INTERNATIONAL PACIFIC SALMON
FISHERIES COMMISSION

PROGRESS REPORT

No. 28

TESTS WITH NI-FURPIRINOL (P7138) TO CONTROL PRESPAWNING MORTALITIES OF FRASER RIVER SOCKEYE

BY

I. V. WILLIAMS

NEW WESTMINSTER, B. C.
CANADA
1973

INTERNATIONAL PACIFIC SALMON
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Appointed under a Convention
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Protection, Preservation and Extension of
the Sockeye and Pink Salmon Fisheries
in the Fraser River System

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ABSTRACT

Ni-Furpirinol (P7138), a nitrofuran compound effective against some fish diseases, was tested for its effectiveness in controlling prespawning mortalities of two races of Fraser River sockeye during 1970. Adult fish captured shortly before spawning were held in 6-ft diameter fiberglass tanks and administered 1 and 3 ppm treatments of Ni-Furpirinol. Experimental results were compared with the natural spawning populations and indicated that Ni-Furpirinol was effective to a degree in controlling Chondrococcus columnaris but did not prevent prespawning deaths of the early fish. An experiment to determine retention time of Ni-Furpirinol in flesh, gonad and skin of both male and female sockeye showed no traces of Ni-Furpirinol 24 hr after a 1 ppm treatment of 1 hr. Although effective in certain respects Ni-Furpirinol is not considered a practical therapeutic agent for prevention of prespawning mortalities of Fraser River sockeye.

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TESTS WITH NI-FURPIRINOL (P7138) TO CONTROL PRESPAWNING MORTALITIES OF FRASER RIVER SOCKEYE

INTRODUCTION

Prespawning mortalities of sockeye salmon (Oncorhynchus nerka) throughout the Fraser River watershed have caused serious losses, primarily to the early segment of races undergoing extensive river migration. The Horsefly River population has had the largest sustained losses since its expansion from a few thousand spawners in 1949 to its present population level of several hundred thousand. Losses have reached as high as 62% with the average mortality for six dominant year-classes being 34.1% (Williams 1973). Chilko River had the largest prespawning mortality recorded for the watershed when 90% or 910,000 fish died without spawning in 1963. Prespawning losses at Chilko have averaged 13.1% from 1945 to 1970.

The International Pacific Salmon Fisheries Commission has been investigating this problem in an attempt to understand and if possible prevent such losses. The commercial fishery might be used to crop the early segment of the runs affected thus removing the segment of the population most subject to prespawning mortalities. This solution has serious handicaps, involving an overlap of timing of the migrations of various races. Thus, investigations of means of preventing these mortalities on the spawning grounds have been made.

On the basis of bacteriological investigations, initiated in 1963, two categories of bacterial infections have been observed in association with prespawning mortality:

1. A myxobacterium, Chondrococcus columnaris has reached epidemic proportions among fish on the spawning grounds in years of high water temperature (Wood 1965; Colgrove and Wood 1966). The afflicted fish appear lethargic with gross body and gill lesions. The large mortalities at Horsefly in 1961 and 1965 and at Chilko in 1963 were associated with C. columnaris.

2. In years of normal water temperatures, various types of bacteria, similar to these associated with bacterial gill disease, have been observed. The fish appear healthy until just before death, and upon death, only a mottled color to the gills can be detected by gross examination. However,

microscopic examination reveals massive damage to the gill tissue. These bacteria have been associated with mortality at Birkenhead and Gates Creek in 1968 and at Horsefly in 1969 (Mead MS 1969).

The evidence that substantial mortalities can occur in relatively cool water plus the positive identification of the viral agent Infectious Hematopoietic Necrosis (IHN), which proliferates at relatively low water temperatures, as responsible for heavy mortalities among hatchery reared fish at Cultus Lake (Amend et al. 1969) prompted the Commission to consider the possibility of a virus causing prespawning mortalities. A survey for viral infections was carried out during 1968 on five spawning populations. The data, which included samples taken from moribund unspawned fish, failed to indicate any viral agent that could be linked to these prespawning mortalities (Williams MS 1972).

Experiments with adult Horsefly sockeye suggested that outbreak of C. columnaris, could be controlled by keeping water temperature lower than 57 to 58°F (Colgrove and Wood 1966). In 1969 the Commission completed a pilot project on McKinley Creek, a tributary of the Horsefly River to test the effectiveness of control of water temperature in preventing prespawning mortality. Although outbreak of C. columnaris was controlled, prespawning mortalities approximating 65 and 50%, respectively, were recorded for McKinley and Horsefly populations (Williams 1973).

Therefore, in 1970 experiments were set up at both the Birkenhead and Chilko Rivers (FIGURE 1) during the spawning season, to test the effectiveness of Ni-Furpirinol (P7138) in controlling prespawning mortalities of Fraser River sockeye salmon.

DESCRIPTION OF THE SPAWNING POPULATIONS

Birkenhead

The sockeye population at Birkenhead in 1970 consisted of 72,760 spawners of which approximately 20,800 were females. The first arrivals on the spawning ground were noted on September 3 and all of the population was present by September 25. Peak of spawning occurred during September 24 to 26.

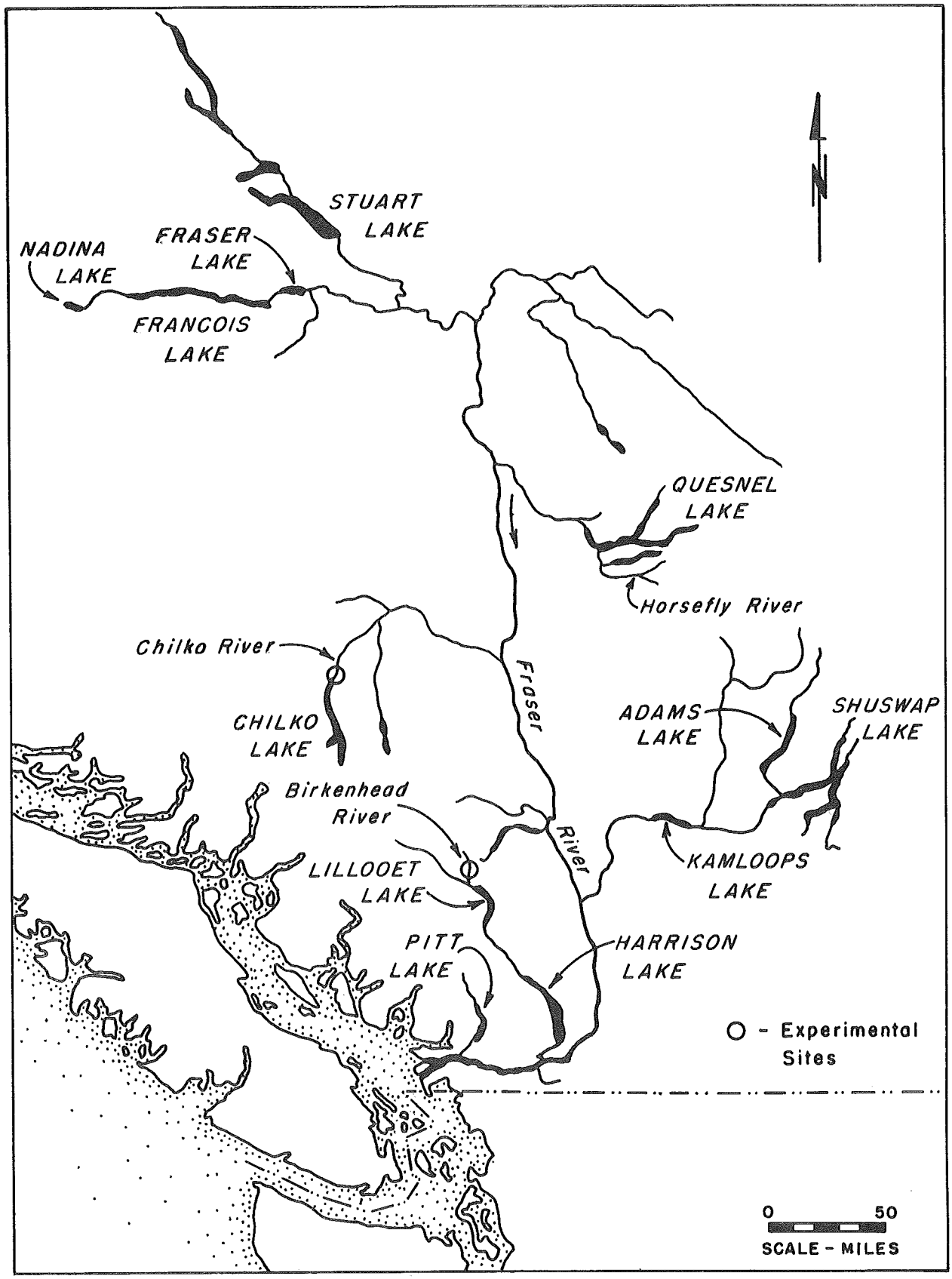

FIGURE 1 - Fraser River watershed showing locations of prespawning control experiments in 1970.

