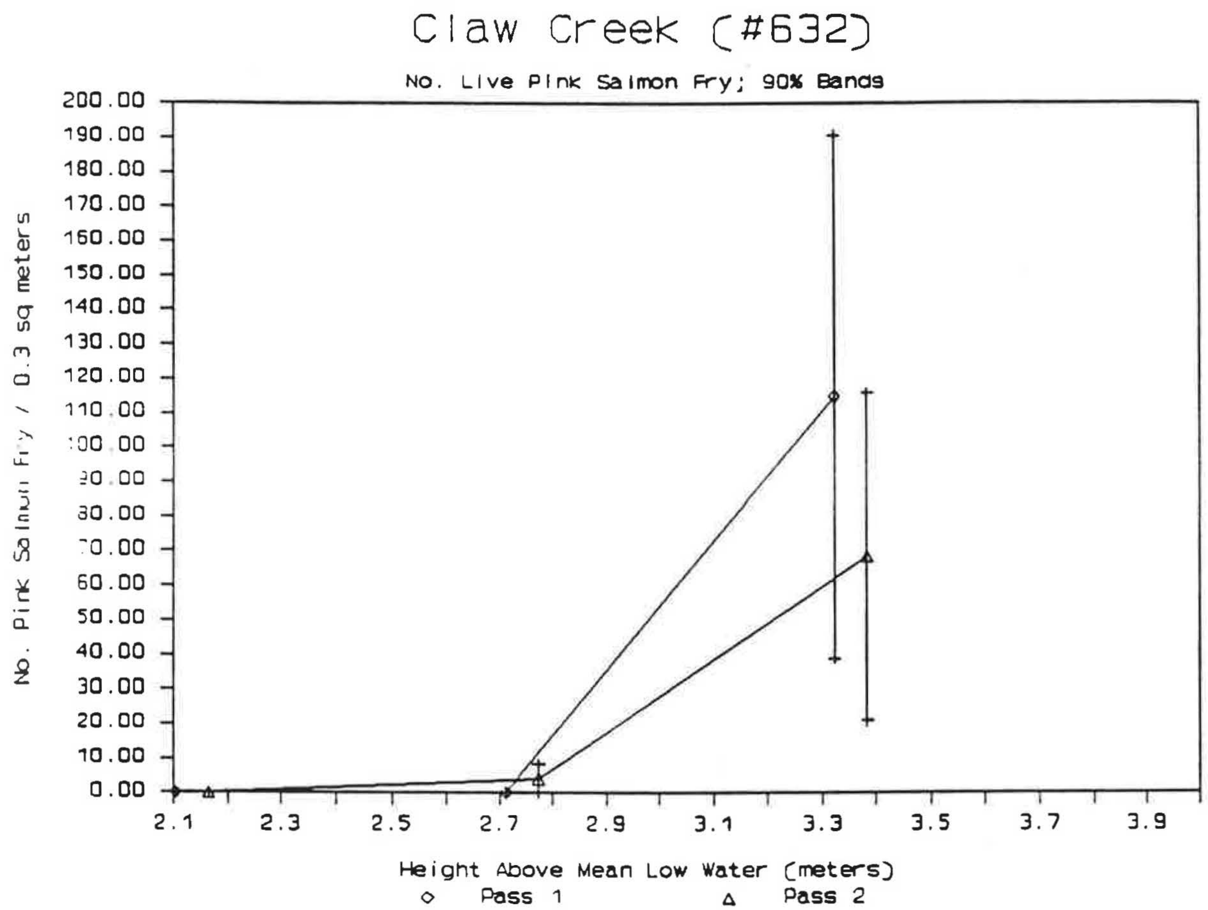

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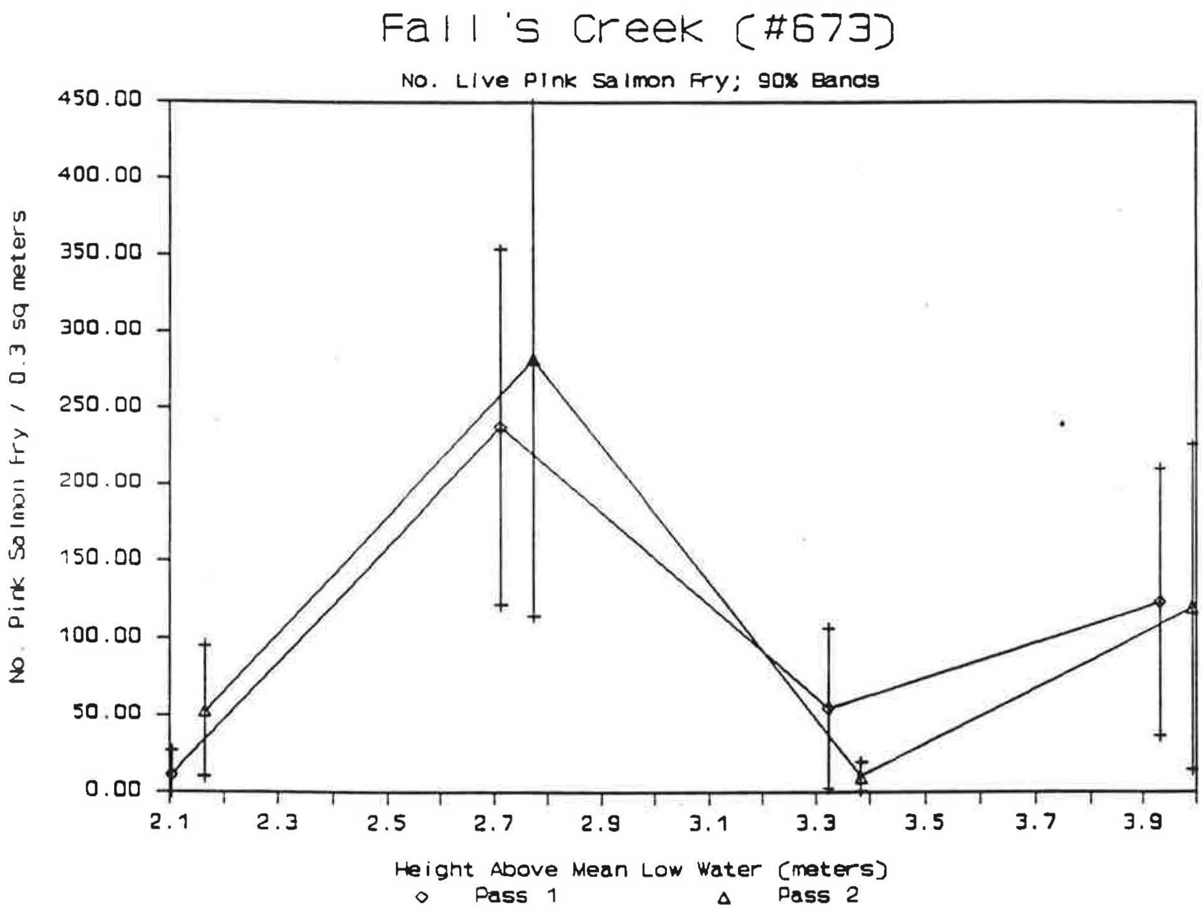
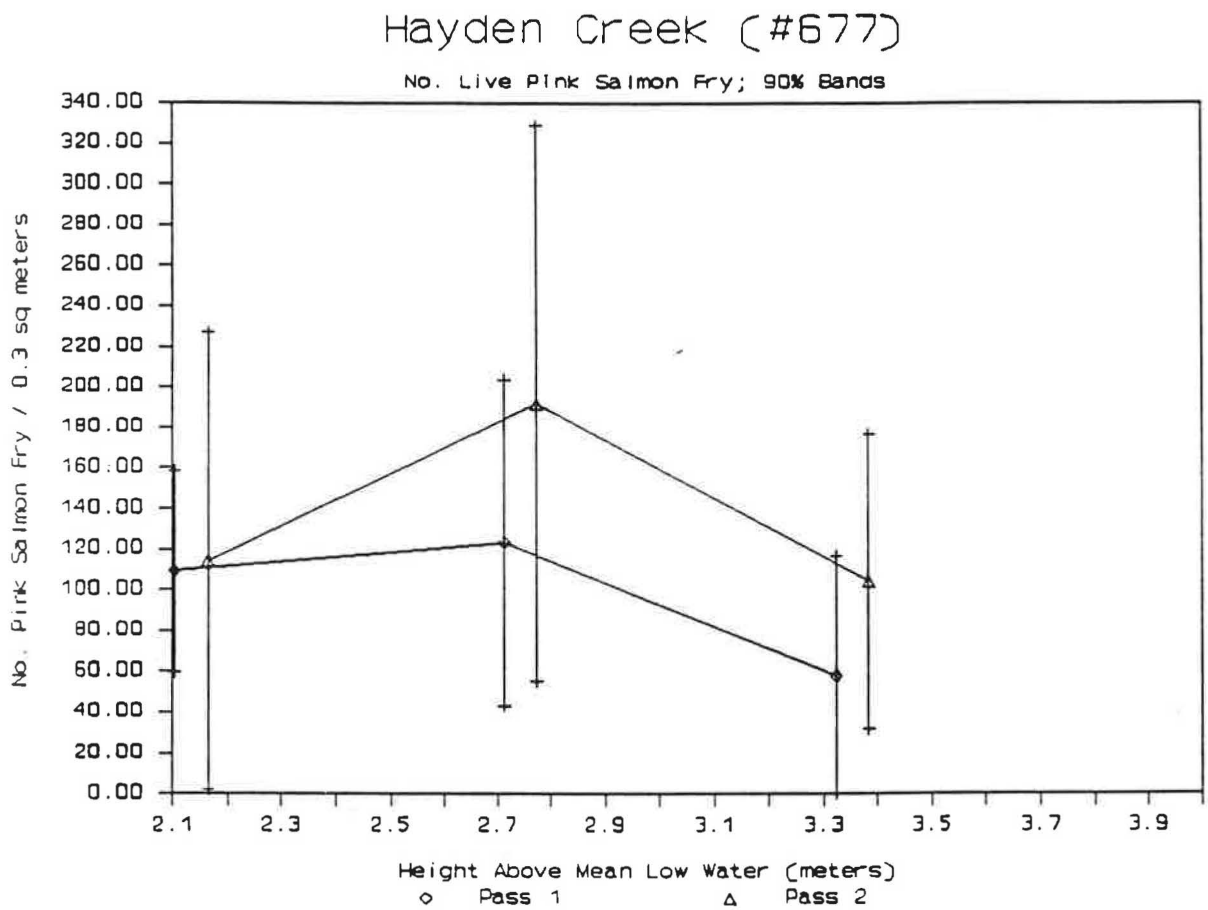
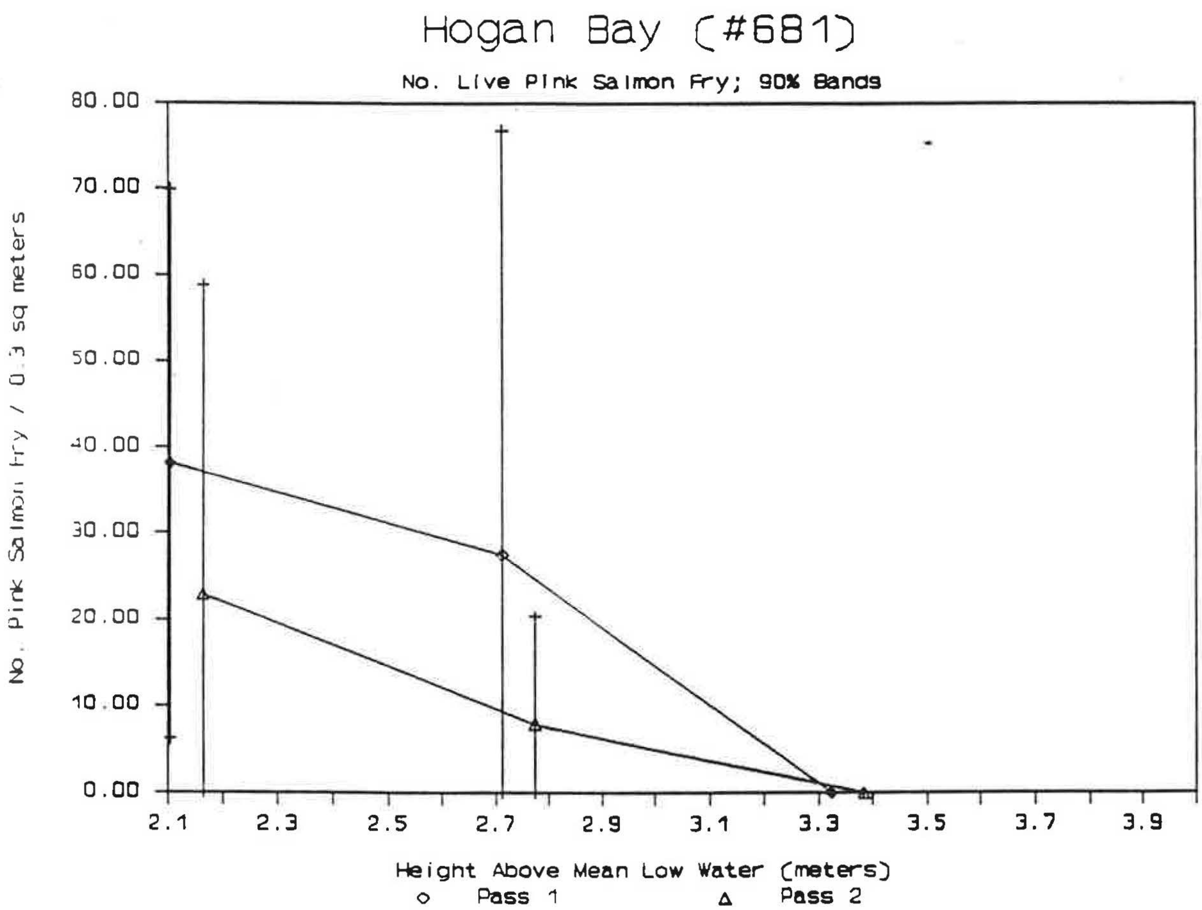
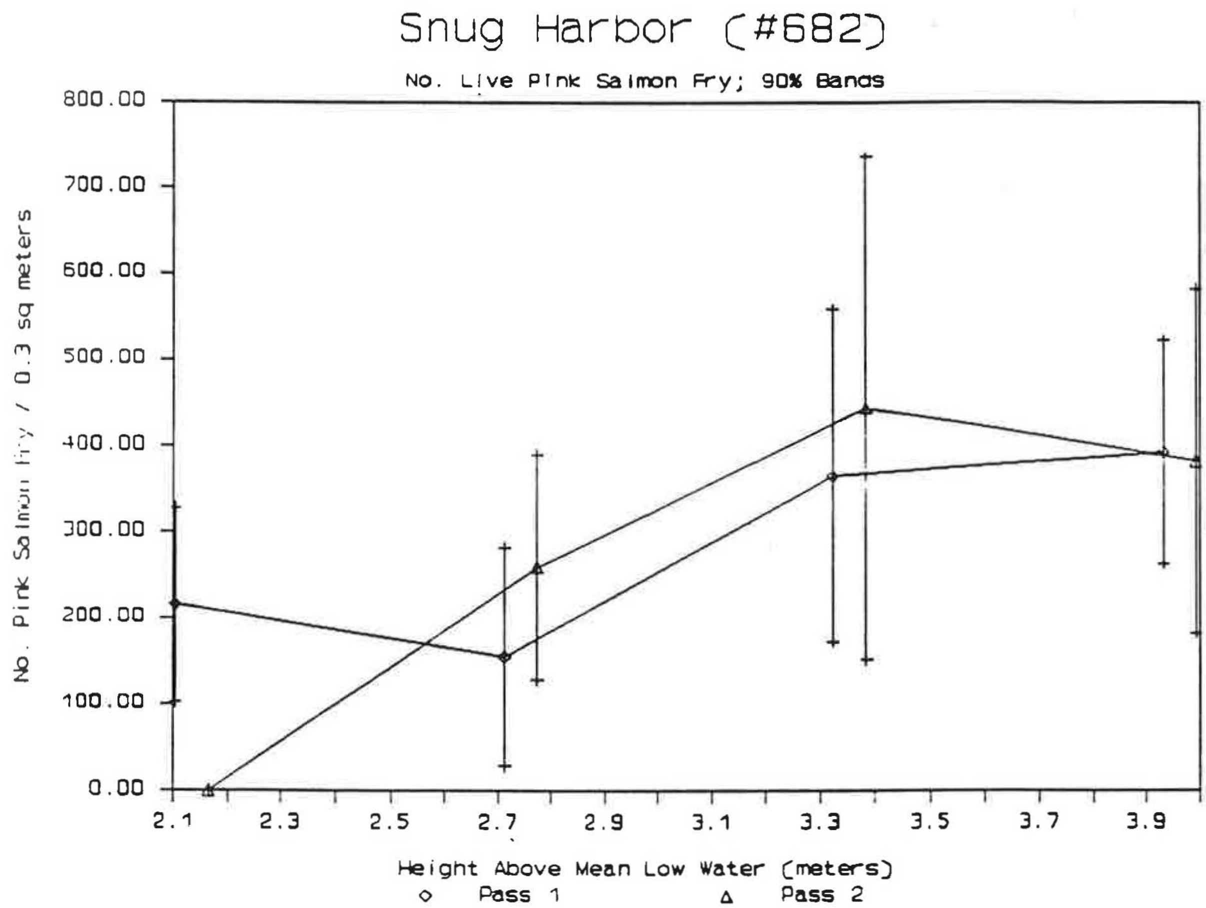
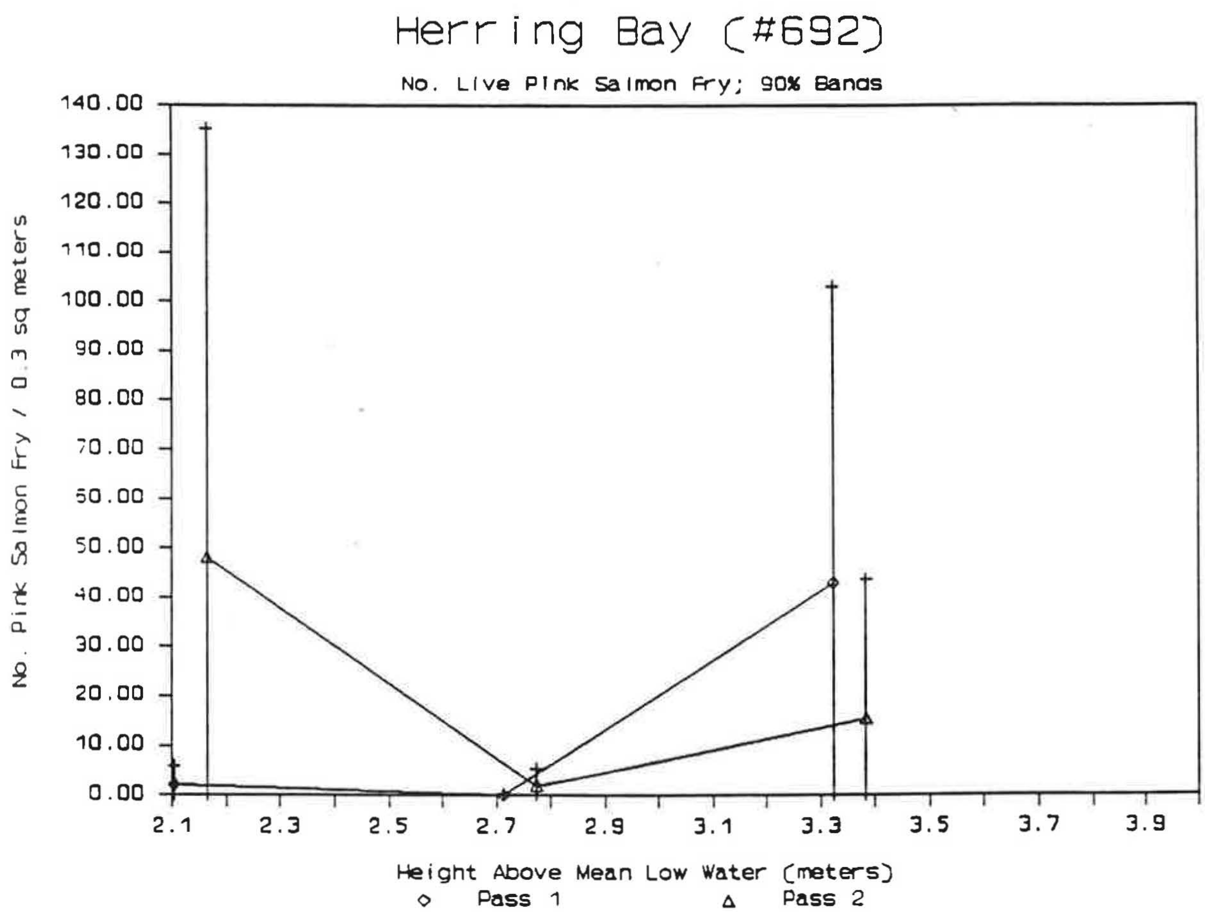
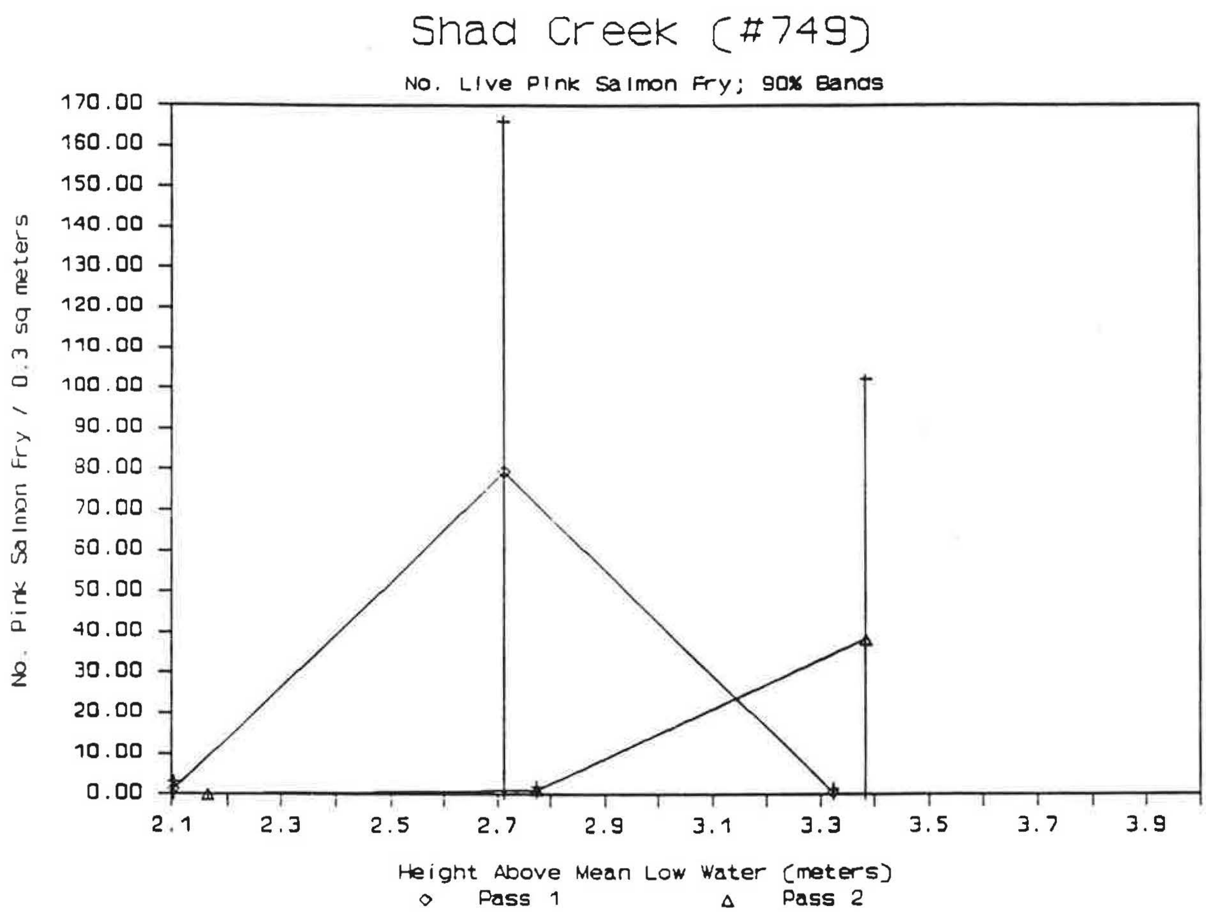

