Erythromycin Phosphate Used to Control Bacterial Kidney Disease in Yellowstone Cutthroat Trout (Salmo clarki)

by
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ABSTRACT

The Yellowstone River Trout Hatchery at Big Timber, Montana is the state brood and production hatchery for the Yellowstone Cutthroat trout. In March of 1977 a two phase treatment was initiated to control bacterial kidney disease. Erythromycin Phosphate is subcutaneously injected into the fish and the eggs are water hardened in a solution of the drug. Methods of giving the injections have been refined to a fast and efficient procedure. Some small problems have been encountered with water hardening, however these problems have been solved.

Results up to this time have been encouraging with a dramatic drop in mortalities, improved eggs, and an increase in hatch and growth rates.

Presented at Great Plains Fishery Workers Meeting
February 12, 13, 14, 1979
"Not published"

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INTRODUCTION

The Yellowstone River Trout Hatchery at Big Timber, Montana is the state brood and production hatchery for the Yellowstone Cutthroat trout. In 1972 the brood stock was destroyed and the hatchery disinfected due to a severe epizootic of Furunculosis. At this time examination of slides taken from adult fish contained some gram positive diplobacillic organisms resembling baterial kidney disease (BKD) bacteria. Due to the lack of knowledge about BKD at that time a confirmatory diagnosis could not be made. The hatchery was restocked with fingerlings which were hatched from eggs taken from the brood stock before the epizootic broke out. The eggs had been shipped to other hatcheries. In June of 1973 Yellowstone Cutthroat eggs from McBride Lake in Yellowstone Park were brought into the hatchery. This is the only strain now at the hatchery. After the epizootic the hatchery still suffered high mortalities in the brood fish but no evidence of Furunculosis or BKD was found during annual disease certification from 1973 to 1975. In March of 1976 a confirmed diagnosis of BKD was made by personnel at the Fish Disease Control Center in Fort Morgan, Colorado using the fluorescent anti-body technique.

It was at this time that a search was started to find a way to control BKD. It was found that Dr. George Klontz at the University of Idaho was having success controling BKD in chinook salmon with injections of Erythromycin Phosphate. He had also started studies of eliminating the

bacteria in the egg by water hardening the eggs in a solution of Erythromycin. Arrangements were made for the Yellowstone River Trout Hatchery to start studies using both the injection and water hardening methods of controlling BKD. The studies are coordinated through the Fishery Resources Program, University of Idaho under an Investigational New Animal Drug permit from the Food and Drug Administration. The first test lot of McBride strain Yellowstone Cutthroat trout were injected on March 2, 1977. The first eggs were water hardened on March 30, 1977.

Bacterial kidney disease is a chronic, systemic bacterial disease of salmonids caused by a corynebacterium. J.E. Sanders and J.L. Fryer from Oregon State University have proposed the name Corynebacterium salmoninus for this gram-positive diplobacillic bacteria. The incubation period has been determined to be 30 to 35 days at 11° C. This is the water temperature at Big Timber.

In the cutthroat BKD doesn't cause an increase in mortalities until the fish are approximatly 15 months old. Age is determined from the time of hatch. At this time the signs and symptoms of the disease appear. Most of the symptoms that have appeared in the cutthroat are typical to other species. Symptoms seen are red blebs on the sides, lesions, liquid filled blisters, occasional hemorrhaging at the base of fins, pop eye, and distended abdomen. A fish may rise to the surface and wiggle frantically for a few seconds then return to normal. Seriously affected fish darken in color and go off feed. They become lethargic and emaciated. The kidney becomes swollen, gray and mushy with small abscesses. Lesions may be found on the walls of the body cavity. The testes or ovaries start hemorrhaging along with other organs. The hindgut is frequently swollen and filled with a yellow

mucoid fluid. It appears that even small numbers of bacteria cause a reduced growth rate. However, a light infection does not result in outward symptoms of the disease.

