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EFFECT OF CERTAIN ELECTRICAL PARAMETERS AND WATER RESISTIVITIES ON MORTALITY OF FINGERLING SILVER SALMON

BY JOHN R. PUGH

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CONTENTS

ABSTRACT

Immediate and delayed effects of certain electrical parameters and water resistivities upon the mortality of fingerling silver salmon (*O. kisutch*) were determined in a statistically controlled experiment. Differences in mortality attributable to differences between the variations of pulse shape, frequency, and water resistivity were noted after 24 hours but not after 30 days. Differences in mortality that could be attributed to differences between the variations of voltage and wiring pattern were not significant at either time.

EFFECT OF CERTAIN ELECTRICAL PARAMETERS AND WATER RESISTIVITIES ON MORTALITY OF FINGERLING SILVER SALMON

By JOHN R. PUGH, *Fishery Research Biologist*

BUREAU OF COMMERCIAL FISHERIES

Biologists have been searching for many years for an effective method of diverting salmon fingerlings from hazardous areas, such as turbine intakes, spillways, and high-velocity channels. In their search, they have explored the possibilities of physical barriers, sound-producing instruments, air bubbles, lights, controlled water velocities, chemicals, and electricity.

To further this research, the electrical guiding project of the Bureau of Commercial Fisheries Biological Laboratory, Seattle, Wash., conducts extensive studies to determine the effectiveness of guiding or diverting salmon fingerlings with electricity. We have progressed from small-scale laboratory studies to field sites on small streams [1] and, currently, to a large-scale field investigation.[2]

With each of these field studies there has been associated laboratory work to determine whether the electrical conditions to be tested are harmful to the fish. Collins, Volz, and Trefethen (1954) found that mortality increased with an increase in total voltage, pulse frequency, duration of exposure, water temperature, or with combinations of these factors. Raymond (1956) noted a slight loss in fish in tests with duty cycles of 0.10 or greater. Maxfield [3] found, over the range of electrical conditions which he tested, that the electric shock did not affect the future reproductive ability of young rainbow trout exposed as yearling fish. Trefethen (1955) and Newman (1959b) conducted laboratory investigations in guiding salmon fingerlings with electricity, but made little or no mention of mortality; however, personal interviews with each investigator revealed that very few mortalities occurred in either experiment.

The potential application of electrical guiding techniques depends on the degree of safety, as well as efficiency, with which electrical fields guide or divert fish. Although observations indicate that the number of injuries and mortalities caused by electrical fields is negligible, the question of mortality invariably arises with each new experimental method of diverting fish with electricity.

The objective of this study was to determine, under controlled laboratory conditions, the immediate and delayed effects of certain electrical parameters and water resistivities on the mortality of fingerling silver salmon (*Oncorhynchus kisutch*).

Rea E. Duncan and Donald D. Worlund assisted in the planning of the experimental design and in making the statistical analyses, and Charles C. Gillaspie was responsible for the operation and maintenance of the electronic equipment.

METHOD AND MATERIALS

Experimental design

The experiment was conducted in Latin square sequence using water resistivity, wiring pattern, pulse shape, voltage, and frequency as variables (see table 1). Throughout the experiment the pulse duration was 8.3 milliseconds, the water depth 1 foot, and the average water velocity 1 foot per second. Some of these experimental conditions were similar to conditions tested in earlier research. This similarity enabled us to correlate the results of this experiment with our previous studies. Other conditions were more rigorous in an attempt to establish the maximum level of electrical intensity that could be used at future installations without causing mortalities among the migrating fish.

NOTE.—Approved for publication, June 21, 1961. Fishery Bulletin 208.

[1] Hunter, Charles J. Manuscript in preparation. Experimental guiding of salmonids by electricity. Bureau of Commerical Fisheries Biological Laboratory, Seattle, Wash.

[2] Mason, James E., and Rea E. Duncan. Manuscript in preparation. Development and appraisal of methods of diverting fingerling salmon with electricity at Lake Tapps. Bureau of Commercial Fisheries Biological Laboratory, Seattle, Wash.

[3] Maxfield, Galen H. Manuscript in preparation. Effect of electricity on reproductive ability of rainbow trout. Bureau of Commerical Fisheries Biological Laboratory, Seattle, Wash.

TABLE 1.—*Latin square experimental design*

[The rows are comprised of water resistivities, the columns of wiring patterns and pulse shapes, and the treatments of voltages and frequencies]

	I	II	I	II
	Half sine-wave	Square-wave	Square-wave	Half sine-wave
5,000 ohm cm	165v.-30 p.p.s.	250v.-30 p.p.s.	250v.-15 p.p.s.	165v.-15 p.p.s.[1]
15,000 ohm cm	250v.-30 p.p.s.	165v.-30 p.p.s.	165v.-15 p.p.s.	250v.-15 p.p.s.
1,000 ohm cm	165v.-15 p.p.s.	250v.-15 p.p.s.	250v.-30 p.p.s.	165v.-30 p.p.s.
10,000 ohm cm	250v.-15 p.p.s.	165v.-15 p.p.s.	165v.-30 p.p.s.	250v.-30 p.p.s.

[1] Pulse per second.

As shown in table 1, a total of 16 tests were conducted with the array energized. In addition, four groups of fish were tested with the power off to serve as controls. Each control group was tested in one of the water resistivity conditions used during the power-on tests. Each group, test or control, consisted of approximately 100 silver salmon fingerlings.

Prior to testing, each group of fish was tattooed with a distinctive mark. The fish were then exposed to electric shock in an experimental tank and, after examination for immediate mortalities, they were transferred to an outdoor holding tank where they were held 30 days for observation. The tattoo marks provided a means of identifying each group of fish during the 30-day holding period. Observations were made daily and a record of mortalities was maintained.

Test fish

The test fish were age-group 0 silver salmon (*O. kisutch*) obtained from the national hatchery at Quilcene, Washington. They were transported to the Bureau of Commercial Fisheries Biological Laboratory, Seattle, Wash., in an aerated tank and held in outdoor holding tanks prior to testing.

The fish ranged in size from 55 to 98 mm., measured from the tip of the snout to the posterior end of the vertebral column (standard length measurement illustrated by Schultz, 1948). The average standard length was 85 mm.