A high prespawning mortality occurred among the very early fish but very little prespawning loss occurred among later fish (FIGURE 2). The early arrivals equivalent to those sampled for treatment had a 21.1% prespawning mortality compared with an overall loss of 7.5% for the entire spawning population.

Chilko

The total 1970 sockeye population on the Chilko River spawning grounds was 145,000 spawners of which approximately 71,900 were females. The first fish were observed on August 12 and nearly all were present by September 15 (FIGURE 3). The peak of spawning occurred during September 22 to 24.

Prespawning mortalities ranged from 100% for the very early fish down to 0% at the peak of dying and averaged 16.9% for the entire run. Dead females recovered during the period September 3 to 25 accounted for 37% of the total females recovered and probably represent the early arrivals on the spawning ground as chronological order of arrival and death are generally maintained (Killick 1955). This group of fish had a mean prespawning mortality of 51.2% compared with a 1.5% prespawning mortality in the group of fish recovered from September 26 to October 9 (FIGURE 3).

MATERIALS AND METHODS

Description of Ni-Furpirinol

The compound 6-hydroxymethyl-2-pyridine, or Ni-Furpirinol (P7138), has a broad antimicrobial spectrum, showing inhibitory activity *in vitro* at low concentrations against at least 25 microorganisms including some strains isolated from fishes. Therapeutic benefits were also demonstrated *in vivo* both by bathing and by oral administration to fishes infected with pathogenic bacteria (Shimizu and Takase 1967). Subsequent testing at the Western Fish Disease Laboratory in Seattle proved the drug to be effective against several bacteria including *C. columnaris in vitro* (Ross 1972). In addition, concentrations as small as 1 ppm controlled columnaris disease *in vivo* using juvenile salmonids as test animals (Amend and Ross 1970). Field tests conducted under hatchery conditions indicated that Ni-Furpirinol may be

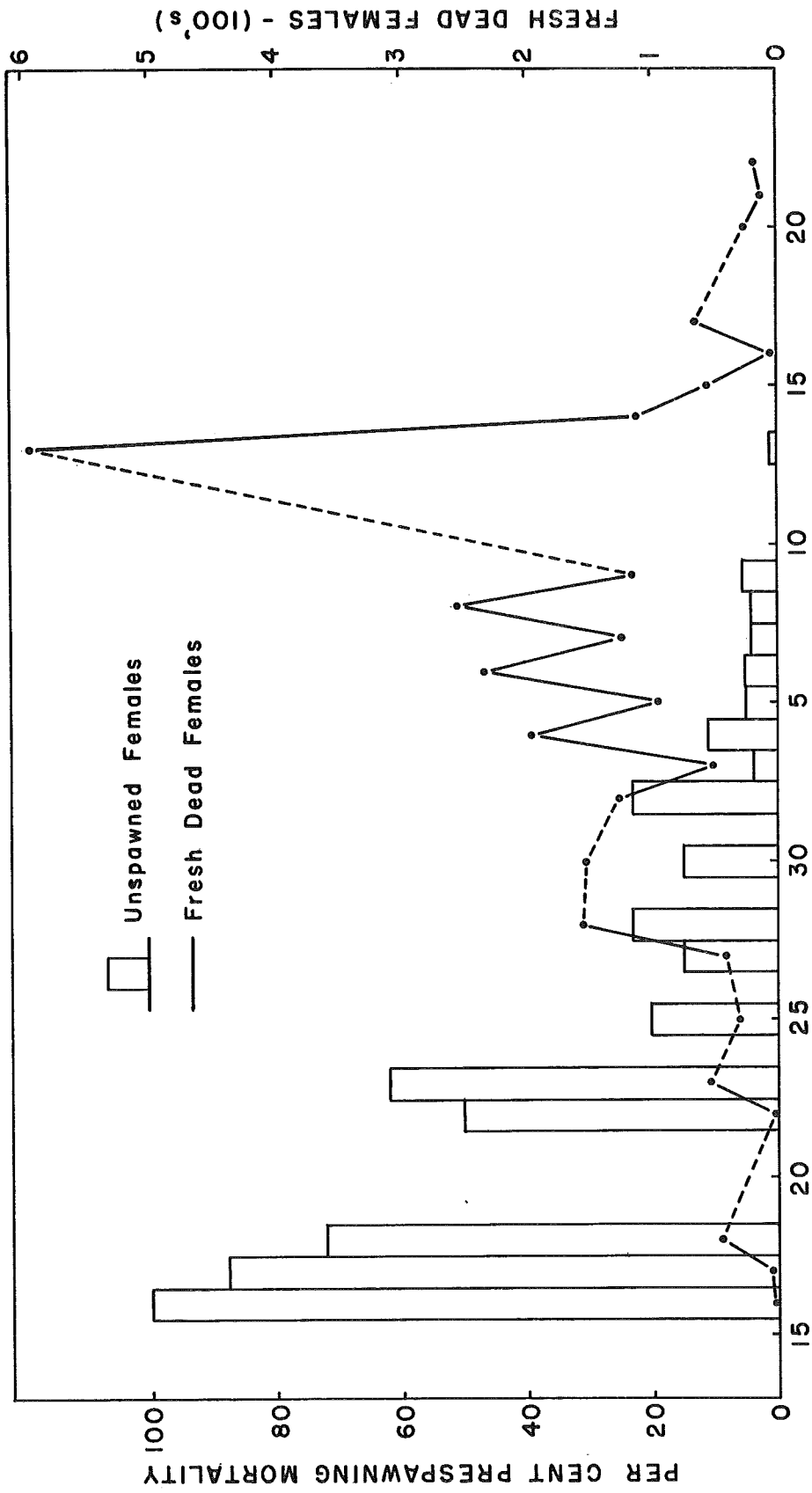

FIGURE 2 - Birkenhead sockeye dead recovery and per cent prespawning mortality of females, 1970.