It has been found that in cutthroat the disease correlates with fungal gill infection. When BKD appears, fungus problems also appear. At the Big Timber hatchery the bacteria has a greater effect on the males. The mortality ratio is 65% males to 35% females. As the testes start to mature they become heavily infected. It appears that the maturing testes provide an ideal medium for KD bacterium growth.

How bacterial kidney disease is transmitted has been controversial. It is widely accepted that it is passed transovarion. Dr. Klontz at the University of Idaho has conducted studies which show that the bacteria is passed in the egg and not in the sperm even though the male is infected. Another one of his studies showed that the bacteria is not transmitted through the water. It is believed however that it can be passed from fish to fish by nipping at open sores where the bacteria can be found.

MATERIALS AND METHODS

The Erythromycin Phosphate used for injections is Erythro-200, an injectable formula from Abbott Laboratories. This can be obtained through a veterinarian. Each ml of Erythro-200 contains 200 mg of active ingredient. A 1 cc tuberculin syringe is used for injecting the fish. A 3 cc syringe may be used for large fish requiring a large dosage. With a 1 cc syringe full it is possible to inject 20 two pound fish. Size of needle depends on the size of the fish. Needles from 7/8 in. 25 ga to 1½ in. 20 ga have been used at Big Timber. To make the fish easier to

handle they are anesthetized by placing them in a solution of MS-222. Holding the fish secure during the injection was a problem. To solve this problem a holding box 18 inches long was made with 1 X 4 boards and foam rubber. The foam rubber glued inside the box greatly limits the movement of fish held in it.

Erythromycin Phosphate is subcutaneously injected into the fish anterior to the dorsal fin at a dosage of 5 milligrams of active ingredient per pound of fish. Care must be taken not to inject into the muscle tissue as this will cause a sterile abcess to form. It was decided that weighing each fish individually to determine the amount of drug to inject was unnecessarily time consuming. Tests at other hatcheries have shown that no adverse affects have resulted from over doses of as much as 10 times the prescribed amount. Taking this into consideration the average weight of the lot of fish to be injected is determined then all the fish are injected with the same amount of drug. When a noticeably larger fish is found it is given a larger amount. With this procedure, an experienced four man crew can inject over 200 fish per hour with no mortalities. Several hours after the injection a darkened area can be seen going down the sides of the fish as the drug starts to disperse from the injection site. At Big Timber fish weighing from 1/3 pound to 4 pounds have been injected.