Laboratory facilities

The experiment was conducted in the fall of 1959 in the main experimental tank of the fish-behavior laboratory described by Newman (1959a). This tank is 20 feet wide, 58 feet 6 inches long, and 5 feet deep. It was divided lengthwise by installing a partition constructed of ¾-inch plywood, creating a channel 10 feet 2 inches wide (fig. 1). Installation of this partition produced the desired conditions (depth of water, 1 foot; average water velocity, 1 foot/second) without altering any of the existing laboratory facilities.

The floor and walls of the experimental channel were coated with asphalt and insulating paint to minimize electrical grounding. Horizontal baffles, designed to produce a uniform flow of water, were installed in the headbox (fig. 2). Both ends of the channel were screened with ¼-inch hardware cloth to confine the fish to the test area.

A release box (fig. 2), 4 feet by 2 feet 10 inches by 2 feet with a removable gate, was placed in the channel approximately 8 feet downstream from the headbox. A block-and-tackle assembly enabled us to remove the box from the water after release of the test fish. A second box with the same design and dimensions, but fitted with lead-in nets, was placed at the downstream end of the channel (fig. 3). This second box was used in the recovery of the fingerlings after they had passed through the test area.

Two troughs, each 66½ by 9½ by 8½ inches, were set up in an area adjacent to the experimental tank (fig. 4). One trough was placed on a rack 4 inches higher than the other and served as a gravity-flow reservoir for the lower trough and a holding area for the fish prior to tattooing. The lower trough served as a table for the tattooing machine and as a holding area for the test fish after they had been tattooed. Water was supplied to the upper trough at a rate of one-half gallon per minute by means of a submersible pump placed in the headbox of the main experimental tank. A standpipe overflow returned the water from the lower trough through a flexible hose to the experimental tank headbox. This system maintained a constant flow of water through the troughs and a stable water level in the experimental tank.

Electrical equipment and test conditions

Array.—The electrode array consisted of a 10- by 5½-foot wooden framework with cross mem-

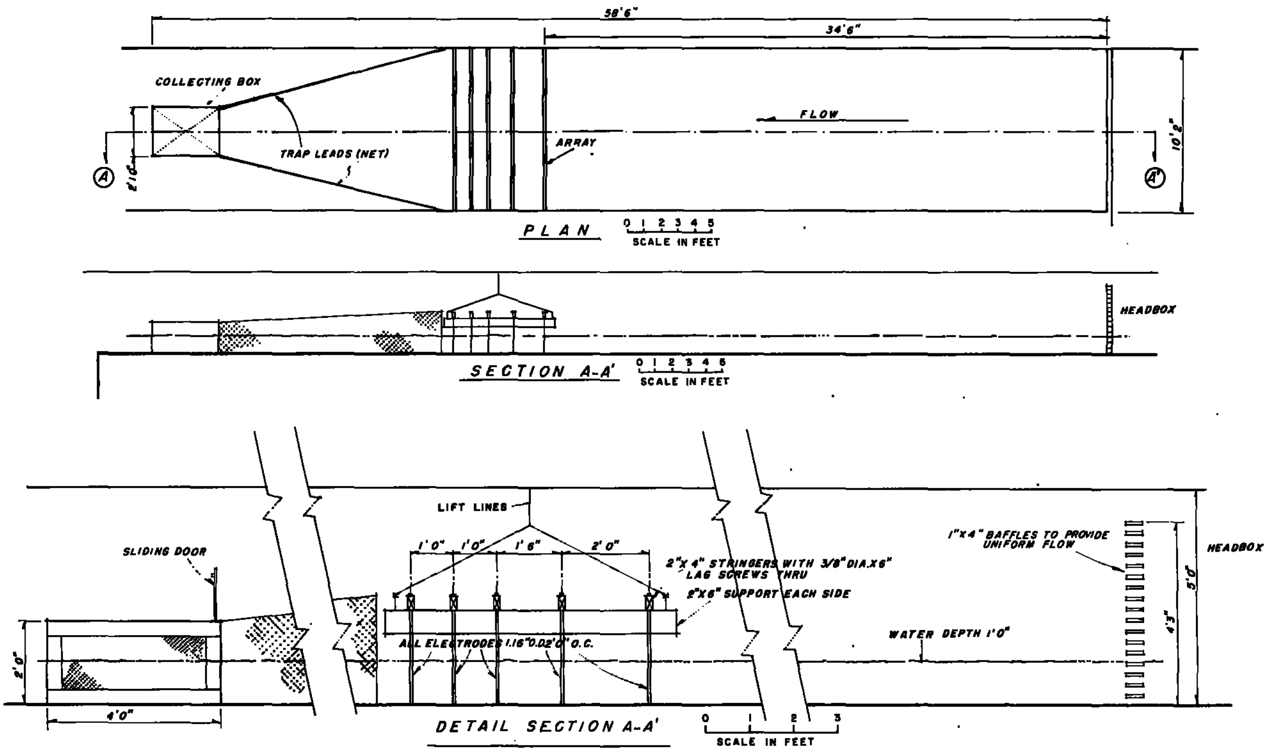

FIGURE 1.—Plan and cross-sectional view of experimental channel.

bers to support the electrodes, their connecting wires, and the electrical-pulse supply cables from the sequential switching equipment (fig. 5). The array was suspended so that the bottoms of the electrodes were ½ inch from the bottom of the tank. The electrodes were 2½-foot lengths of galvanized electrical conduit with an outside diameter of 1.163 inches. They were arranged in five staggered rows with 2-foot spaces between the electrodes in each row. The upstream row of electrodes was designated row A and the downstream row, row E. The spacing was 2 feet between rows A and B, 1½ feet between rows B and C, 1 foot between rows C and D, and 1 foot between rows D and E (fig. 6). The applied voltage to all electrodes was the same; hence, the decreasing distance between electrode rows created an area of higher voltage gradient on the downstream side of the array.

Water resistivity.—Four levels of water resistivity, 1,000, 5,000, 10,000, and 15,000 ohm centimeters, were tested.

Pulse shape.—The tests included two variations of pulse shape: square-wave and half sine-wave (fig. 7).

Pulse amplitude.—Two levels of applied peak voltage were tested: 165 and 250 volts.

Pulse duration.—The pulse duration for both the square-wave and the half sine-wave pulses was 8.3 milliseconds.

