LEADING ADULT SQUAWFISH
(*Ptychocheilus oregonensis*)
WITHIN AN ELECTRIC FIELD

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ABSTRACT

The nature of squawfish predation on hatchery-reared salmon fingerlings requires an economical and practical method for continuously removing the squawfish from the areas of releases. Research has been directed toward the development of an electrical trapping device employing the principle of electrotaxis. The objectives of the study were to determine by systematic testing, (A) the optimum values of the electrical variables of (1) potential (60, 75, and 90 volts), (2) pulse frequency (2, 5, and 8 pulses per second), and (3) pulse duration (10, 20, and 30 milliseconds) for leading adult squawfish within an electrical array; and (B) the possible significance of the direction of movement of the electrical fields. Pulse frequency was found to be the most critical variables (optimum, 2 pulses per second). Potential was also significant (optimum, 60 volts), but pulse duration was not significant, at least in the range tested. The direction of the electrical fields in the laboratory tank was a highly significant variable. The fish showed a greater response when the electrical fields moved toward the north end of the laboratory tank than toward the south. The reason for this reaction was not determined.

INTRODUCTION

For the past five years, the Columbia River fishery for salmon and steelhead has been valued at $17,000,000 annually. In addition, this fishery has provided an inestimable amount of pleasure to sports fishermen. The fishery is sustained, in part, by some 600,000 Columbia River salmon and steelhead that annually ascend the fish ladders at Bonneville Dam. However, reduction in spawning areas, due to construction of dams, has caused increasing dependence on hatchery-reared fish.

In 1956, about 58,000,000 salmon fingerlings, reared in hatcheries at a cost of $732,000, were released into the Columbia River and its tributaries below McNary Dam. Since the fingerling salmon are released in dense concentrations, thus becoming easy prey for predatory fish, the success of the salmon-rearing program is greatly affected by the predators. Biologists and hatchery personnel have often observed at times of release of fingerling salmon that the squawfish (Ptychocheilus oregonensis (Richardson), is the most destructive of these predators.

A study by the U. S. Fish and Wildlife Service 1/ has shown that almost all of the predation by squawfish occurs immediately after release of the fingerlings. This fact indicates that the most effective way to control squawfish predation is to reduce the numbers of squawfish in the areas where the fingerlings are released.

Management biologists have used dynamite and gill nets to reduce the populations of resident squawfish in the release areas for periods of several weeks prior to the release of the fingerlings. Neither of these control measures has been effective, however, as the release of fingerlings soon attracts more squawfish.2/ Once the fingerlings are released, dynamite no longer

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1/ Thompson, Richard B. Food of the squawfish, Ptychocheilus oregonensis (Richardson), of the Lower Columbia River. United States Fish and Wildlife Service. Fish. Bull. No. 158 (In press)

2/ Zimmer, Paul D. Observations on hatchery releases and squawfish predation in Little White Salmon River in spring of 1953. United States Fish and Wildlife Service, Mimeo. Report, August 1953. 14 pp. Portland, Oregon.

can be used; and the manpower required for continuous control of squawfish population by gill netting makes that method impractical also. The failure of these controls makes clear that there is need for an economical and practical method for continuously removing squawfish from the area in which the fingerlings are released.

In an effort to meet this need, Maxfield and Volz in 1953 [3] conducted a laboratory study of controlling squawfish by electricity. This study involved the use of a single electrical field as a barrier to block the entrance of sqawfish into a designated area. Direct current was supplied to two rows of electrodes, the positive row being located downstream. The individual squawfish swimming up to the barrier would feel the electrical field and, usually, would be deterred from entering the strong field between the two rows of electrodes. If, however, a squawfish did enter the strong field between the two rows, its reaction was to swim back toward the positive row and out of the array. This reaction of the fish can be explained by the principle of electrotaxis--movement of the fish toward the positive pole in a direct-current field. Fingerling salmon, being shorter than the adult squawfish, were less affected by the potential difference in the field and passed safely through this electrical barrier when released on the upstream, or negative, side.

Preliminary Experiment

In recent laboratory studies at Seattle, we attempted to use electrotaxis to lead the squawfish into a net enclosure or trapping area. The square-wave form of pulsating direct current was used with an array of several rows of electrodes in which the electrical fields were sequentially created in one direction between successive rows of electrodes.

However, initial attempts to lead individual squawfish into a trapping area in the laboratory required basic knowledge of the effect on squawfish of the electrically variable factors of potential, pulse frequency, and pulse duration. A preliminary experiment, therefore, was set up to determine the extreme ranges of these three variable factors.

The results showed that the following conditions should be the limits and ranges of further experiments:

Potential: The range of potentials studied extended from (1) the minimum potential at which the squawfish responded to (2) the minimum potential at which the squawfish were in obvious distress. This range was 60 to 90 volts, respectively, when applied to two rows of electrodes spaced 17 inches apart in rows 18 inches apart.