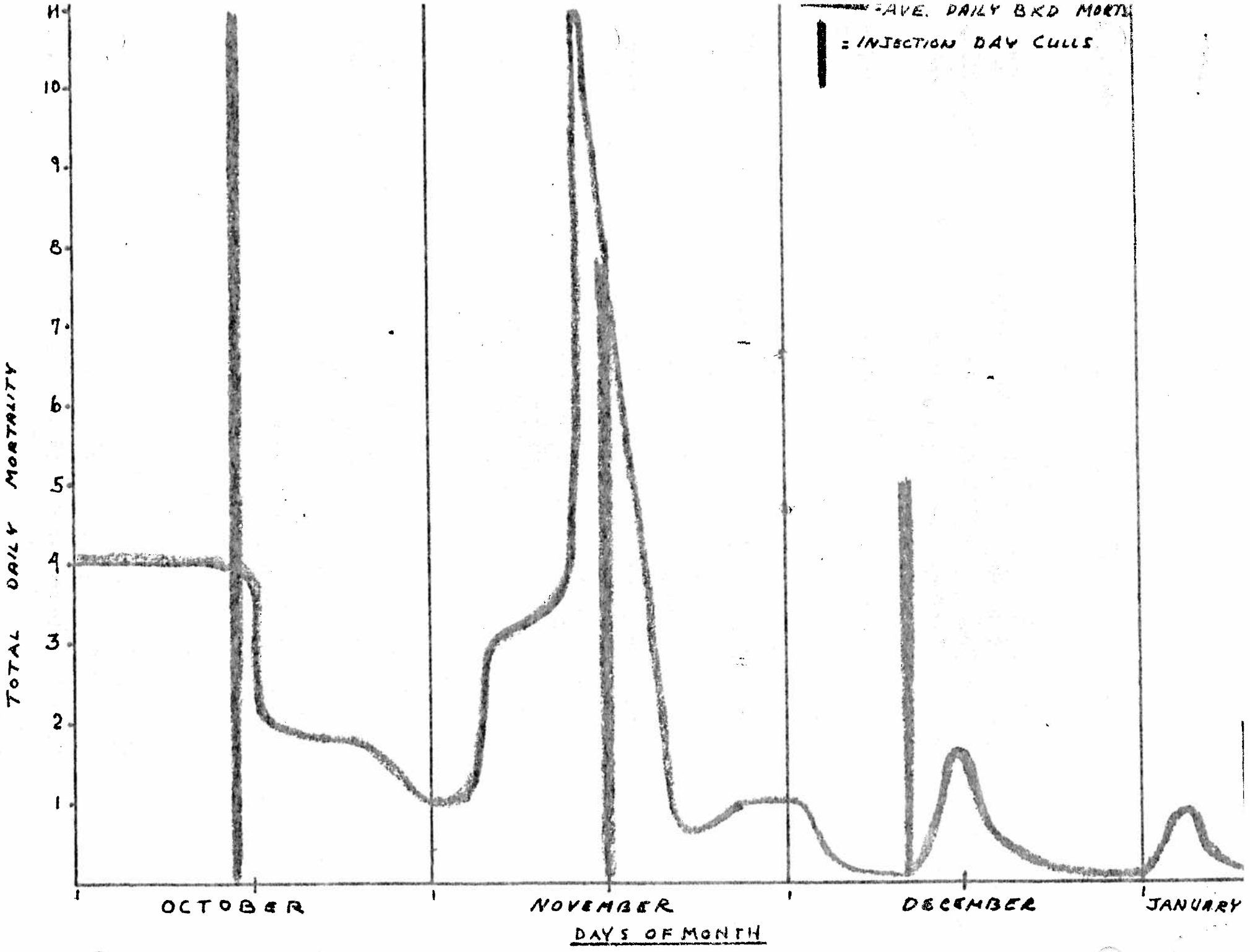
An Erythromycin Phosphate solution is also used for water hardening eggs. Several different forms of the drug have been used. Up to this time we have received our drug from Dr. Klontz. In 1977 a liquid form that had to be kept frozen was used. In 1978 a powder form of the drug was used. Whatever form is used it is mixed with water so as to have 2 ppm of active ingredient. Eggs should be water hardened in

this solution for at least thirty minutes.

RESULTS

The differences between the anticipated effects of these two forms of treatment must be understood. Injections are a means for controlling the kidney disease bacteria by retarding their growth, however, this does not eliminate the disease. Water hardening the eggs in a solution of Erythromycin is meant to eliminate the bacteria permanently in the fish by killing any bacteria on the surface or within the egg.

Results from the injections have been very encouraging. A definite improvement can be seen in the fish the day following the injection. There is an increase in activity, more vigorous feeding and a movement of the fish away from the lower end of the pond. Within a week there is an improvement in color and general appearance. Some of the blebs fade away. The reduction in the number of mortalities is amazing. Graph 1 shows the number of mortalities per day for a four month period starting with fish 16 months old. Age is determined from the time of hatch. There was a steady increase in daily mortalities up to the day of injection. The graph shows high mortalities on the day of injections due to the removal of culls and moribund fish. After injections the daily mortalities dropped rapidly. As an example, in the month of November there were 30 mortalities in the first fifteen days of the month but only 3 mortalities the rest of the month after the injection. The number of daily mortalities remained low for 30 to 35 days and then increased rapidly. At this time the fish were again injected. In December the fish were injected before there was any significant


AVE. DAILY BRD MORTL
= INJECTION DAY CULLS
TOTAL DAILY MORTALITY
11
10
9
8
7
6
5
4
3
2
1
OCTOBER
NOVEMBER
DECEMBER
JANUARY
DAYS OF MONTH

increase in mortalities. This proved to be an effective control as there was no significant rise in mortalities in December.