Sequential switching equipment.—The electronic equipment that supplied the square-wave pulses consisted of a pulse generator that sequentially switched the output of a direct current generator to various groups of electrodes. The d.c. generator was powered by commercially available 60-cycle alternating current. Figure 8 is a block diagram of this equipment. A detailed description and schematic diagrams of the switching equipment are being prepared and will be published at a later date.[4]

The equipment that supplied the half sine-wave pulses was essentially the same as that which supplied the square-wave pulses, except that the d.c. generator, the overload protector, and the exciter were replaced by 60-cycle alternating

[4] Volz, Charles D., and H. P. Dale. Manuscript in preparation. A high-power pulse generator for electrical fish-guiding research. Bureau of Commercial Fisheries Biological Laboratory, Seattle, Wash.

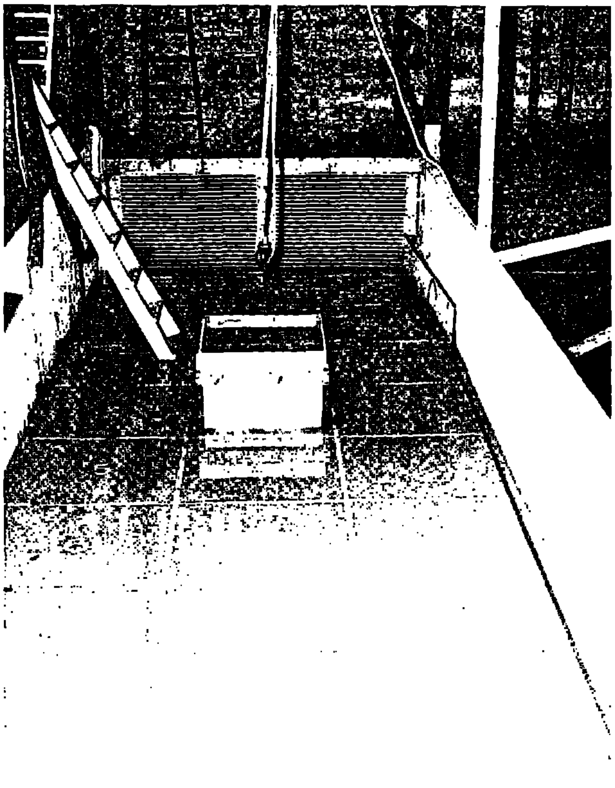

FIGURE 2.—Release box with sliding gate closed. Horizontal baffles and headbox are shown in background.

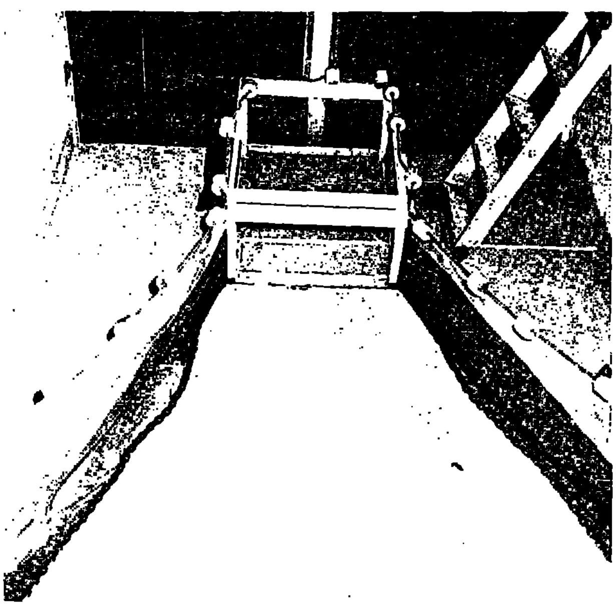

FIGURE 3.—Recovery box with sliding gate removed. Lead-in nets are shown in foreground; downstream screens in the background.

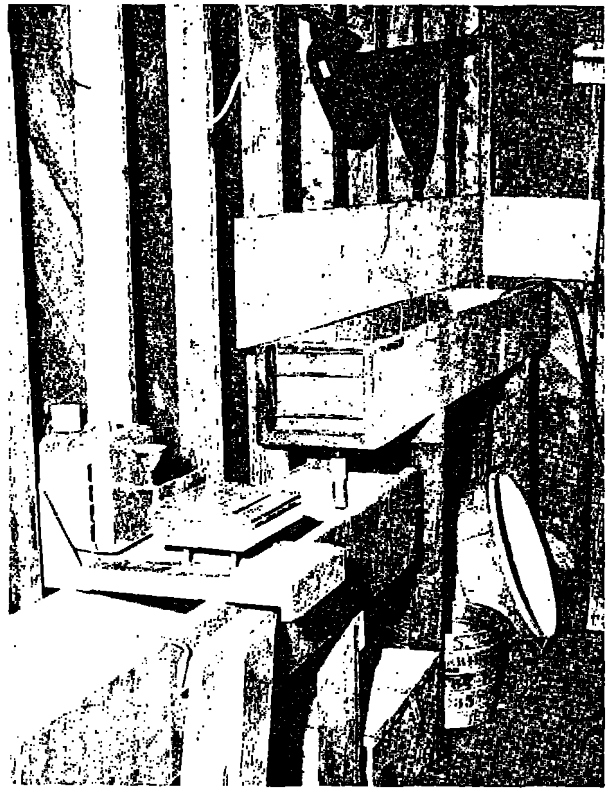

FIGURE 4.—Tattooing facilities.

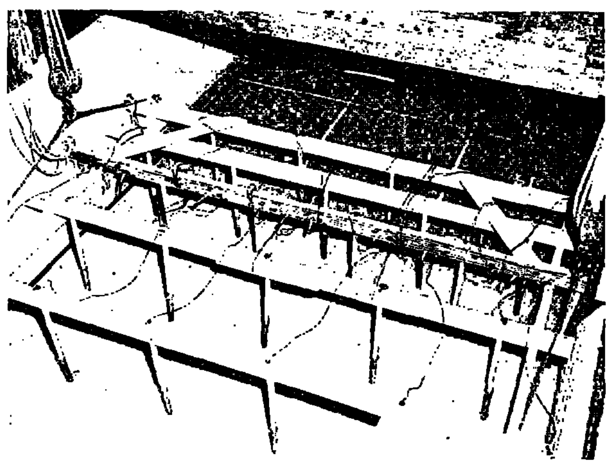

FIGURE 5.—Staggered electrode array used in this experiment.

current, supplied by a line transformer. Figure 9 is a block diagram of this modified equipment.