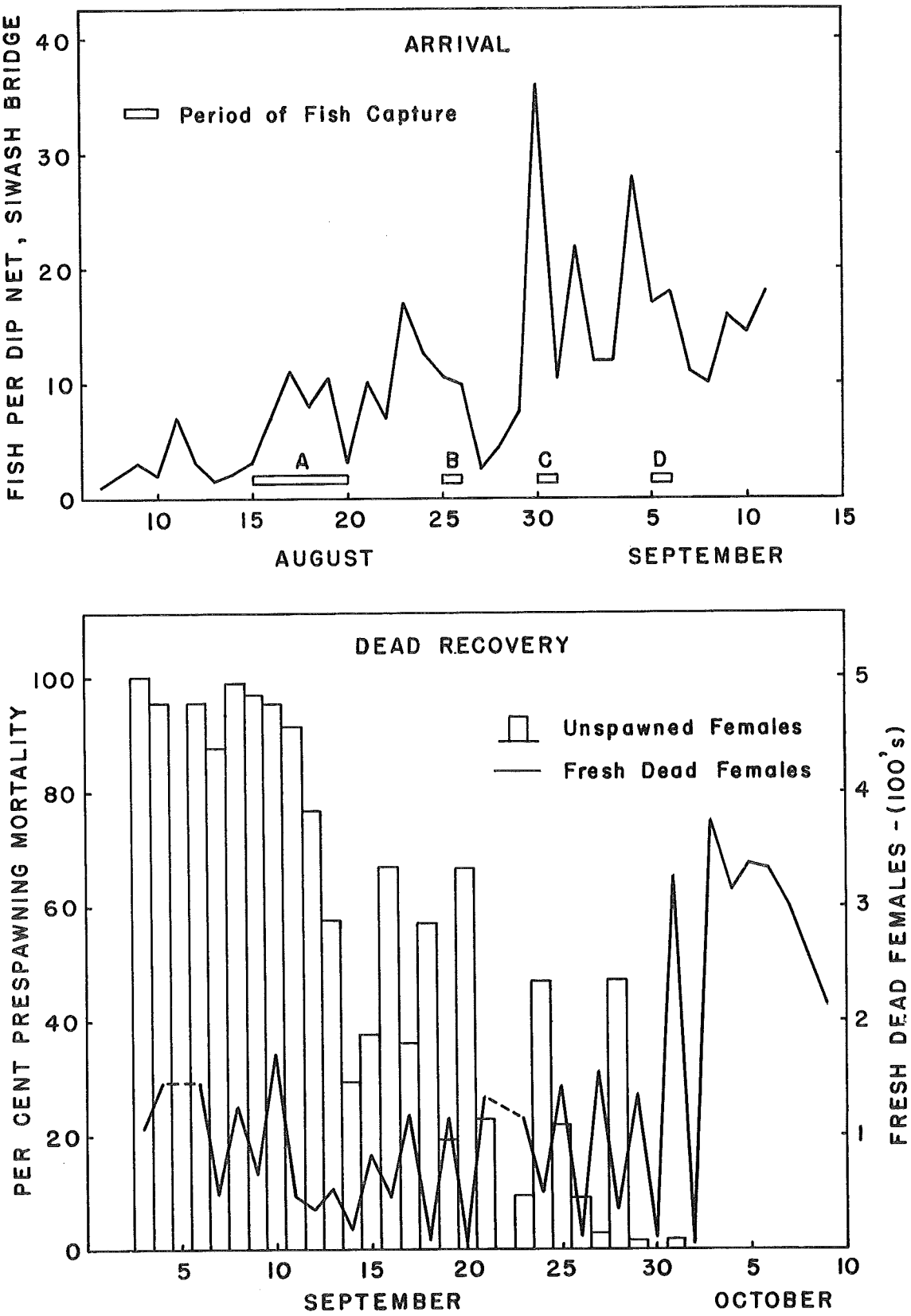

FIGURE 3 - Chilko sockeye arrival, dead recovery and per cent of dead females unspawned, 1970.

effective in controlling at least some types of bacterial gill infection as well as columnaris on juvenile salmon (Wood personal communication).

The drug Ni-Furpirinol has a half-life of approximately four days when exposed to the ultraviolet component of natural light and therefore would tend to break down very quickly after treatment, thus minimizing any possible detrimental effects on streams or lakes. Preliminary cost estimates indicated that it was also inexpensive compared with other drugs.

Capture of Fish

At Birkenhead, a total of 40 adult sockeye (20 male and 20 female) from the early segment of migration were seined from the river approximately 6 miles below their spawning grounds (FIGURE 4).

At Chilko a portion of the very early as well as the early and peak sockeye were seined from the Chilko River approximately 3 miles below the spawning grounds. The remaining very-early fish were sampled on arrival at the spawning grounds (FIGURE 5). A total of 50 adult sockeye were captured from the very-early segment of the migration; 96 fish were sampled from the early segment, and 60 sockeye were captured from the peak segment. In addition, 52 spawners were seined from the spawning grounds and transported to the experimental site (TABLE 1).

TABLE 1 - Chilko sockeye sampled in 1970 for drug treatment and retention experiments.

Segment of Population	Date Captured	Experiment	No. Males	No. Females	Total
Very-Early	Aug. 15-20	Drug Treatment	25	25	50
Early	Aug. 21	Drug Retention	18	18	36
Early	Aug. 25-26	Drug Treatment	24	36	60
Peak	Aug. 30-31	Drug Treatment	24	36	60
Spawning	Sept. 5	Drug Treatment	16	24	40
Spawning	Sept. 6	Drug Retention	4	8	12
Total			111	147	258

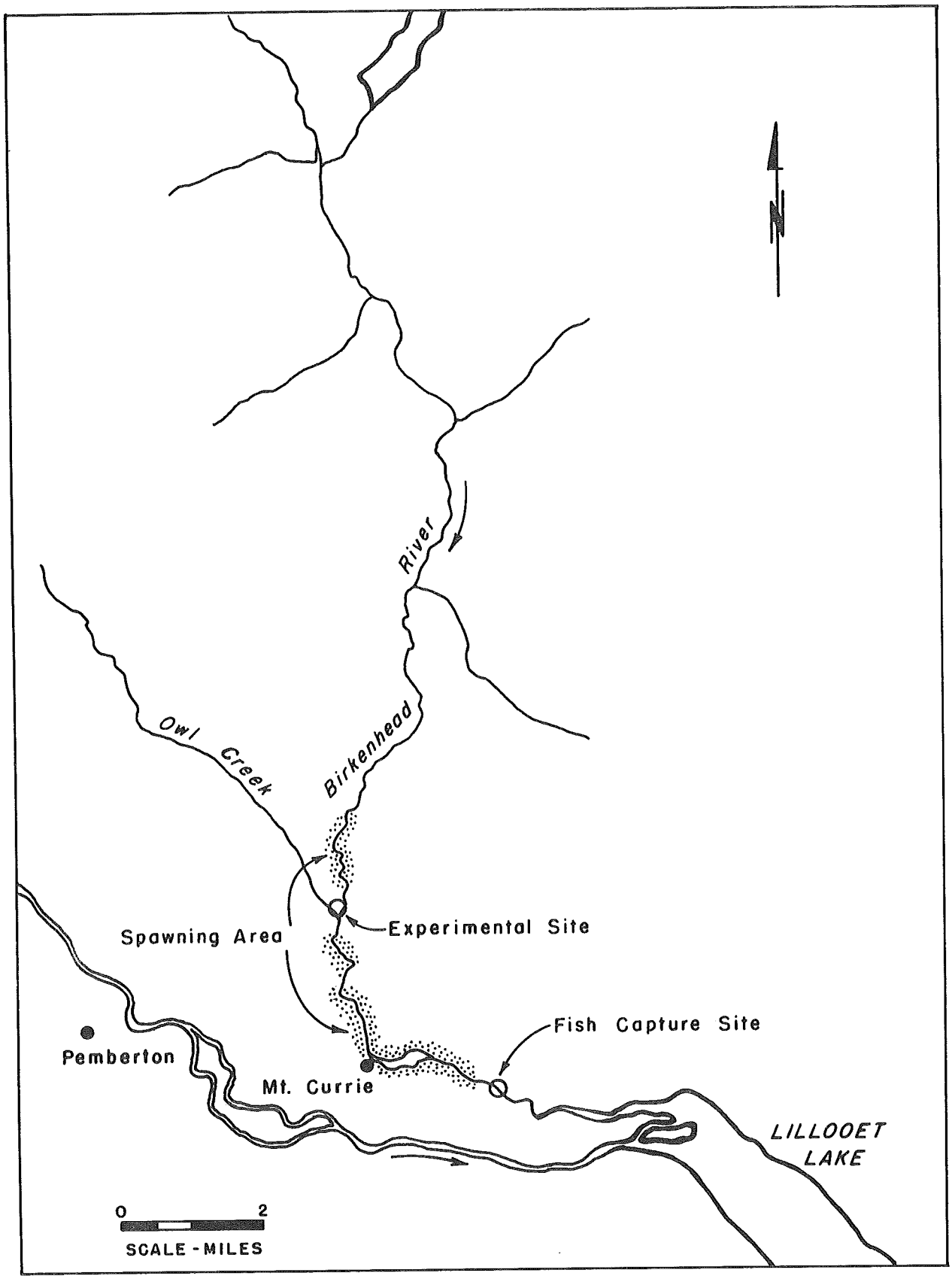

FIGURE 4 - Spawning areas, experimental area, and fish capture site on the Birkenhead River.