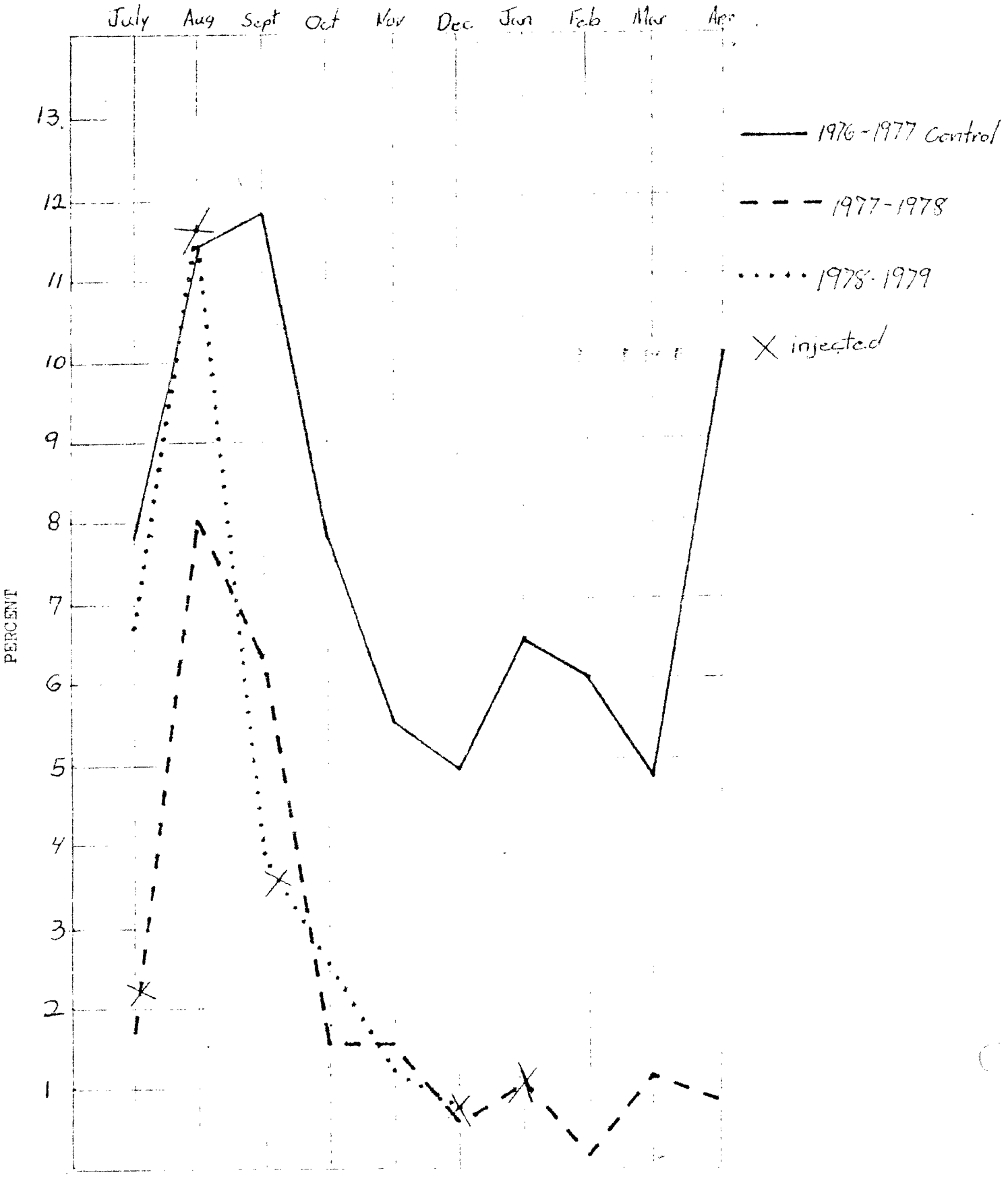
A comparison of the mortalities over the last three years shows a dramatic decrease in the percent of mortalities on the monthly bases. It also shows a definite cycle that the disease follows in the cutthroat. Graph 2 shows the percent mortality per month for the brood fish starting each year when the fish were 26 months old until they were three years old. This period is from July to April of the following year. The data from July 1976 to April 1977 is used as the control since this group of fish was not injected. The following years the fish were injected when the mortalities started to increase. As the graph shows there has been a rapid decrease in the mortalities with a 4 to 8 percent decrease some months. July, August and September are the months with the highest mortality rate. During this time a majority of the mortalities have a fungus gill infection. Although the fungus has been treated with malachite green and formalin it continues to be a chronic problem. Erythromycin Phosphate injections have had much effect on the fungus problem except in August of 1978 when the situation was compounded by a problem with the feed. We feel that the fungus is definitely associated with BKD.