Wiring patterns.—The two wiring patterns tested are illustrated in figure 10. With each of these patterns, the electrodes were energized by the sequential switching equipment in such a

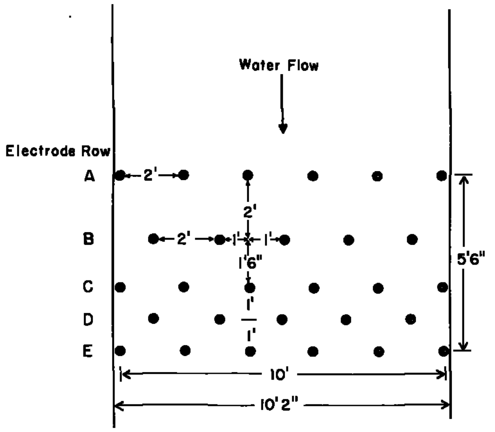

FIGURE 6.—Staggered electrode array with spacing as indicated. (Heavy dots represent the electrodes.)

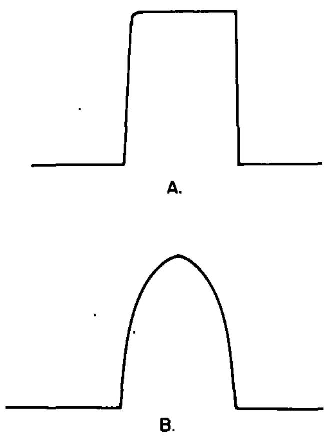

FIGURE 7.—Square-wave (A) and half sine-wave (B) pulses.

manner that five electrical fields were produced. These fields will be discussed later in the text.

Pulse frequency.—Frequencies of 15 and 30 pulses per second were tested. Since both wiring patterns produced five electrical fields, each field was pulsed either three or six times per second,

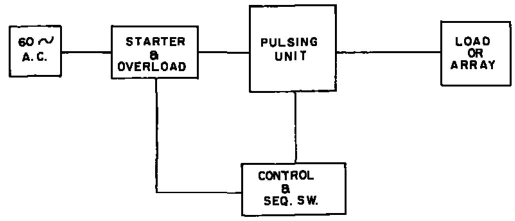

FIGURE 9.—Block diagram of sequential switching equipment used to supply half sine-wave pulses,

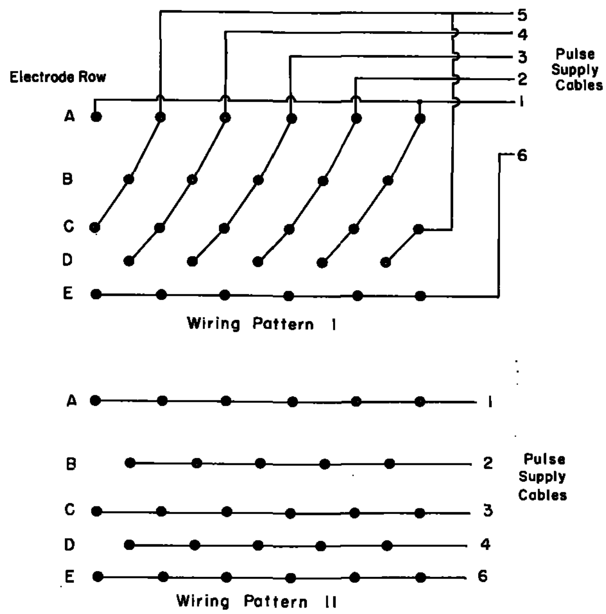

FIGURE 10.—Wiring patterns used in the experiment. (Heavy dots represent the electrodes.)

depending on the frequency being used, i.e., frequency divided by number of electrical fields.

Sequential pulsing—When the array was wired according to wiring pattern I (fig. 10) and energized with square-wave pulses, the polarity of the

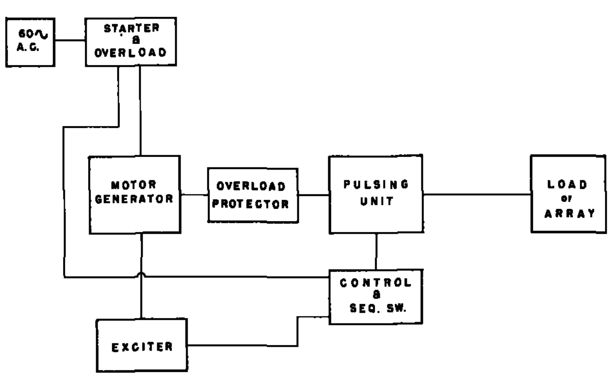

FIGURE 8.—Block diagram of sequential switching equipment used to supply square-wave pulses.

electrodes was alternated with each successive pulse and a sequence of pulses, moving from left to right looking downstream, was established. Table 2 shows this pulsing sequence.

When the first pulse was delivered, the electrodes connected to pulse supply-cable 1 became positive and the electrodes connected to pulse supply-cable 6 (row E) became negative. On the second pulse, the electrodes connected to pulse supply-cable 2 became negative and the electrodes in row E became positive. This sequence of pulses and alternating polarity continued through pulse supply-cables 3, 4, and 5 to complete the cycle. Then, on the first pulse of the second cycle, the electrodes connected to pulse supply-cable 1 became negative and the electrodes in row E became positive. On the second pulse of the second cycle the electrodes connected to pulse supply-cable 2 became positive and the electrodes in row E became negative. Again, this succession of pulses and alternating polarity continued through pulse supply-cables 3, 4, and 5 to complete the second cycle. In the third cycle, the electrodes were energized and the polarity alternated as they were during the first cycle. This process was an automatic function of the sequential switching equipment.

TABLE 2.—*Pulsing sequence and polarity changes when the electrodes were wired according to wiring pattern I and energized with square-wave pulses*

Pulse supply-cable	Pulsing sequence and polarity changes					
	Pulse 1	Pulse 2	Pulse 3	Pulse 4	Pulse 5	Pulse 1 (second cycle)
1	(+)	0	0	0	0	(−)
2	0	(−)	0	0	0	0
3	0	0	(+)	0	0	0
4	0	0	0	(−)	0	0
5	0	0	0	0	(+)	0
6 (row E)	(−)	(+)	(−)	(+)	(−)	(+)

Because of technical limitations of the existing sequential switching equipment, polarity of the electrodes was not alternated when the array was wired according to wiring pattern I and energized with half sine-wave pulses. The sequence for this switching operation is shown in table 3.