Fall's Creek (#673)
No. Live Pink Salmon Fry; 90% Bands
No. Pink Salmon Fry / 0.3 sq meters
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
3.7
3.9
Height Above Mean Low Water (meters)
◇ Pass 1
△ Pass 2


Hayden Creek (#677)
No. Live Pink Salmon Fry; 90% Bands
No. Pink Salmon Fry / 0.3 sq meters
340.00
320.00
300.00
280.00
260.00
240.00
220.00
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
3.7
3.9
Height Above Mean Low Water (meters)
Pass 1
Pass 2


Hogan Bay (#681)
No. Live Pink Salmon Fry; 90% Bands
No. Pink Salmon Fry / 0.3 sq meters
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
3.7
3.9
Height Above Mean Low Water (meters)
Pass 1
Pass 2


Snug Harbor (#682)
No. Live Pink Salmon Fry; 90% Bands
No. Pink Salmon Fry / 0.3 sq meters
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
3.7
3.9
Height Above Mean Low Water (meters)
Pass 1
Pass 2


Herring Bay (#692)
No. Live Pink Salmon Fry; 90% Bands
No. Pink Salmon Fry / 0.3 sq meters
140.00
130.00
120.00
110.00
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
3.7
3.9
Height Above Mean Low Water (meters)
Pass 1
Pass 2



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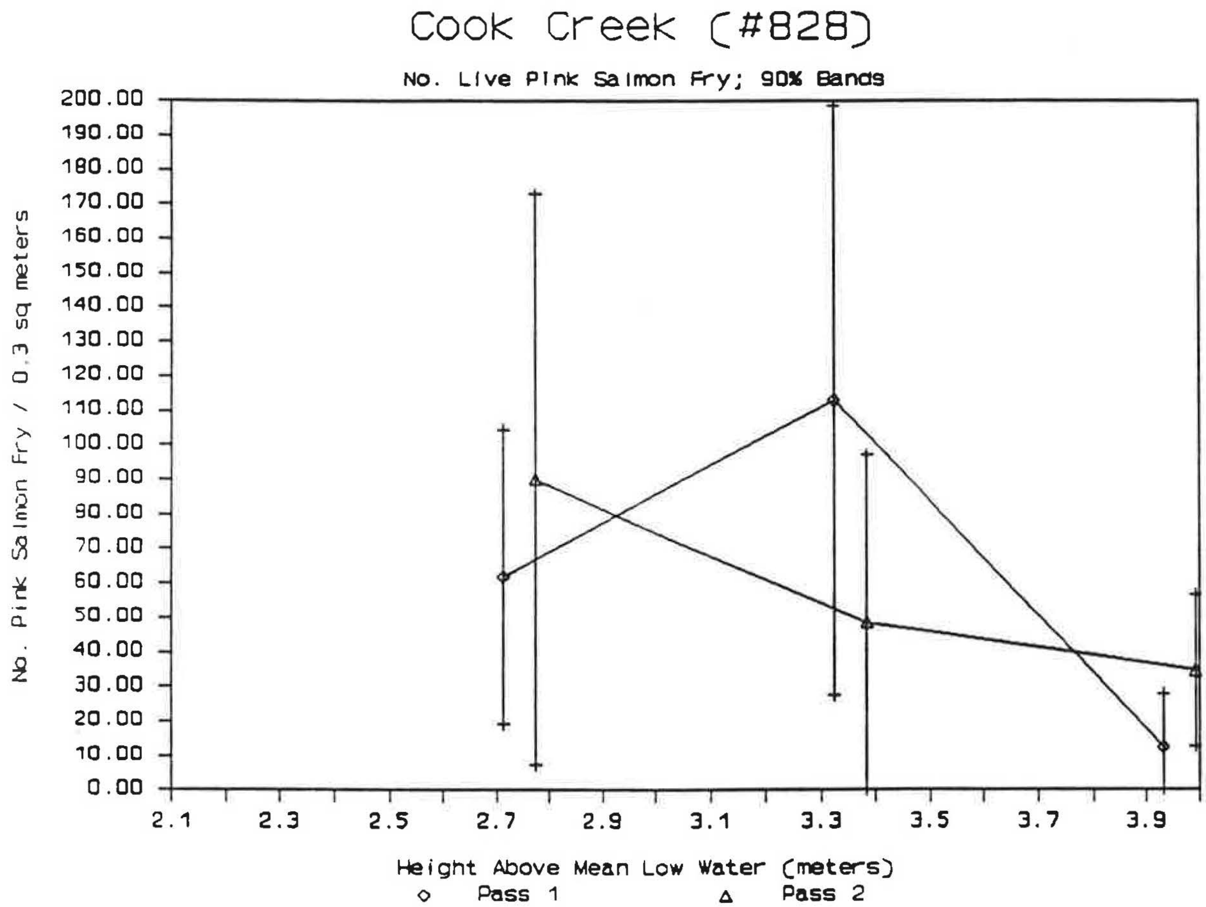
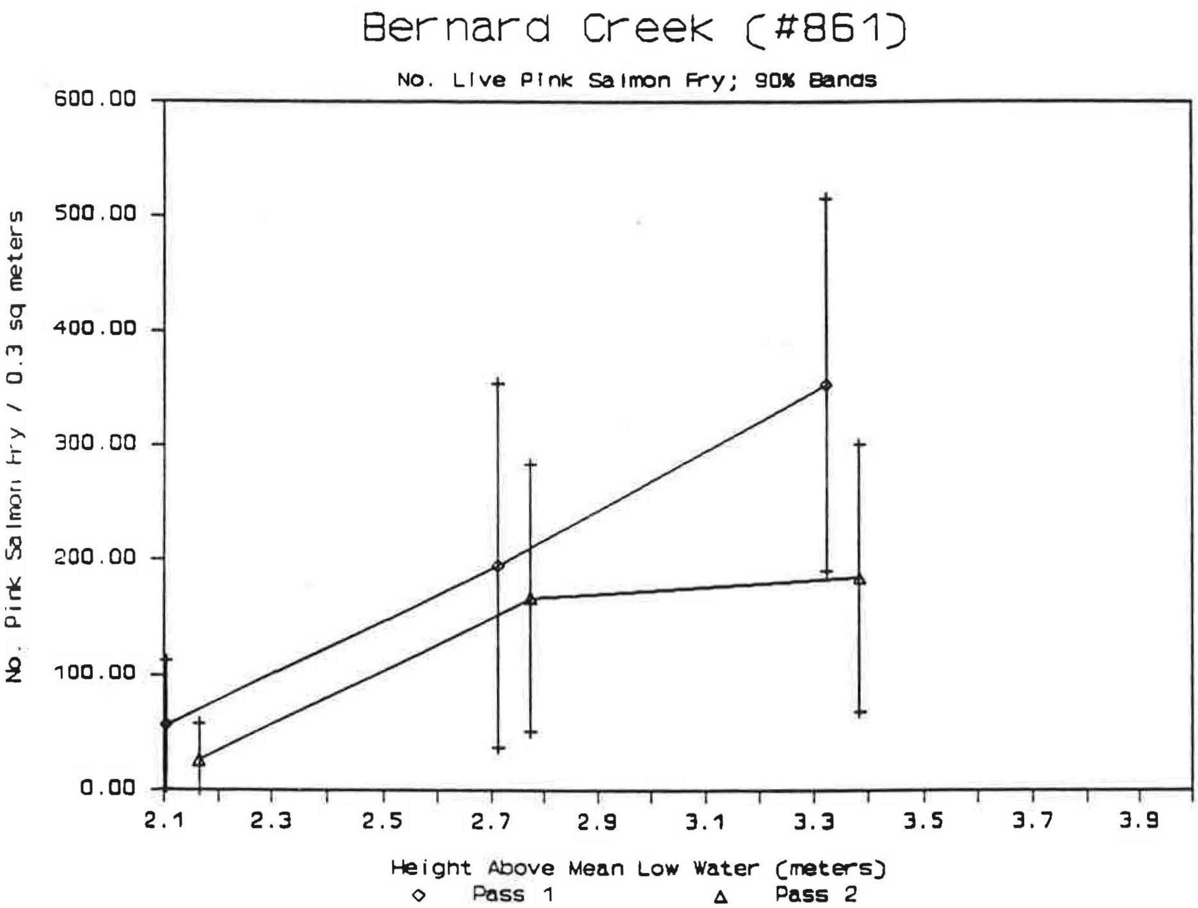


Cook Creek (#828)
No. Live Pink Salmon Fry; 90% Bands
No. Pink Salmon Fry / 0.3 sq meters
200.00
190.00
180.00
170.00
160.00
150.00
140.00
130.00
120.00
110.00
100.00
90.00
90.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
3.7
3.9
Height Above Mean Low Water (meters)
◇ Pass 1
△ Pass 2


Bernard Creek (#861)
No. Live Pink Salmon Fry; 90% Bands
600.00
500.00
400.00
300.00
200.00
100.00
0.00
No. Pink Salmon Fry / 0.3 sq meters
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
3.7
3.9
Height Above Mean Low Water (meters)
◇ Pass 1
△ Pass 2

Appendix B.1 Salmon fry samples for hydrocarbon analysis. Collected from gravel at intertidal spawning riffles in salmon streams of Prince William Sound, 1989.