The growth rate has improved in the brood stock fish. In December of 1978 they weighed approximately one pound each more then the same age fish in December 1976.

After injections with Erythromycin were started there have been markedly fewer females with bloody eggs at spawning time. The same is true with the males and sperm. Eggs are taken from three year olds in April.

Graph 2

Percent Brood Mortalities per Month



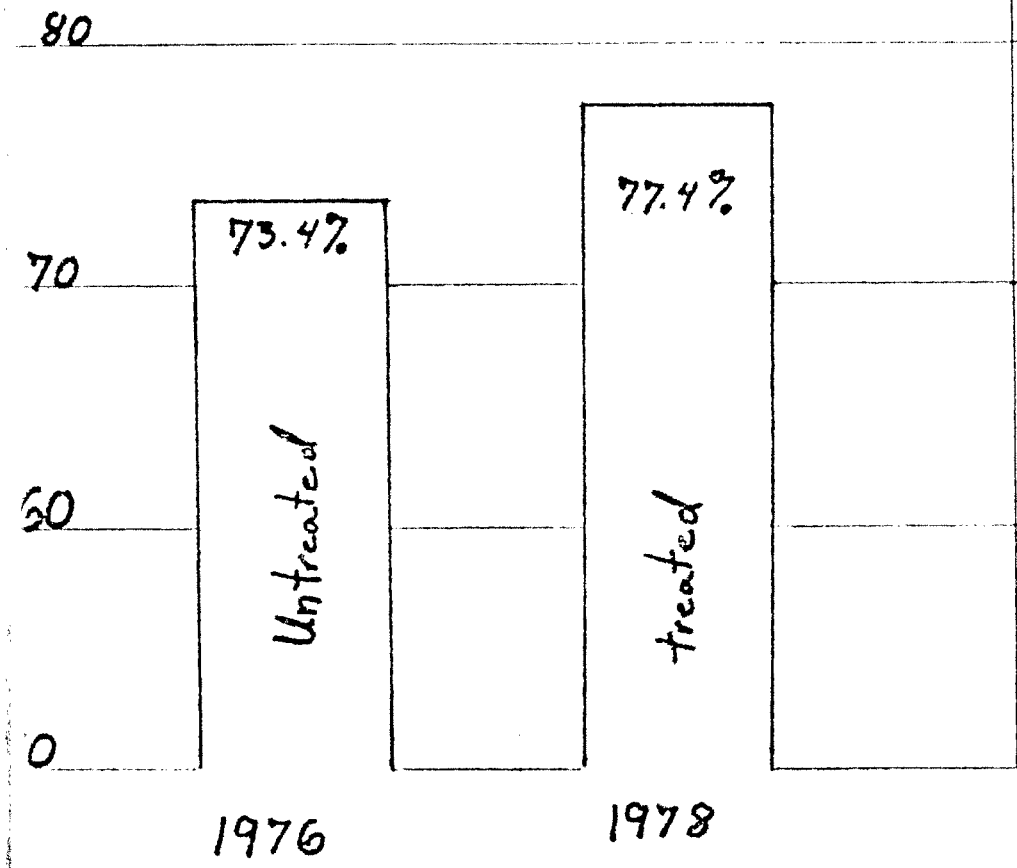
Now that enough data has been collected for a pattern to develop in the disease we are starting to inject the fish before the mortalities increase. Fish over two years old have been more able to tolerate the disease then those under two years old For those under two, injections have to be given about every 34 days to control the bacteria. Those over two are injected before the expected rise in mortalities. The fish should not be injected any closer then 30 days prior to spawning.