The first pulse from the switching equipment energized only the electrodes connected to pulse supply-cable 1, making them positive, and the electrodes in row E, making them negative. The second pulse energized the electrodes connected to pulse supply-cable 2, making them positive and again the electrodes in row E became negative. This sequence continued through pulse supply-cables 3, 4, and 5, until five successive pulses had been delivered. The electrodes in row E were of negative polarity with each pulse. When the fifth pulse had been delivered, the cycle was completed and the sequence was automatically repeated.

When the array was wired according to wiring pattern II, the pulsing sequence and polarity changes were the same for both the square-wave and the half sine-wave pulses. Table 4 illustrates the pulsing sequence for wiring pattern II.

The first pulse energized rows D and E, making row D positive and row E negative. On the second pulse, row D became negative and row C positive. On the third pulse, row C was negative and row B positive. On the fourth pulse, row B became negative and row A positive; and on the fifth pulse, row A became negative and row E positive. When this sequence was completed, the cycle was automatically repeated.

Voltage gradients.—The electrical fields created by the two wiring patterns were determined by analog gradient plotting and are shown in figures 11 and 12. The plotting interval is 10 percent of

TABLE 3.—*Pulsing sequence when electrodes were wired according to wiring pattern I and energized with half sine-wave pulses*

[Polarity not alternated with this configuration]

Pulse supply-cable	Pulsing sequence					
	Pulse 1	Pulse 2	Pulse 3	Pulse 4	Pulse 5	Pulse 1 (second cycle)
1	(+)	0	0	0	0	(+)
2	0	(+)	0	0	0	0
3	0	0	(+)	0	0	0
4	0	0	0	(+)	0	0
5	0	0	0	0	(+)	0
6 (row E)	(−)	(−)	(−)	(−)	(−)	(−)

TABLE 4.—*Pulsing sequence and polarity changes when electrodes were wired according to wiring pattern II and energized with either square-wave or half sine-wave pulses*

Electrode row	Pulsing sequence and polarity changes					
	Pulse 1	Pulse 2	Pulse 3	Pulse 4	Pulse 5	Pulse 1 (second cycle)
A	0	0	0	(+)	(−)	0
B	0	0	(+)	(−)	0	0
C	0	(+)	(−)	0	0	0
D	(+)	(−)	0	0	0	(+)
E	(−)	0	0	0	(+)	(−)

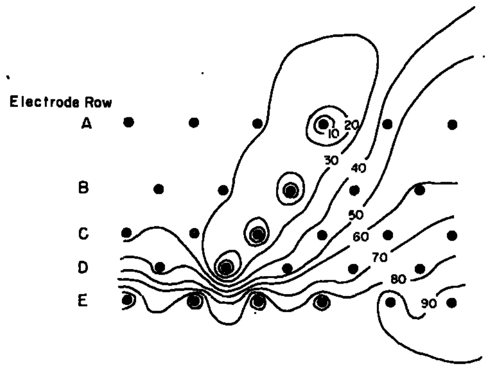

FIGURE 11.—Electrical field created on the third pulse of the cycle when the array was wired according to wiring pattern I. (Lines connect points of equal potential; numbers show percentage of the applied voltage. Heavy dots represent the electrodes.)

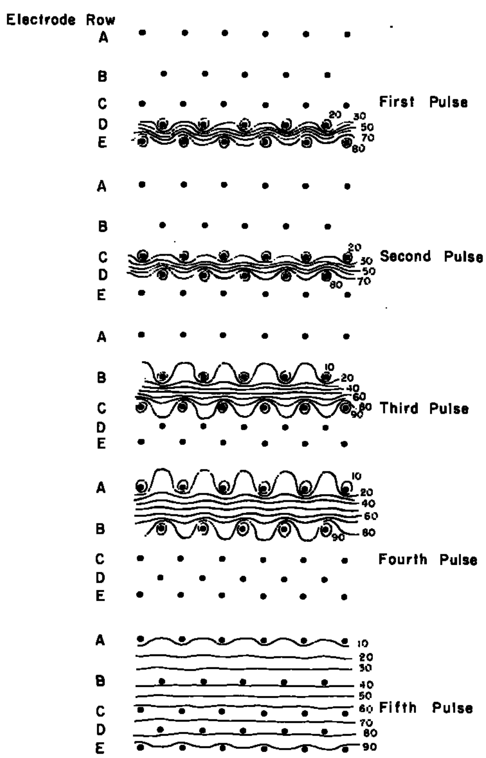

FIGURE 12.—Electrical fields created during one complete cycle when the array was wired according to wiring pattern II. (Lines connect points of equal potential; numbers show percentage of the applied voltage. Heavy dots represent the electrodes.)

the applied voltage. Figure 11 shows the electrical field produced when the array was wired according to wiring pattern I. Only one pulse of the five-pulse cycle is illustrated, but it is representative of the electrical fields produced with each pulse, since the wiring pattern was uniform with respect to electrode spacing.

Wiring pattern II was not uniform with respect to electrode spacing, and therefore each pulse created a slightly different electrical field. The electrical fields established when the array was wired in this pattern are shown in figure 12.

Experimental procedure

Before the start of each test the electrodes were connected to create the desired wiring pattern. The sequential switching equipment was turned on and, with a calibrated oscilloscope as a monitor, was adjusted to supply the correct pulse parameters. When the switching equipment was functioning properly, it was turned off until the test fish had been placed in the release box.

Water resistivity was measured by means of a commercial conductivity bridge and regulated to the desired level by adding rock salt or by draining the tank and adding fresh water. Facilities were not available to control water temperature, and it varied from 63° F. at the beginning of the experiment (September 16, 1959) to 59.5° F. at the end of the experiment (October 14, 1959). Temperature readings were taken with a standard mercury thermometer.

Before each test, approximately 100 fish were transferred from an outdoor holding tank to the rectangular troughs in the fish-behavior laboratory for marking. Then, in groups of 10, the fish were anesthetized in a solution of tricaine methanesulfonate (M.S. 222) at a concentration of 1:20,000, and tattooed with mineral pigments. When approximately 100 fish had been marked, and all had recovered from the anesthetic, they were placed in the release box in the test channel. In order to observe and remove mortalities due to handling, the fish were held in the release box from 10-15 minutes before the tests were initiated.