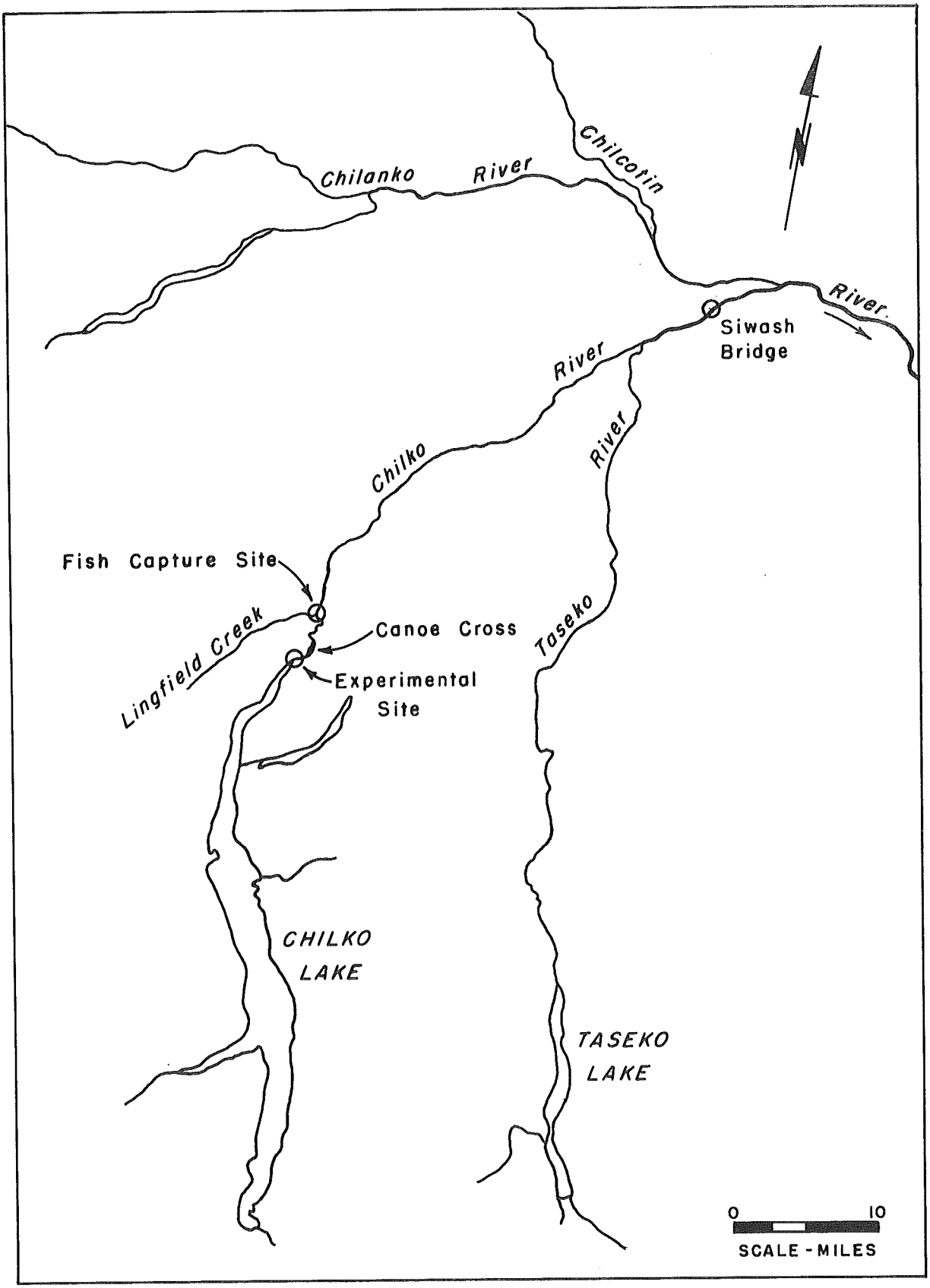

FIGURE 5 - Experimental and fish capture sites on the Chilko River.

Physical measurements were collected from additional small samples of early and peak fish as they arrived on the Chilko spawning grounds. Measurements included fork length, total weight, viscera weight, gonad weight and eviscerated body weight. Physical measurements were not obtained from the Birkenhead population.

Transport and Holding of Fish

All sockeye captured from Birkenhead River were transported to the experimental site in a 300-gal wooden transportation box at a maximum density of 30 fish per trip. Oxygen, diffused through the water with air-stones, was used to sustain the fish. The Birkenhead experimental site was located on the right bank of the Birkenhead River, 7 miles upstream from the river's outlet into Lillooet Lake, adjacent to the spawning ground of the early population (FIGURE 4). Approximately 11% of the spawners were also located upstream from the experimental water intake in Birkenhead River. Only the early segment of the Birkenhead migration sampled September 9 to 10 was used for this experiment.

The Chilko experimental site was located on the left bank of the river approximately 1.5 miles from the outlet of Chilko Lake (FIGURE 5). The spawning area at Chilko extends from the outlet of the lake to Canoe Cross with the majority of the spawning occurring upstream from the experimental site. Sockeye captured below the spawning grounds at Lingfield Creek were transported to the experimental site in a 300-gal wooden transportation box similar to the method at Birkenhead; those from the spawning grounds were placed in a wire-mesh live box and floated down the river to the experimental site.

At both experimental sites the fish were held in 6-ft diameter fiberglass tanks (capacity 300 gal) at a density of 10 fish per tank or 1 lb fish in 50 lb water. Centrifugal pumps powered by a diesel electric generator at Birkenhead and by diesel engine at Chilko were used to draw water from their respective rivers, supplying a flow of 10 gpm per tank.

Thermographs were used to record temperatures on the spawning grounds and in the experimental tanks at both Birkenhead and Chilko. Water temperatures in the Birkenhead experimental tanks varied between a maximum of 56.5°F and a minimum of 48.5°F with a mean diurnal variation of 3.9°F.

At Chilko, temperatures in experimental tanks varied between a maximum of 62°F and a minimum of 46°F, with a mean diurnal variation of 2°F. Daily maximum temperatures in the tanks were similar to the river water temperatures at both areas (FIGURES 6 and 7). Analysis of water samples taken from Chilko River near the pump intake showed a dissolved oxygen concentration of 8.73 mg/l or 99% saturation, and total alkalinity of 21 mg/l $CaCO_3$ at hardness of 33.2 ppm.

The Birkenhead fish were held for a total of 12 days and on September 22 the fish remaining were spawned. All surviving Chilko fish, except those for tissue retention studies, were held until September 22 when they were spawned.

Treatment with Ni-Furpirinol

Test groups of sockeye were treated at concentrations of 1 or 3 ppm "active" Ni-Furpirinol (the drug is supplied in granules containing 10% active Ni-Furpirinol agent). The required concentrations in each tank were obtained initially by adding 11.5 or 34.5 gm of Ni-Furpirinol (10% active) for the 1 and 3 ppm tanks, respectively. A stock solution of 10 ppm active Ni-Furpirinol was then mixed with the incoming water to supply the concentration required for the 1-hr treatment period. During treatment periods, effluent from the tanks was diverted to a large reservoir for retention. Thereafter, the tanks were supplied with fresh water and were drained directly into the river.

At Birkenhead, a "treatment" consisted of two doses of the drug administered 24 hr apart, and in two cases this treatment was repeated after an interval of four days. In all, three test groups of Birkenhead sockeye received treatment and one group was held as a control (TABLE 2).

TABLE 2 - Distribution and treatment of experimental fish from early segment of the run at Birkenhead.

Segment of Run	Date Captured	Date Treated	No. Tanks		
			1 ppm Treatment	3 ppm Treatment	Control
Early	Sept. 9-10	Sept. 12-13 only	1	-	1
		Sept. 12-13 and Sept. 17-18	1	1	-

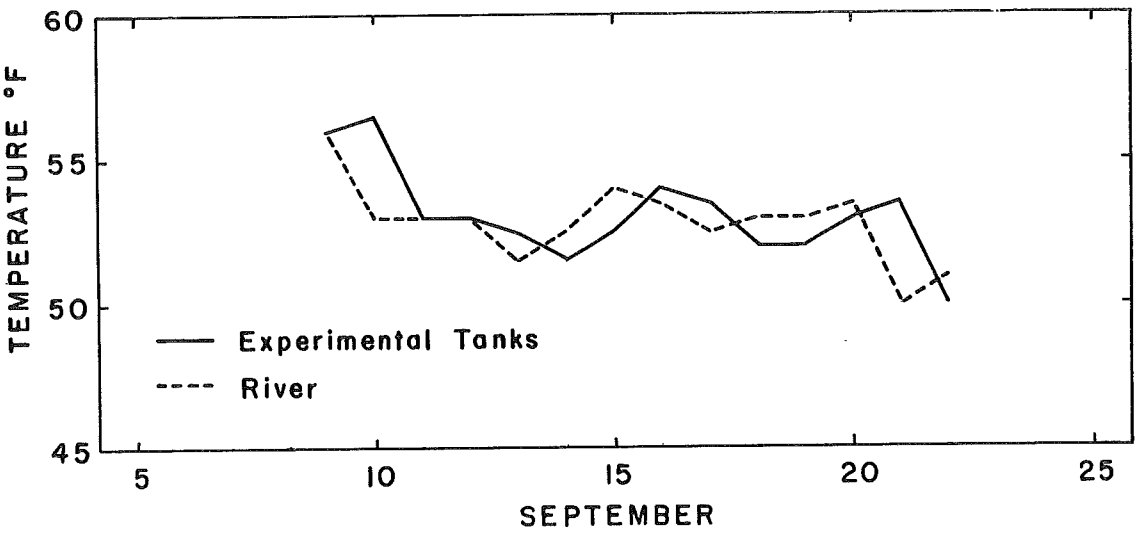

FIGURE 6 - Daily maximum water temperatures for Birkenhead River tests.