Pulse frequency: Employing the two experimentally determined values of potential of 60 and 90 volts, we used the above procedure to arrive at a suitable range of pulse frequencies: 2 to 8 pulses per second.

Pulse duration: Finally, employing combinations of the two potentials and the two pulse frequencies, we also used the same procedure in an attempt to arrive at a suitable range of pulse durations. Variations in pulse duration, however, in the range of 10 to 90 milliseconds did not have any observable different effects on the individual squawfish. A range of pulse duration of 10 to 30 milliseconds, therefore, was selected arbitrarily for use in the following experiments.

After gaining knowledge of the extreme ranges, we wished to determined the optimum electrical conditions for leading adult squawfish. Therefore, we designed the experiments reported below in which we tested, under rigorously controlled conditions, the effects on squawfish of extreme and intermediate values of the electrical variables. In addition, we had observed in the preliminary work that the direction of movement of the electrical fields in the laboratory tank might be a significant variable. Accordingly, we also tested the effect of this variable on squawfish.

Objectives

The objectives of the present study were to determine by systematic testing, (A) the optimum values of (1) potential,

3/ Maxfield, Galen H., and C. D. Volz. An electrical barrier for controlling squawfish (Ptychocheilus oregonensis) predation. U. S. Fish and Wildlife Service, Seattle, Washington. (Unpublished ms.)

(2) pulse frequency, and (3) pulse duration for leading adult squawfish within an electrical array; and (B) the possible significance of the direction of movement of the electrical fields.

Acknowledgments

The research reported here, conducted in 1956, was made possible with funds for squawfish control studies from the Lower Columbia River Program, U. S. Fish and Wildlife Service. Acknowledgment is made to personnel of the Fish and Wildlife Service hatcheries of the Lower Columbia River for use of holding facilities. Joseph Gauley and Ben Pulliam collected adult squawfish at Bonneville Dam, and Clifford Davidson cooperated in the collection of squawfish at Rock Island Dam. Archie Anderson, Oregon Fish Commission, gave the use of a holding pond at Bonneville Hatchery; Dr. Lauren R. Donaldson, University of Washington, permitted the use of several holding ponds at the School of Fisheries in Seattle.

Especial appreciation is expressed to Dr. Douglas G. Chapman, Department of Mathematics, University of Washington, for reviewing the statistical treatment of the data and to Paul Zimmer, Fish and Wildlife Service, Portland, Oregon, for his efforts in furthering the research.

METHOD

We designed these experiments (1) to proceed from the results of our preliminary experiments, (2) to use most advantageously a limited supply of squawfish, (3) to overcome the inability to reverse readily the direction of the electrical fields, and (4) to use standard experimental procedures in measuring the leading effectiveness of electrical conditions.

Experimental Design

1. Preliminary observations on individual fish defined the working ranges of potential, pulse frequency, and pulse duration, as follows: Potential, 60 to 90 volts; pulse frequency, 2 to 8 pulses per second; and pulse duration, 10 to 30 milliseconds. Intermediate values were tested in each range to determine whether the ranges included optimum electrical conditions for leading adult squawfish. Inclusion of these intermediate values increased the number of possible combinations of values of potential, pulse frequency, and pulse duration from 8 to 27. These 27 combinations in the experimental design were set up as a series of tests.

2. As more than one test at each combination in the series was needed to determine whether the effects of one variable factor depended upon the values of one or both of the other variable factors, the number of tests required in relation to the number of fish available for use in the tests was such as to require using each group of fish twice. Furthermore, it was not possible to test every combination of the three variable factors with both unshocked and once-shocked fish before proceeding to the next combination.

3. As we had no facilities to control the temperature of the water in the experimental tank, we anticipated a temperature decrease throughout the experiments from late summer to fall.

4. It was not possible readily to reverse the direction of movement of the electrical fields in the laboratory tank, but it was of some interest to determine, if possible, whether the direction of the electrical fields had a significant effect.

5. On the basis of readings taken at release areas, the resistance of the water was maintained at 15,000 ohms per cubic centimeter throughout the experiments.

Based on our preliminary work, the possibility existed that the fish could show a preference for the north or south end of the experimental area. This could be determined readily if the direction of the electrical fields could be reversed quickly--a combination could be tested with the movement of the electrical fields first in one direction and then in the other. However, preliminary observations indicated that, with the power off, a prohibitive number of fish would be required to demonstrate the presence or absence of a preference of the fish for one end of the laboratory tank.

The use of the north and south blocks of tests arose from the time-consuming procedure that would have been necessary in order to alternate the direction of movement of the electrical fields. All combinations were tested in separate series with unshocked and once-shocked fish in each of the two blocks: (1) positive fields moving to the north, and (2) positive fields moving to the south. The assumption that the relative effect of each combination would be the same in each block, even though the tests in one block would be performed later than those in the other, was tested by graphically comparing the trends of the effects between blocks and by analyzing interaction effects.