The array was then energized with the preset electrical conditions and a pump was started to provide a flow of water through the channel. Next, the sliding gate on the release box was raised, and the fish were released. When all of the fingerlings had left it, the box was hoisted clear of the water by means of the block-and-tackle assembly.

To ensure that all of the fish were exposed to the electrical energy, they were forced to swim through the array by crowding them with a seine. They were then captured in the recovery box below the electrical array, counted, and examined for mortalities or any sign of visible injury. The average time required to conduct each test, from the time the fish were released until they were recaptured, was 25 minutes.

After all of the fish had been examined and the data recorded, they were transferred to an outdoor holding tank where they were held 30 days for observation. The dead fish were picked out daily and carefully examined for marks or bruises. At the termination of the experiment, the live fish were also examined for any signs of visible injury and the results were tabulated.

After every fourth power-on test, but before the water resistivity was changed for the next series of tests, approximately 100 control fish were tattooed and released into the experimental tank. The control fish were handled in exactly the same manner as the test fish, except that the array was not energized while they were in the experimental tank.

The statistical analysis that follows is based on a comparison of the differences in mortality in the fish tested under the various electrical conditions and also on a comparison of the mortality of the test fish with that of the control fish. These comparisons were made for the differences that occurred during the first 24 hours following the testing and also for the differences that occurred during the second to thirtieth day after testing.

RESULTS AND DISCUSSION

The experimental results are summarized in table 5, and include the number and percentage of fish dead after one day and the cumulative mortalities, by number and percentage, from the second to the thirtieth day. The data from table 5 were transformed from percentages to arc sin $\sqrt{\text{percentage}}$ and the entire analysis was made with the transformed data.

Immediate effect

Analysis of variance (table 6) to determine the immediate effect (during the first day) of the variables tested reveals that at the 5-percent significance level a higher mortality resulted from the square-wave pulses than from the half sine-wave pulses, and that a higher mortality resulted from

TABLE 5.—*Immediate (1st day) and delayed (2d to 30th day) mortalities, by number and percentage, of the test fish and control fish*

Test No.	Test conditions [1]	Number of fish released	Died 1st day		Died 2d-30th day	
			Number	Percent	Number	Percent
	5,000 ohm cm.					
Test fish:						
1	I, HSW, 165 v.-30 p.p.s.	96	8	8.3	17	19.3
2	II, SW, 250 v.-30 p.p.s.	96	17	17.7	17	21.5
3	I, SW, 250 v.-15 p.p.s.	96	5	5.2	15	16.5
4	II, HSW, 165 v.-15 p.p.s.	99	1	1.0	13	13.3
Control: 1-C		100	2	2.0	15	15.3
	15,000 ohm cm.					
Test fish:						
5	I, HSW, 250 v.-30 p.p.s.	99	4	4.0	16	16.8
6	II, SW, 165 v.-30 p.p.s.	95	4	4.2	5	5.5
7	I, SW, 165 v.-15 p.p.s.	98	6	6.1	18	19.6
8	II, HSW, 250 v.-15 p.p.s.	99	3	3.0	3	3.1
Control: 2-C		99	2	2.0	9	9.3
	1,000 ohm cm.					
Test fish:						
9	I, HSW, 165 v.-15 p.p.s.	98	4	4.1	9	9.6
10	II, SW, 250 v.-15 p.p.s.	99	12	12.1	25	28.7
11	I, SW, 250 v.-30 p.p.s.	98	18	18.4	8	10.0
12	II, HSW, 165 v.-30 p.p.s.	98	6	6.1	9	9.8
Control: 3-C		100	4	4.0	5	5.2
	10,000 ohm cm.					
Test fish:						
13	I, HSW, 250 v.-15 p.p.s.	108	1	0.9	9	8.4
14	II, SW, 165 v.-15 p.p.s.	108	2	1.9	11	10.4
15	I, SW, 165 v.-30 p.p.s.	99	7	7.1	2	2.2
16	II, HSW, 250 v.—30 p.p.s.	108	6	5.6	10	9.8
Control: 4-C		104	2	1.9	7	6.9

[1] Explanation of test condition symbols:
I—Wiring pattern I.
II—Wiring pattern II.
SW—Square-wave pulses.
HSW—Half sine-wave pulses.
p.p.s.—Pulses per second.

the high frequency (30 pulses per second total or 6 p.p.s. per field) than from the low frequency (15 p.p.s. total or 3 p.p.s. per field).

TABLE 6.—*Analysis of variance of the immediate effect (1st day) of water resistivity (rows), wiring pattern and pulse shape (columns), and voltage and frequency (treatments), on mortality of fingerling silver salmon*

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Water resistivity	129.9289	3	43.3096	3.23
Wiring pattern and pulse shape	123.9093	3		
Wiring pattern	1.5813	1	1.5813	0.118
Pulse shape	122.1578	1	122.1578	*9.109
Interaction	0.1702	1	0.1702	0.013
Voltage and frequency	175.6544	3		
Voltage	43.9238	1	43.9238	3.275
Frequency	127.8596	1	127.8596	*9.534
Interaction	3.8710	1	3.8710	0.289
Residual	80.4669	6	13.4112	
Total	509.9595	15		

*Significant at 5-percent level.

As previously mentioned, polarity of the electrodes was not alternated when the array was wired according to wiring pattern I and energized with half sine-wave pulses. The polarity of the electrodes was alternated, however, when the array was wired according to wiring pattern I and energized with square-wave pulses. Therefore, the difference in mortality which in table 6 is attributed to the difference between pulse shapes could, in wiring pattern I, be due to the effect of alternating polarity, or to a combination of alternating polarity and the difference between pulse shapes, rather than to the difference between pulse shapes alone.

If the difference in mortality rates was due to alternating polarity, either alone or in combination with pulse shape, the analysis would be expected to show a significantly higher mortality resulting from wiring pattern II than from wiring pattern I, since polarity of the electrodes was alternated for both pulse shapes when the array was wired according to pattern II. Also, the analysis would be expected to show significance for the interaction of wiring patterns and pulse shapes. In both of these analyses, however, the differences are not significant. Therefore, we have concluded that the difference in mortality was due to the difference between pulse shapes and not to the effect of alternating polarity. Figure 13 is a comparison of the two pulse shapes and substantiates this conclusion. The figure shows that the total electrical energy of a square-wave

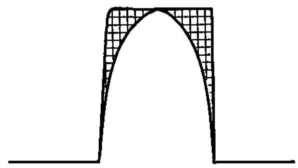

FIGURE 13.—Comparison of square-wave and half sine-wave pulses. Shaded area indicates additional amount of electrical energy available to the fish with each square-wave pulse.

pulse is approximately 30 percent greater than that of a half sine-wave pulse at any constant value of voltage, frequency, and duration.