Up to this time water hardening has been encouraging even though there has been some minor setbacks. The percentage of hatch and the percent of eggs planted out as fish has increased. In 1976 when the eggs were not treated the percent hatch was 73.4 compared to 77.4 in 1978 when all eggs were treated (Graph 3). The percent of eggs taken that were planted out as fish was 62.8 in 1976 compared to 74.2 in 1978 (Graph 4). Most of the fish are planted out when they are about two inches long. In 1977 the growth rate of fish from treated and untreated was compared. Although the treated eggs were taken two weeks after the untreated eggs the treated fish were larger three months after hatch and continued to outgrow the untreated ones. As graph 5 shows, at nine months the fish from the treated eggs were more than .6 of an inch longer. The daily change of length of the fish from treated eggs was 0.0245 compared to 0.0218 of an inch in the untreated.

A setback occured when the fish from the treated eggs started showing symptoms of BKD with an increase in mortalities when they were 15 months old. It was determined that the liquid form of drug that was used to treat the eggs had been thawed and frozen many times before we received it. Therefore, it was not at the strength it was thought to be and the eggs were treated at a lower dosage then recommended. The level

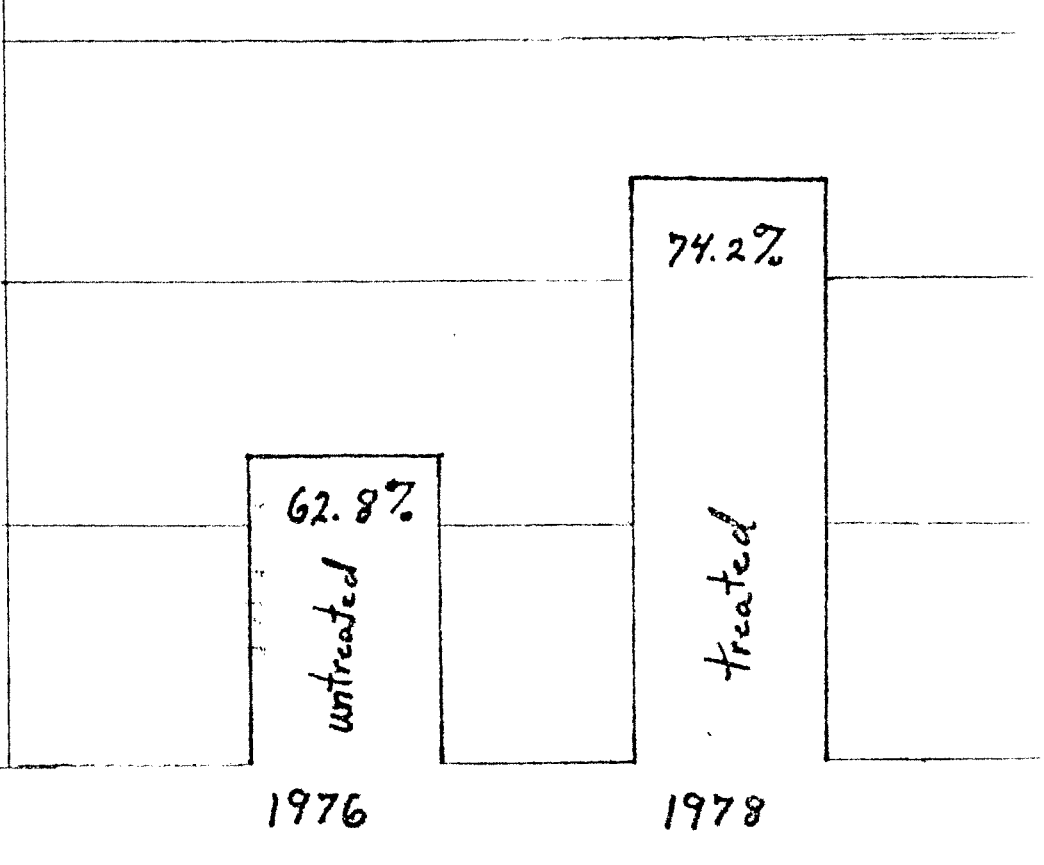
Graph 3

Percent Hatch

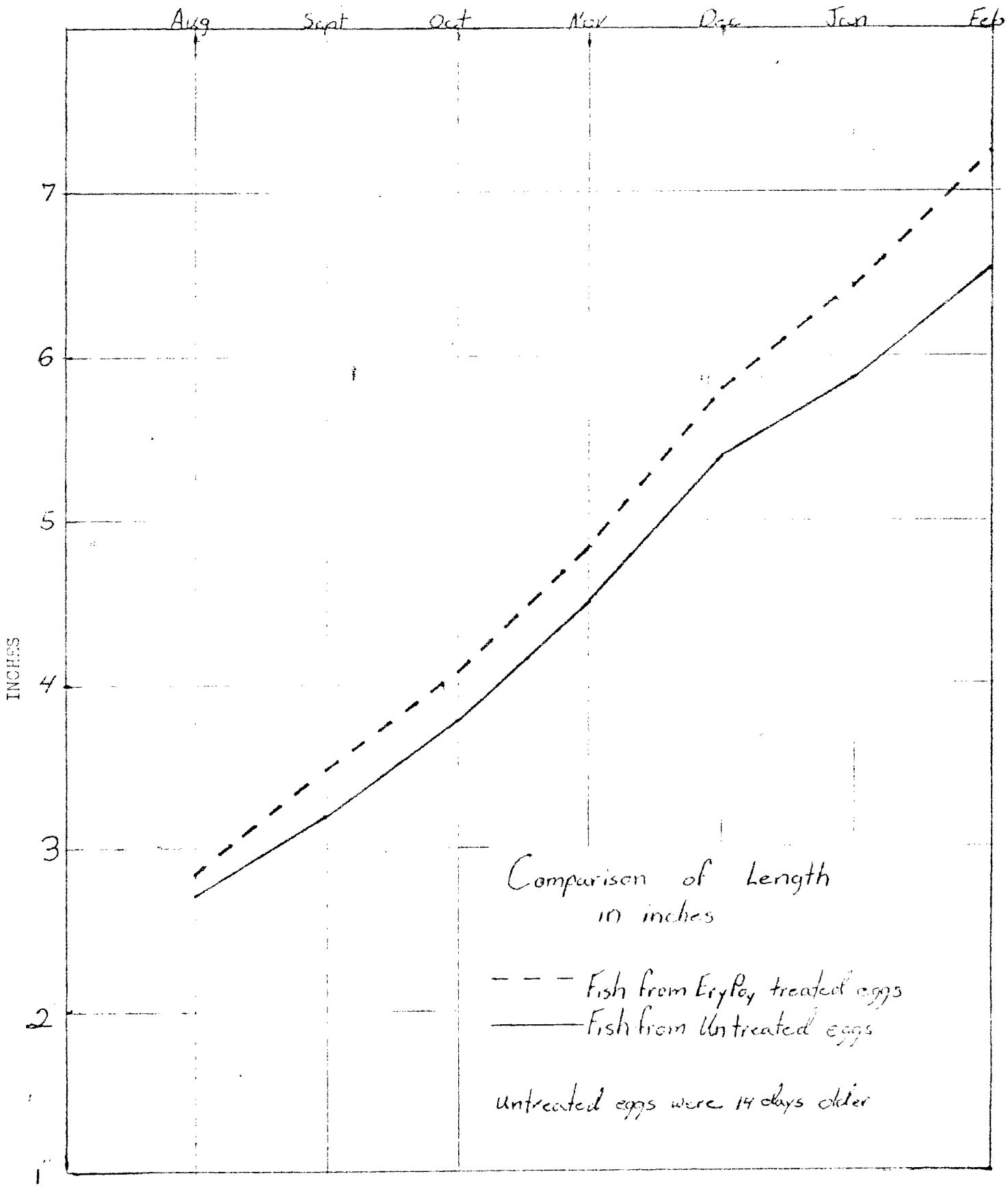


Graph 4