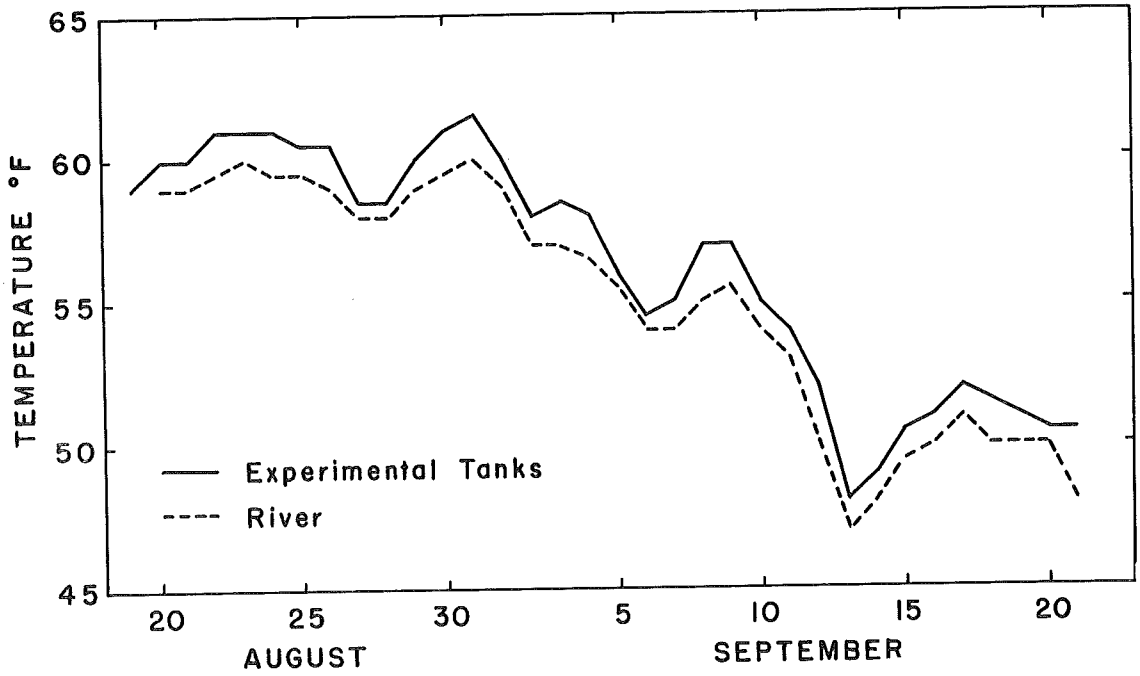

FIGURE 7 - Daily maximum water temperatures for Chilko River tests.

At Chilko only one dose of the drug was administered per treatment, and treatment was repeated at 5- or 6-day intervals. A total of 11 test groups of 10 fish each received treatment and 7 groups of 10 fish each were held as controls (TABLE 3).

TABLE 3 - Distribution and treatment of experimental fish at Chilko.

Segment of Run	Sample	Date Captured	Date Treated	No. Tanks		
				1 ppm Treatment	3 ppm Treatment	Control
Very-Early Arrival	A	Aug. 15-20	Aug. 21 only	1	-	2
			Aug. 21,27 and Sept. 1	1	1	
Early Arrival	B	Aug. 25-26	Aug. 27 only	1	1	2
			Aug. 27, Sept. 1 and Sept. 6	1	1	
Peak Arrival	C	Aug. 30-31	Sept. 1 and 6	1	1	2
Mixed Segments from Spawning Grounds	D	Sept. 14	Sept. 15 only	1	1	1

Ni-Furpirinol concentrations used in treatment were checked by taking a water sample from each tank approximately 30 min past initiation of treatment for a cylinder plate assay (Bennet et al. 1966).

Bacterial Investigations

The Birkenhead River was monitored for bacteria from water samples taken immediately upstream of the pump intake. The sampling period extended from August 26 to September 22. A tenfold dilution of river water was plated onto a modified cytophaga media (Fijan 1969) and incubated at 20°C for 72 hr. A peptonized milk media (Carlson and Pacha 1968) was also used.

Before being placed in the experimental tank, each fish at both Birkenhead and Chilko Rivers, was examined grossly for visible lesions and the gills were cultured on cytophaga, tryptic soy agar (T.S.A.), and peptonized milk agar media. The very-early Chilko fish and the Birkenhead fish were also

tagged so that individual fish could be followed through to death. As mortalities occurred, the date, tank number, sex and state of maturity were recorded. The fish were then examined once again for visible lesions, and the gills, and in some cases the kidneys, were cultured for pathogens. Gills from mortalities with no apparent gill damage were kept for histological examination. In addition, fresh dead fish from the spawning grounds on Chilko and Birkenhead Rivers were examined for indications of disease and spawning success.

Tissue Retention of Ni-Furpirinol

Studies of tissue retention of Ni-Furpirinol were carried out at Chilko River. A total of 18 adults were exposed to 1 ppm active Ni-Furpirinol for 1 hr while an additional 18 adults were held in a control tank. Fish were sampled at 0, 1, 24 and 48 hr past treatment. At each sample time three fish were taken from both the test tank and the control tank. Samples of flesh, skin and gonad were immediately taken and the Ni-Furpirinol was then extracted from the tissue with dimethyl sulfoxide and water. The extract was then subjected to a cylinder plate assay as described by Bennet et al. (1966). This basically involved a Bacillus subtilus spore suspension in a nutrient agar. Four wells were punched in the agar plate and the tissue extract placed in these wells. The plate was then incubated for 24 hr at 30°C and the diameter of the zone inhibiting the growth of B. subtilus was measured. This was then compared to a standard curve from which the concentration of Ni-Furpirinol was obtained. Preparation of standard curves and analysis of unknown samples followed the methods described by Kramer et al. (1968).

BIRKENHEAD RIVER RESULTS

Bacterial Investigations

Viable bacteria were demonstrated by culture from the free-flowing Birkenhead River water at the experimental site from August 26 to September 22. Myxobacteria counts from samples of Birkenhead River water increased from 0 cells/ml on August 26 to a high of 7,900 cells/ml on September 22. Bi-polar rods also increased during this same period from 100 cells/ml to 56,000 cells/ml (TABLE 4).

TABLE 4 - Bacteria counts from Birkenhead River water.

Date	Myxobacteria cells/ml	Bi-Polar Rods cells/ml
Aug. 26	0	100
Sept. 3	200	3,700
Sept. 7	400	5,300
Sept. 12	700	12,400
Sept. 16	2,300	31,800
Sept. 19	4,100	43,000
Sept. 22	7,900	56,000

An evaluation of the extent of disease organisms was carried out on the Birkenhead spawning grounds beginning with the arrival of the fish on September 3 and ending September 22. The initial cultures and gram stains taken upon capture of experimental fish indicated that numerous strains of bacteria were infecting the gill surfaces of the early fish as they arrived on the spawning grounds (TABLE 5). Only one sockeye possessed a gill lesion and the causative agent was probably a myxobacteria similar to, but not C. columnaris.

TABLE 5 - Bacterial flora of the experimental Birkenhead sockeye.

Gram's Stain	Size	Motility	Shape	Per Cent Fish Infected	
				Initially	At Death
-	0.4μ x 1.6μ	None	Bi-Polar	100	100
-	0.5μ x 5-7μ	Gliding	Rod-Chains	75	85
-	0.4μ x 1μ	None	Single Rods	60	20
+	0.8μ	None	Cocci	15	30
-	0.5μ x 2μ	None	Curved Rods	35	40

Investigations during the spawning period revealed that C. columnaris was probably not present but other bacteria could be demonstrated. Culture smears taken from 27 lethargic adults found in the river between September 3 and 22 revealed 44% were harboring myxobacteria, and 100% had bi-polar rods

present. Only one lesion was found on 115 prespawning mortalities examined on the spawning grounds during the same period of time.

It would appear that there were many bacteria present on the Birkenhead fish upon arrival and that these bacteria probably increased in number during residence on the spawning grounds. However, pathogenicity of these bacteria has not been established as yet.