Total electrical energy is directly proportional to frequency, providing pulse shape, voltage, and pulse duration remain constant. Therefore, the fish subjected to the high frequency experienced twice as much electrical energy as the fish subjected to the low frequency. This difference in total electrical energy explains the higher mortality that resulted from the high frequency.

Although no significant differences in mortality resulted from the differences between the variations of water resistivity, wiring pattern, and voltage, certain indications are apparent from the table of means (table 7) and are summarized as follows:

Water resistivity.—Mortalities that resulted from the four levels of water resistivity were combined into two groups: those resulting from the low levels (1,000 and 5,000 ohm cm.) form one group and those resulting from the high levels (10,000 and 15,000 ohm cm.) form the other. Under the conditions tested, it is apparent that the low levels of water resistivity resulted in a higher percentage of losses than the high levels. The analysis of variance shown in table 8 reveals that this difference is significant at the 5-percent level.

Wiring pattern.—The difference in mortality that can be attributed to the difference between wiring patterns I and II is only 0.5 percent.

Voltage.—High voltage (250 volts) appears to have resulted in a higher percentage of losses than low voltage (165 volts).

Analysis of variance shows that significant differences in mortality resulted from the differences between the variations of pulse shape, frequency, and water resistivity (when the mortalities resulting from the four levels of water resistivity were combined into a high and a low group and the variance test was between groups). However, it

TABLE 7.—*Immediate (first day) mean percentage mortality resulting from each of the test conditions*

[Table of means]

Test conditions	Arc sin √percentage	Percent	Group percent
Water resistivity:			
1,000 ohm cm	17.94	9.5	8.1
5,000 ohm cm	15.14	6.8	
10,000 ohm cm	10.62	3.4	3.8
15,000 ohm cm	11.91	4.3	
Wiring pattern:			
I	14.22	6.0	
II	13.59	5.5	
Pulse shape:			
Square wave	16.67	8.2	
Half sine wave	11.14	3.7	
Voltage:			
Low (165 v.)	12.24	4.5	
High (250 v.)	15.58	7.2	
Frequency:			
Low (15 p.p.s.)	11.08	3.7	
High (30 p.p.s.)	16.73	8.3	

TABLE 8.—*Analysis of variance of the immediate effect (1st day) of low (1,000 and 5,000 ohm cm.) and high (10,000 and 15,000 ohm cm.) levels of water resistivity on the mortality of fingerling silver salmon*

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Water resistivity [1]	129.9289	3	43.3096	3.23
Low vs. high	110.9336	1	110.9336	*8.2717
Remainder	18.9953	2	9.4976	0.708
Residual	80.4669	6	13.4112	

[1] See table 6.
* Significant at 5-percent level.

does not show whether the least-harmful variations of these test conditions caused a significant number of mortalities in the test fish compared with that in the control fish. Therefore, a chi-square analysis was conducted to determine whether a difference in mortality existed between the test fish and the control fish.

In this analysis, the mortalities resulting from the low and high levels of water resistivity and each variation of pulse shape and frequency were grouped into eight combinations. Each combination included the mortalities resulting from one variation of each of these three variables. The total percentage mortality of each combination was then compared with the total percentage mortality of the controls. The results of this analysis are shown in table 9.

Table 9 reveals that the least-harmful test variations (high water resistivity, half sine-wave pulses, and low frequency) did not cause a significantly higher mortality of test fish than of the control fish. The table also shows that the test variations caused a high mortality only when two or more of the harmful variations were combined.

TABLE 9.—*Chi-square comparison of the immediate (1st day) mortality of the test fish with that of the control fish*

Comparison [1]	Sample size	No. of deaths	Percent	Degrees of freedom	X^2
Control	403	10	2.48	1	0.00
LR, LF, HSW	197	5	2.54		
Control	403	10	2.48	1	*7.62
LR, HF, HSW	194	14	7.22		
Control	403	10	2.48	1	*11.85
LR, LF, SW	195	17	8.71		
Control	403	10	2.48	1	*45.60
LR, HF, SW	194	35	18.04		
Control	403	10	2.48	1	0.205
HR, LF, HSW	207	4	1.93		
Control	403	10	2.48	1	2.437
HR, HF, HSW	207	10	4.83		
Control	403	10	2.48	1	0.958
HR, LF, SW	206	8	3.88		
Control	403	10	2.48	1	*3.97
HR, HF, SW	194	11	5.67		

[1] Explanation of symbols:
LR—Low levels of water resistivity (1,000 and 5,000 ohm cm.).
HR—High levels of water resistivity (10,000 and 15,000 ohm cm.).
LF—Low frequency (15 p.p.s.).
HF—High frequency (30 p.p.s.).
HSW—Half sine-wave pulses.
SW—Square-wave pulses.
* Significant at 5-percent level.

This analysis is based on the assumption that the variations of wiring pattern and voltage did not cause a significant number of deaths among the test fish when compared with the control fish. However, since voltage and wiring pattern were integral parameters of the experiment, they were factors in the results of the analysis. Therefore, they would have caused the entire analysis to show significance if the variations of either had caused a significant number of mortalities in the test fish as compared with that in the control fish.

The analysis is also based on the assumption that no differences in mortality existed among the four groups of control fish. To test this assumption, another chi-square analysis was made, and it showed that the differences in mortality among control groups were not significant at the 5-percent level. The chi-square value for this test was 1.273 with 3 degrees of freedom.