Percent Eggs Planted out as Fish



Graph 5


Aug
Sept
Oct
Nov
Dec
Jan
Feb
7
6
5
4
3
2
1
INCHES
Comparison of Length in inches
Fish from EryPay treated eggs
Fish from Untreated eggs
Untreated eggs were 14 days older

of treatment was enough to cause an increase in hatch and growth but not enough to eliminate the bacteria. In 1978 a powder form of the drug provided by Dr. Klontz was used for water hardening. After spawning was completed it was found that the powder had less active ingredient then believed. Because of this the fish from the 1978 eggs may come down with BKD even though there was an excellent hatch and they are growing well.

In 1979 we are going to use a different form of Erythromycin from a different source. It is called Gallimycin Poultry Formula Improved. It has the amount of active ingredient written on the package. We plan also to water harden the eggs in a 5 ppm solution to leave room for error.

DISCUSSION

Precautions are being taken to minimize the chances of reinfecting the fish from the treated eggs. They are held inside the hatchery building for one year. No equipment that is used in the outside ponds is taken into the hatchery. Any time the fish in the outside ponds are handled we thoroughly disinfect our hands before feeding or working with the fish in the hatchery. All equipment that is used in one outside pond is completely disinfected before used in the other pond.

The goal at the Yellowstone River Trout Hatchery is to completely eliminate bacterial kidney disease with the use of Erythromycin Phosphate. If the lot of fish from 1978 doesn't develop BKD , then it should be possible to have BKD free fish in 1981. If they do develop BKD it will be 1982 before it could possibly be eliminated. Other studies have shown that at the right dosage the drug does eliminate the bacteria in the egg therefore, we believe it will work with the cutthroat. As long as the

fish show symptoms of the bacteria, injections will be continued. If we reach the stage where the fish from the treated eggs are free of the bacteria then the injections will no longer be needed for control, but water hardening will continue to help prevent reinfection.

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Data for Graph 1
Daily Mortalities

	OCT	NOV	DEC	JAN
1				
2		1		
3	3			1
4	2	2		
5	2	1		
6	2	3		
7	1	3	5E	
8	2			
9	3			1
10				2E
11	4			
12	12E	1		
13	3			
14	1	11		
15	2	8E	2	
16	1			
17				
18	2			
19		1		
20	1			
21	2		1	
22	1			
23	1		3	
24				
25	2	1		
26				
27				
28				
29		1		
30				
31				

E - day of injection

Data for Graph 5
Length in Inches

	TREATED	UNTREATED
AUG	2.82	2.70
SEPT	3.47	3.19
OCT	4.08	3.79
NOV	4.84	4.51
DEC	5.81	5.39
JAN	6.44	5.86
FEB	7.26	6.56

Data for Graph 2
Brood Mortalities
% loss per month

Month	1976 - 1977	1977 - 1978	1978 - 1979
July	7.8	1.7	6.7
August	11.4	8.0	11.7
September	11.8	6.3	3.9
October	7.8	1.5	1.7
November	5.5	1.5	1.3
December	4.9	0.57	0.74
January	6.5	1.0	
February	6.0	0.14	
March	4.8	1.1	
April	10.1	0.95	