Therapeutic Treatment

The birkenhead fish captured September 9 and 10 were first treated with Ni-Furpirinol on September 12 and 13. The first mortalities, two from the 3 ppm tank and one from a 1 ppm tank, occurred within 8 hr following treatment. Tissue cultures of the gills of these fish revealed the presence of bi-polar rods many times more numerous than at the onset of holding. Myxobacteria present on two of these fish prior to treatment were cultured from one of the mortalities taken from the 3 ppm treatment group. However, none of the dead fish exhibited lesions. A coccus strain of bacteria was also present in small numbers on the gills of these fish. Microscopic examination of five treated fish which died prior to the second treatment on September 17 and 18 showed that the gills were heavily infected with bi-polar bacteria and moderately infected with myxobacteria (TABLE 5).

The first fish in the control tank died September 14. It was heavily infected with bi-polar bacteria and myxobacteria, and gill lesions were evident which had not been present four days prior to death.

On September 17, within 9 hr following the first dose of the second 1 ppm treatment, five fish expired; three fish also died in the tank which did not receive a second 1 ppm treatment. Although gill lesions were absent from all of these fish, myxobacteria were abundant on all of the fish that did not receive the second treatment, and on two of the five dead fish from the tank receiving the treatment. All dead fish were heavily infected with bi-polar bacteria. The mortality per tank through September 17 was: 1 ppm (1 treatment) 5 (50%), 1 ppm (2 treatments) 6 (60%), 3 ppm (2 treatments) 5 (50%) and only 1 (10%) in the control tank (TABLE 6).

TABLE 6 - Mortalities of experimental Birkenhead sockeye.

Time of Mortality	Date	Days Past Treatment	No. of Fish Dying			
			Control	1 ppm 1 treatment	1 ppm 2 treatments	3 ppm
Prior to pump failure	Sept. 9-17	5	1	5	6	5
Mortality during pump failure	Sept. 17-18	6	5	5	3	0
Total mortality at end of experiment	Sept. 18-21	9	6	10	9	5

During the night of September 17-18 clogging of the intake screen severely reduced the flow of water to some of the tanks. Eight fish were lost in the 1 ppm tanks, five in the control group, but none in the 3 ppm tank. Tissue smears from the gills of these dead fish indicated that all were heavily infected with bi-polar bacteria and moderately infected with myxobacteria. Three of these five control fish exhibited gill lesions but none of the treatment mortalities possessed lesions. The stage of maturity of these fish indicated that they were ready to be spawned. These were the last deaths to occur during the experiment (TABLE 6).

Gill smears taken from the remaining fish on September 22 revealed that the control fish were heavily infected with bi-polar rods, myxobacteria, and cocci. All of the controls had small lesions or white-tipped gill filaments in addition to being very lethargic. The 3 ppm treated fish were all heavily infected with bi-polar bacteria but only one carried myxobacteria on its gills. These fish were free of gill lesions and were very active. The sole surviving fish from the 1 ppm treatment tank was heavily infected with myxobacteria and bi-polar rods and large lesions were present on its gills.

Results prior to equipment failure indicated that use of Ni-Furpirinol to control prespawning mortalities would be of little value as there was 50 to 60% mortality among treated fish compared to 10% in the controls. However, those fish remaining in the 3 ppm tank did show an increased vigor and a much reduced incidence of myxobacteria, indicating a positive effect of treatment for at least some of the early Birkenhead fish.

CHILKO RIVER RESULTS

Physical Measurements

Physical measurements were taken from the early and peak segments of the Chilko population (Birkenhead fish were not measured). The late segment was not sampled due to the extreme difficulty in obtaining a pure sample of late fish. A progression in the state of maturity of these fish is shown by the percentage weight of gonads which increased from 12.4% on August 23 to 13.7% by September 2 (TABLE 7).

TABLE 7 - Physical measurements of 1970 Chilko female sockeye salmon.

Sample Date	Sample Size	Fork Length cm	Mean Weight - gm				% Gonads
			Total	Eviscerated Body	Viscera	Gonad	
Aug. 23 (early)	5	58.9	2,015	1,736	56.4	216	12.4
Aug. 25 (early)	10	59.5	2,084	1,785	77.3	240	13.4
Sept. 2 (peak)	10	60.0	2,647	2,179	89.1	297	13.7

Bacterial Investigations

The incidence of columnaris on the spawning grounds of Chilko River fish varied from the first arrivals to peak of spawning. Of the 66 very-early fish examined, 16 had gill lesions indicative of columnaris. Columnaris cultures were isolated from 10 of the fish with lesions and 5 of the fish without lesions were positive for C. columnaris, indicating that at least 22.7% of the early fish had viable columnaris bacteria present on their gills.

Twelve (20%) of the peak fish captured August 30 and 31 had gill lesions indicative of columnaris and 10 gave positive cultures of C. columnaris from their gills.

Seven lethargic fish from the upper 3 miles of the Chilko spawning ground were cultured on September 3. Two fish had columnaris gill lesions and both these fish cultured positive for columnaris. The gross appearance of the other five fish appeared normal and both gill and kidney smears cultured on cytophaga and T.S.A. were negative for viable bacteria.

Visual examination of fresh dead fish on the Chilko spawning grounds indicated an average of 29% of the females and 31% of the males examined had columnaris lesions (TABLE 8).

TABLE 8 - Incidence of columnaris-type lesions on Chilko River spawning ground mortalities.

DATE	FEMALES					MALES		
		0% Spawned		100% Spawned				
	No. Examined	% With Lesions	% Without Lesions	% With Lesions	% Without Lesions	No. Examined	% With Lesions	% Without Lesions
Sept. 15	52	15	72	1	12	46	18	82
19	74	20	59	10	11	29	38	62
20	38	24	21	21	34	18	56	44
27	34	6	32	0	62	53	36	64
29	56	2	2	37	59	52	25	75
30	34	3	3	26	68	25	32	68
Total No. and Mean Per Cent	288	13	35	16	36	223	31	69

Sixty-five per cent of the early segment females with lesions (recovered September 15 to 20) were unspawned while only 6% of the peak females with lesions (recovered September 27 to 30) were unspawned (TABLE 9).

TABLE 9 - Prespawning mortality of fresh dead Chilko females with and without lesions.

Date	No. Examined	With Lesions		Without Lesions	
		No.	% Unspawned	No.	% Unspawned
Sept. 15-20	164	49	65	115	77
Sept. 27-30	124	34	6	90	14
Total	288	83	41	205	49

In addition 77% of the early females without lesions were unspawned while 14% of the peak females without lesions were unspawned.

These data suggest that columnaris was present in a significant portion of the 1970 Chilko spawning population. Although many sockeye bearing evidence of columnaris did spawn successfully, the data suggest that columnaris could have been a factor in the large prespawning mortality (51%) among the early arrivals on the spawning grounds.

Therapeutic Treatment

Sample A sockeye treated with 1 and 3 ppm Ni-Furpirinol had a reduced incidence of columnaris during the experimental period, whereas the controls had an increased incidence of columnaris, as evidenced by severe lesions (FIGURE 8) and by culturing (TABLE 10).

TABLE 10 - Incidence of columnaris in sample A experimental fish by culture method.

	Per Cent of Gill Cultures Positive for Columnaris		
		Treated With Ni-Furpirinol	
	Control	1 ppm	3 ppm
Prior to Treatment	15	35	10
End of Experiment (Mortality Cultures Included)	89	20	0

Furthermore, of the sample A fish yielding positive cultures for columnaris prior to treatment, 60% of the fish treated with 1 ppm Ni-Furpirinol and 100% of the fish treated with 3 ppm were cultured negative for columnaris at death or termination of the experiment. In addition, of the fish cultured negative for C. columnaris prior to treatment, only 25% of the fish treated with 1 ppm and 0% of the fish treated with 3 ppm yielded positive cultures at death or termination of the experiment (TABLE 11). These results were in distinct contrast to the untreated controls, of which 89% of those initially negative (and all of those initially positive) were cultured positive for C. columnaris at the end of the experiment.