Delayed effect

Analysis of variance to determine the delayed effect (second to thirtieth day) reveals that at the 5-percent significance level there were no differences in mortality that could be attributed to the differences between the test variations (table 10). Again, however, certain indications are apparent from a table of means (table 11) and are summarized as follows:

General.—With the exception of frequency, the variations of the test conditions resulted in approximately the same differences in mortality

TABLE 10.—*Analysis of variance of the delayed effect (2d to 30th day) of water resistivity (rows), wiring pattern and pulse shape (columns), and voltage and frequency (treatments), upon the mortality of fingerling silver salmon*

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Water resistivity	188.5606	3	62.8535	1.377
Wiring pattern and pulse shape	82.4236	3		
Wiring pattern	0.2450	1	0.2450	0.005
Pulse shape	16.8921	1	16.8921	0.357
Interaction	65.2864	1	65.2864	1.430
Voltage and frequency	71.8659	3		
Voltage	27.4052	1	27.4052	0.600
Frequency	10.5300	1	10.5300	0.231
Interaction	33.9306	1	33.9306	0.743
Residual	273.9149	6	45.6525	
Total	616.7650	15		

after 30 days as they did after 1 day. These differences, however, were not significant because of an increase in residual error.

Pulse frequency.—The difference in mortality which resulted from the difference between levels of frequency actually reversed in sign. This is presumably another indication of the increase in residual error.

Water resistivity.—Mortalities which resulted from each of the four levels of water resistivity were combined in a low and a high group as they were for the analysis of the immediate effect. Analysis of variance (table 12) reveals no significant difference in mortality between the two groups after 30 days.

TABLE 11.—*Cumulative mean percentage mortality (2d to 30th day), resulting from each of the test conditions*

[Table of means]

Test conditions	Arc sin √percentage	Percent	Group percent
Water resistivity:			
1,000 ohm cm.	21.78	13.8	15.6
5,000 ohm cm.	24.76	17.5	
10,000 ohm cm.	15.61	7.2	8.6
15,000 ohm cm.	18.54	10.1	
Wiring pattern:			
I	20.30	12.0	
II	20.05	11.8	
Pulse shape:			
Square wave	21.20	13.1	
Half sine wave	19.15	10.8	
Voltage:			
Low (165 v.)	18.86	10.4	
High (250 v.)	21.48	13.4	
Frequency:			
Low (15 p.p.s.)	20.98	12.8	
High (30 p.p.s.)	19.36	11.0	

Chi-square analysis showed that after the 30-day holding period there was no longer a significant difference in mortality between the control fish and the test fish. The chi-square value of this comparison was 3.476, with 1 degree of freedom. In this analysis the total percentage mortality of the control fish, measured from the second through the thirtieth day, was compared with the corresponding mortality of the test fish. A preliminary chi-square analysis showed no significant differences in mortality between the control groups ($X^2=6.8737$, with 3 degrees of freedom) and analysis of variance (table 10) showed that no differences in mortality had resulted from differences in the test variations.

TABLE 12.—*Analysis of variance of the delayed effect (2d to 30th day) of the low (1,000 and 5,000 ohm cm.) and high (10,000 and 15,000 ohm cm.) levels of water resistivity on mortality of fingerling silver salmon*

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Rows [1]	188.5606	3	62.8535	1.377
Low vs. high	153.5121	1	153.5121	3.363
Remainder	35.0485	2	17.5242	0.384
Residual [1]	273.9149	6	45.6525	

[1] See table 10.

CONCLUSIONS

Although analysis of the immediate effect (effect in the first 24 hours) of the conditions tested shows that a significant number of mortalities occurred when the fish were subjected to combinations of two or more of the deleterious variables (low water resistivity, high frequency, and square-wave pulses), it should be remembered, before reading the conclusions of this manuscript, that the fish tested in this experiment were forced to swim completely through the electrical fields created by the array. This situation is not likely to occur in our more successful field experiments, since our method of diverting fish with electricity is based on the principle of an avoidance reaction.

Immediate effect

1. Of the two pulse shapes tested, the square-wave pulses resulted in a higher mortality than the half sine-wave pulses.

2. High frequency (30 p.p.s.) caused a significantly higher mortality than low frequency (15 p.p.s.).

3. Low levels of water resistivity (1,000 and 5,000 ohm cm.) resulted in a higher percentage mortality than the high levels (10,000 and 15,000 ohm cm.).

4. Of the two levels of applied voltage tested, the high voltage (250 v.) resulted in a higher percentage mortality than the low voltage (165 v.). This dif-

ference, however, was not significant at the 5-percent level.

5. The difference between wiring patterns did not cause a significant difference in mortality.

6. A significant difference in mortality between the test fish and the control fish resulted only when two or more of the deleterious variables (square-wave form, low water resistivity, and high frequency) were combined in the tests.

Delayed effect

1. The differences in mortality that could be attributed to the differences between the test variations were not significant after the 30-day holding period.

2. There was no significant difference in mortality between the test fish and the control fish after the 30-day holding period.

In view of these conclusions, it seems logical to infer that, if the electrical shock is going to cause mortalities, most of these mortalities will occur within the first 24 hours after exposure.

SUMMARY

Electricity has been used with some success as a method of diverting fish. However, since its use invariably raises the question of mortality, this experiment was conducted to determine the effect of certain electrical parameters and water resistivities on the mortality of fingerling silver salmon.

A Latin square experimental design was used in the investigation with water resistivity, wiring pattern, pulse shape, voltage, and frequency as variables.

In groups of approximately 100, the fish were subjected to preset electrical and water conditions and then transferred to an outdoor tank where they were held 30 days for observation. Four control groups were also tested for continuous comparison of their mortality with that of the tested fish.

Statistical analysis of the data to determine the immediate (first day) effect of the conditions tested revealed that (1) square-wave pulses resulted in a significantly higher mortality than half sine-wave pulses; (2) high frequency (30 p.p.s.) caused a higher mortality than low frequency (15 p.p.s.); (3) low levels of water resistivity (1,000 and 5,000 ohm cm.) resulted in a higher percentage mortality than high levels (10,000 and 15,000 ohm cm.); (4) although the difference was nonsignificant, high voltage (250 v.) resulted in a higher percentage mortality than low voltage (165 v.); (5) there was no significant difference in mortality that could be attributed to the difference between the two wiring patterns.

A chi-square comparison of the test fish with the control fish revealed that a significant difference in mortality existed only when two or more of the three variables which proved to be deleterious (low water resistivity, square-wave pulses, and high frequency) were combined in the tests.

Effect of the electric shock diminished after the first day; the statistical analysis to determine the delayed effect (second to thirtieth day) revealed that (1) there were no significant differences in mortality that could be attributed to differences between the test variations, and (2) difference in mortality between the test fish and control fish was nonsignificant.

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