Sample Number	Sample Date	Stream Number	Stream Name	Location
1	20 April 1989	695	None	Drier Bay, East shore of Knight Is., Prince William Sound, Ak.
2	20 April 1989	699	None	Drier Bay, East shore of Knight Is., Prince William Sound, Ak.
3	21 April 1989	740	Kelez Creek	Northwest shore of Montague Is. Prince William Sound, Ak.
4	23 April 1989	828	Cook Creek	Anderson Bay,North shore of Hinchinbrook Is., Prince William Sound, Ak.
5	23 April 1989	861	Bernard Creek	Windy Bay, North shore of Hawkins Is., Prince William Sound, Ak.
6	24 April 1989	455	Paulson Creek	Cochrane Bay, South end of Port Wells, Prince William Sound, Ak.
7	24 April 1989	485	None	West Finger Inlet, Port Nellie Juan Prince William Sound, Ak.
8	24 April 1989	480	Mink Creek	Mink Harbor, Port Nellie Juan Prince William Sound, Ak.
9	24 April 1989	604	Erb Creek	Ewan Bay, Dangerous Passage Prince William Sound, Ak.
10	24 April 1989	621	Totemoff Creek	West shore of Chenega Island, Prince William Sound, Ak.
11	24 April 1989	637	None	Point Countess, Knight Is. Passage, Prince William Sound, Ak.
12	24 April 1989	673	Falls Creek	West shore of Latouche Island, Prince William Sound, Ak.
13	24 April 1989	677	Hayden Creek	West shore of Latouche Island, Prince William Sound, Ak.
14	24 April 1989	682	None	Snug Harbor, East shore of Knight Is. Prince William Sound, Ak.

Appendix B.2 Salmon fry samples for pathology analysis. Collected from gravel at intertidal spawning riffles in salmon streams of Prince William Sound, 1989.

Sample Number	Sample Date	Stream Number	Stream Name	Location
1	14 April 1989	485	None 1/	West Finger Inlet, Port Nellie Juan Prince William Sound, Ak.
2	14 April 1989	485	None	West Finger Inlet, Port Nellie Juan Prince William Sound, Ak.
3	15 April 1989	495	Chimevisky Creek	McClure Bay, Port Nellie Juan Prince William Sound, Ak.
4	15 April 1989	506	Loomis Creek	Mainland shore North of Eshamy Bay Prince William Sound, Ak.
5	16 April 1989	604	Erb Creek	Ewan Bay, Dangerous Passage Prince William Sound, Ak.
6	16 April 1989	621	Totemoff Creek	West shore of Chenega Is. Prince William Sound, Ak.
7	16 April 1989	630	Bainbridge Creek	West arm of Whale Bay, Prince William Sound, Ak.
8	17 April 1989	632	Claw Creek	Between East and West arms of Whale Bay Prince William Sound, Ak.
9	17 April 1989	637	None	Point Countess, east of Whale Bay, Prince William Sound, Ak.
10	17 April 1989	673	Falls Creek	West shore of Latouche Island, Prince William Sound, Ak.
11	18 April 1989	677	Hayden Creek	West shore of Latouche Island, Prince William Sound, Ak.
12	18 April 1989	678	None	Sleepy Bay, North end of Latouche Is., Prince William Sound, Ak.
13	18 April 1989	663	None	Shelter Bay, North end of Evans Is., Prince William Sound, Ak.
14	19 April 1989	628	None	East shore of Chenega Is. Prince William Sound, Ak.
15	19 April 1989	692	None	Herring Bay, Knight Island, Prince William Sound, Ak.
16	20 April 1989	695	None	Port Audrey, Drier Bay, Knight Is., Prince William Sound, Ak.
17	21 April 1989	682	None	Snug Harbor, Knight Is., Prince William Sound, Ak.
18	21 April 1989	681	None	Hogan Bay, Knight Is., Prince William Sound, Ak.
19	21 April 1989	740	Kelez Creek	Northwest shore of Montague Is. Prince William Sound, Ak.
20	22 April 1989	749	Shad Creek	Port Chalmers, Montague Is., Prince William Sound, Ak.
21	23 April 1989	828	Cooke Creek	Anderson Bay, Hinchinbrooke Is., Prince William Sound, Ak.
22	23 April 1989	861	Bernard Creek	Windy Bay, Hawkins Is., Prince William Sound, Ak.
23	24 April 1989	35	Koppen Creek	Sheep Bay, Prince William Sound, Ak.

1/ The first sample collected at stream 485 (sample #1) was collected above the intertidal zone. all other samples were collected approximately 9 feet elevation above mean low tide.