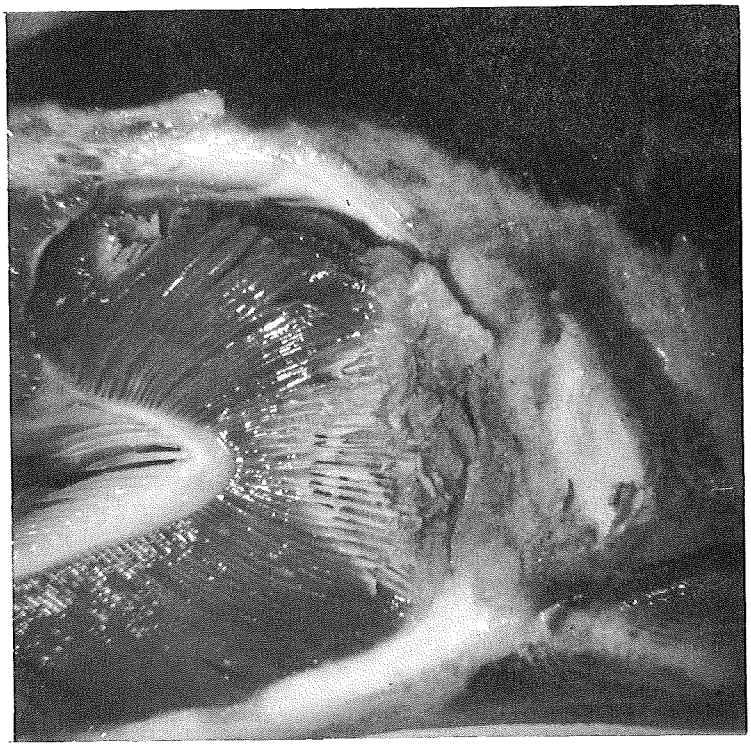

FIGURE 8 - Columnaris lesion on moribund adult sockeye removed from control tank.

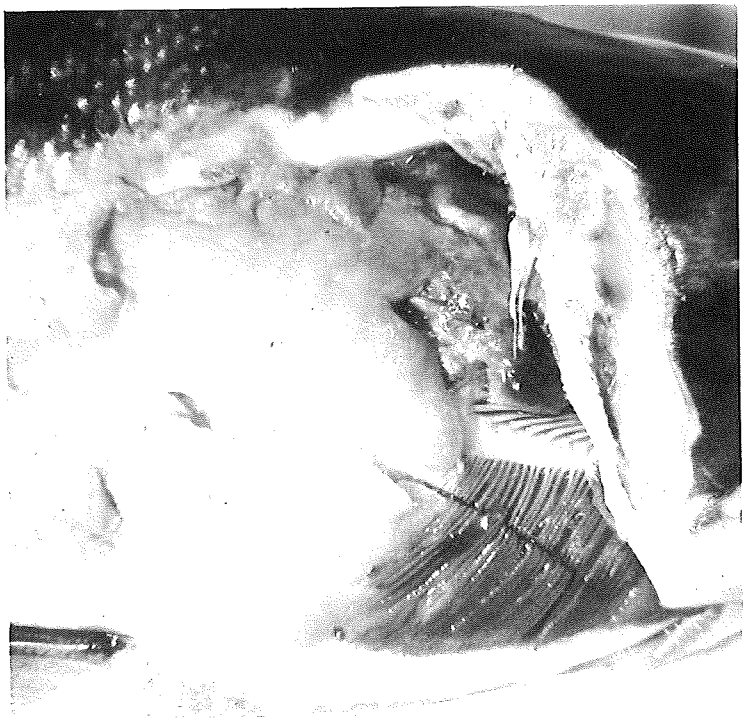

FIGURE 9 - Extensive fungal growth covering gill of sockeye treated with 3 ppm Ni-Furpirinol.

TABLE 11 - Effectiveness of Ni-Furpirinol treatment in controlling C. columnaris in sample A Chilko sockeye.

	Percentage of Positive Fish Cultured Negative at Death	Percentage of Negative Fish Cultured Positive at Death
Control	0	89
1 ppm	60	25
3 ppm	100	0

Even though the treatment provided considerable control of columnaris, it had a very limited effect on the prespawning mortalities of the sockeye from sample A under experimental conditions. In sample A, the controls and the fish treated with 1 ppm Ni-Furpirinol had similar rates of death (TABLES 12 and 13). The 3 ppm treatment appeared to delay death of very-early fish approximately four days (TABLE 12), however the fish suffered 80% mortality over a 20-day period (TABLE 13) and many died with heavy fungus on the gills (FIGURE 9). The remaining fish lived up to termination of the experiment on September 22.

TABLE 12 - Number of days to first mortality, Chilko.

Segment of Run	Sample	Control	Control	1 ppm	1 ppm	3 ppm	3 ppm
Very-Early	A	1	4	4	4	8	-
Early	B	3	-	5	2	3	4
Peak	C	6	-	6	-	2	-
Mixed Segments from Spawning Grounds	D	3	-	1	-	3	-

TABLE 13 - Per cent mortality upon termination of Chilko experiment.

Segment of Run	Sample	Control	Control	1 ppm	1 ppm	3 ppm	3 ppm
Very-Early	A	80	90	80	80	80	-
Early	B	100	-	90	100	80	70
Peak	C	100	-	40	-	50	-
Spawners	D	20	0	10	-	20	-

The sockeye from sample B showed even less response to the Ni-Furpirinol treatment than did those from sample A. In one case, the 1 ppm treatment of early fish was extended to 2 hr but even this treatment was not very effective. The 3 ppm treatment did reduce the incidence of columnaris with only 15% of the fish developing new lesions compared with 30% in the 1 ppm and the controls (TABLE 14), however their rate of dying was very similar to the 1 ppm and control groups (TABLE 13).

TABLE 14 - Summary of incidence of columnaris-type gill lesions in experimental Chilko sockeye.

SEGMENT OF RUN	SAMPLE	CONTROL		TREATED WITH NI-FURPIRINOL			
				1 ppm		3 ppm	
		% With Lesions	% Developing New Lesions	% With Lesions	% Developing New Lesions	% With Lesions	% Developing New Lesions
Very-Early	A	15	45	20	40	20	10
Early	B	20	30	20	30	20	15
Peak	C	20	20	20	10	20	10

In contrast, sockeye from sample C showed a positive response to both the 1 and the 3 ppm treatments of Ni-Furpirinol. The mortality rate was much less, only 50% in 3 ppm and 40% in 1 ppm, during the 23-day holding period compared with the 100% mortality in the controls within a 20-day period (TABLE 13). Also, the incidence of columnaris was somewhat lower among treated fish (10% developed new lesions) than in the controls (20% developed new lesions) (TABLE 14).

Fish from sample D captured on the spawning grounds September 14 and held until September 22 suffered very little mortality (only 5 of 40 fish died), with no significant difference between treated and control groups. It is impossible to state what segment of the run these fish belong to as the fish from all segments are spread over the spawning grounds.

These data indicate that Ni-Furpirinol treatment of Chilko sockeye at both 1 and 3 ppm concentrations provided a significant therapeutic influence of C. columnaris but had a very limited effect on the prespawning mortalities of sockeye under experimental conditions.

Ni-Furpirinol Retention

The concentration of Ni-Furpirinol residue in various tissues was calculated from the standard curves shown in FIGURE 10, using the same methods described for the water samples. Traces of Ni-Furpirinol residue were detected in the gonads, skin and flesh samples of adult sockeye taken 1 hr past a 1 ppm Ni-Furpirinol treatment. There was no trace of Ni-Furpirinol in flesh, gonad or skin 24 hr past treatment (TABLE 15). Experiments analyzing serum of treated adults (carried out later at the Sweltzer Creek Research Laboratory) indicated that Ni-Furpirinol is readily absorbed into the blood of the fish and conforms to previous findings (Takase et al. 1968; Amend and Ross 1970).

TABLE 15 - Ni-Furpirinol residue in tissues of treated Chilko sockeye; Ni-Furpirinol residue (ppm) calculated from diameter of inhibition zone recorded in "Test".

Date	Time (hr)	Diameter of Inhibition Zone - mm			Calculated Ni-Furpirinol Residue ppm	Diameter of Inhibition Zone - mm			Calculated Ni-Furpirinol Residue ppm
		Control	0.5 ppm Standard	Test		Control	0.5 ppm Standard	Test	
			FLESH				SKIN		
Aug. 23	0	0	18.21	0	0	0	21.47	0	0
	1	0	18.23	0	0	0	21.56	8.16	0.18
	24	0	19.35	0	0	0	22.51	0	0
	48	0	20.50	0	0	0	24.31	0	0
Sept. 6	0	0	17.43	0	0	0	20.76	0	0
	1	0	17.82	5.6	Present	0	20.79	12.53	0.41
	24	0	18.73	0	0	0	21.78	0	0
			EGGS				SPERM		
Aug. 23	0	0	17.91	0	0	0	19.58	0	0
	1	0	19.02	7.5	Present	0	20.45	5.9	Present
	24	6.54	17.90	6.40	?	0	19.16	0	0
	48	0	19.06	0	0	0	20.24	0	0

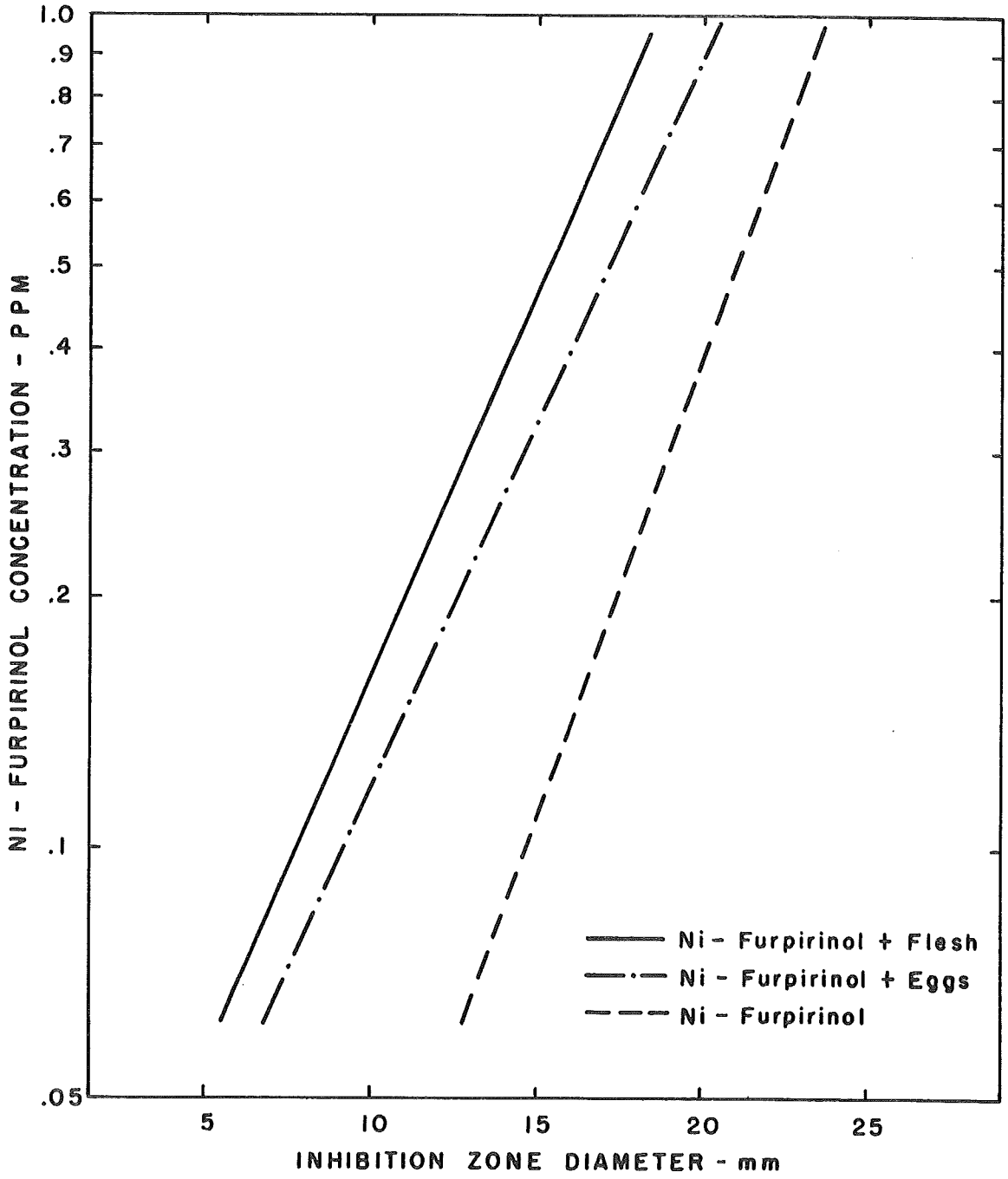

FIGURE 10 - Standard curves for Ni-Furpirinol residue study.

DISCUSSION

Use of the chemical therapeutic Ni-Furpirinol to control prespawning mortality is questionable at this time. Data from the Birkenhead experiment, where columnaris is virtually nonexistent, indicated that Ni-Furpirinol had little effect on the bacterial flora present on the gills of Birkenhead sockeye. There was very little evidence of any beneficial effect of Ni-Furpirinol on the fish and, initially, there appeared to be a detrimental effect as evidenced by the higher mortalities among treated fish. At five days past treatment the treated fish suffered a 50 to 60% mortality while the controls had only a 10% mortality. In comparison, recoveries from the spawning ground indicated only 21% premature deaths among the early fish. The significant increase in mortality among the treated fish prior to pump failure may have resulted from the elimination of one of the normal flora bacterium by Ni-Furpirinol, subsequently allowing a pathogenic organism to grow unrestrained.

Nevertheless, the results of the Chilko experiment suggest that Ni-Furpirinol has a potential for controlling columnaris. One of the difficulties encountered in these experiments was the effect of the artificial environment created within 6-ft diameter fiberglass tanks during the extended holding periods for samples A, B and C. The number of new gill lesions developing on Chilko fish was reduced significantly after treatment in the 3 ppm tanks (TABLE 14), as were the number of gill cultures positive for columnaris (TABLE 11). In spite of this the Ni-Furpirinol treatments did not check the premature death of the early sockeye under experimental conditions. Treatment did provide some therapeutic effect for the peak fish where there was 100% mortality in the controls but only 40 to 50% mortality in the treated groups (TABLE 13). Therefore there may be other factors which are capable of causing premature death and are unaffected by Ni-Furpirinol.

Because Fraser sockeye runs maintain a relatively consistent chronological order during migration, spawning and death (Killick 1955), fish from the Chilko River spawning grounds provide a "natural" control for comparison with experimental groups. The experimental fish can be compared with their respective segments of the spawning run by examining, chronologically, the mortalities on the spawning grounds. Upon arrival on the Chilko River spawning grounds, both the very-early and early groups of sockeye had a 20% incidence of columnaris as evidenced by gill lesions and cultures of gill tissue smears. Forty per cent of these experimental controls developed new lesions during the holding period; similarly, early deaths from the spawning grounds had an incidence of columnaris lesions ranging from 16% up to 66% with a mean of 36%. Mortalities for the very-early experimental controls upon termination of the experiment were 80 to 90% whereas the prespawning mortality on the spawning grounds, although as high as 100% for the first arrivals on the spawning grounds, dropped to a mean of 51% for the first one third of the dead recovery. Among peak fish, all the untreated controls died, compared with a mean prespawning loss of only 1.5% on the spawning grounds. Therefore it becomes obvious that the environment within the circular tanks was conducive to severe prespawning mortality. Under these conditions there were two indications of a beneficial effect of Ni-Furpirinol. First, treatment of very-early fish with 3 ppm for 1 hr appeared to delay death for up to four days. However eventually 80% of these fish died. Second, the treatment of peak fish with 1 and 3 ppm Ni-Furpirinol for 1 hr appeared to reduce mortalities considerably.

In summary, the early segments of the 1970 Birkenhead and Chilko sockeye populations had substantial prespawning mortalities and the peak segments had little prespawning mortality. The Ni-Furpirinol treatments did not reduce prespawning mortality among the early segments of the Birkenhead and Chilko populations under experimental conditions. Treatments were effective in reducing the mortalities of the peak Chilko fish held under experimental conditions. On the basis of these results Ni-Furpirinol is not considered a practical therapeutic agent for prevention of prespawning mortalities of Fraser River sockeye.

CONCLUSIONS

1. Bacterial flora present on the Birkenhead sockeye were not sensitive to Ni-Furpirinol treatments of 1 and 3 ppm.

2. Ni-Furpirinol was effective to a degree in controlling C. columnaris bacteria on Chilko sockeye within the experimental environment.

3. Ni-Furpirinol did not reduce prespawning mortalities of very-early and early Chilko sockeye, or early Birkenhead sockeye under experimental conditions.

4. Ni-Furpirinol was only partially successful in controlling prespawning mortalities of peak Chilko sockeye under experimental conditions.

5. There were traces of Ni-Furpirinol in flesh, eggs and skin of adult sockeye salmon 1 hr past a 1 ppm treatment, however tests conducted 24 hr past treatment indicated no Ni-Furpirinol was retained.

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