If the proportion of fish that entered the north end with power off had changed within a block, then the estimates of relative effects of test conditions run at different times in the block would have been biased. To avoid such a bias the order of testing each combination was random within each block; a new random order was used for each series with unshocked and once-shocked fish.

While designing the experiment, we surmised that a difference between north and south blocks of tests would represent a possibility of the following effects: (1) a preference by the fish for the north or south end; (2) differences in the pattern of electrical current flow associated with the reversal of direction of the electrical fields; and (3) changes in the experimental environment, such as the water temperature changes which did occur between blocks. Furthermore, if differences between blocks due to the pattern of electrical current flow were assumed to be negligible, and if the effect of temperature also were shown to be insignificant, then a pronounced difference between blocks could be ascribed to a preference of the fish for one end of the laboratory tank. During the experiment, we did assume the differences between blocks due to the pattern of electrical current flow to be negligible, and the effect of temperature was insignificant. Although we do not know the cause for the preference, we conclude that the difference between blocks was due to a preference of the fish for one end of the laboratory tank.

The experiment was, therefore, designed as a 3^3 factorial with the following values of electrical variable factors: potential, 60-75-90; pulse frequencies, 2-5-8; and pulse durations, 10-20-30. Eight series of tests (4 on unshocked, and 4 on once-shocked fish) of these 27 combinations were run at random. Four series (2 on unshocked, and 2 on once-shocked fish) were tested in each of the two experimental blocks: (1) positive fields moving to the north, and (2) positive fields moving to the south.

Source of Fish

The adult squawfish (*Ptychocheilus oregonensis*) used in these experiments were transported in an aerated tank from U. S. Fish and Wildlife Service hatcheries at Little White Salmon and Leavenworth, Washington, and from the Bonneville hatchery of the Oregon Fish Commission at Bonneville Dam, Oregon. The fish had been held in outdoor rearing ponds until sufficient numbers had been obtained for transportation

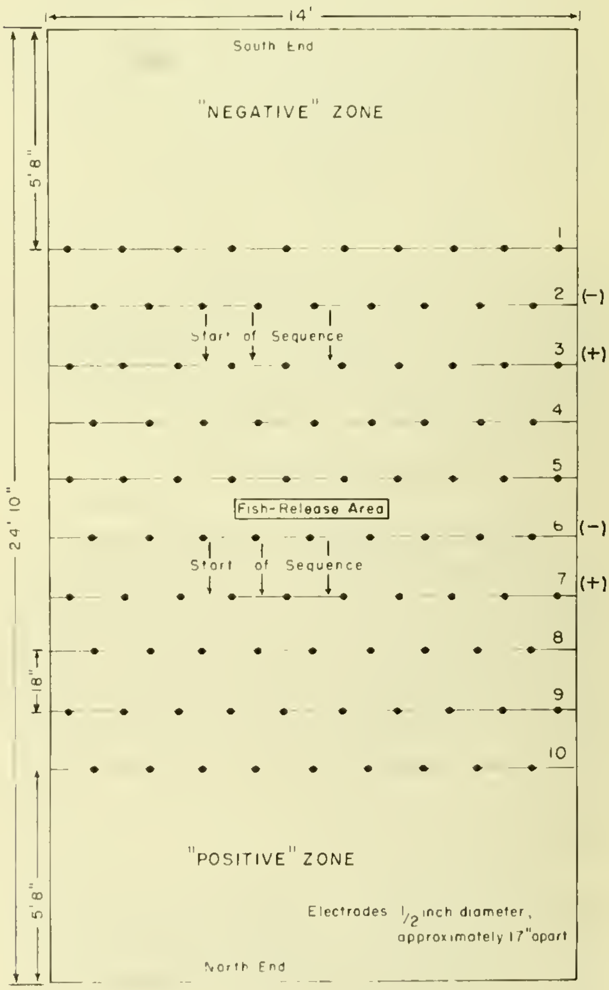

Figure 1.--Laboratory experimental area with staggered electrode array. Arrows indicate start of sequence of electrical fields moving toward north end of laboratory tank.

to Seattle. The fish stored at Little White Salmon hatchery had been obtained (1) by seining and by hook-and-line fishing in Drano Lake, (2) by hook-and-line fishing in the Columbia River near the Spring Creek hatchery, and (3) by seining in a drainage ditch at Echo, Oregon. The fish stored at Leavenworth hatchery had been trapped both in the forebay and in the fishway at Rock Island Dam, Washington. The fish stored at Bonneville hatchery had been trapped in the fingerling bypass at Bonneville Dam. These fish were transported to Seattle in 100-fish lots, and most lots of the fish were placed in screen traps in lake water near the laboratory. Some lots were held in outdoor hatchery raceways in lake water at the School of Fisheries, University of Washington, Seattle.

Apparatus and Measurements

The experimental area (fig. 1) in the laboratory was a portion of a larger insulated tank constructed for electrical studies on salmon fingerlings. The concrete floor and construction block walls were equipped with an electrically insulated lining of several coats of Amercoat paint. The inside dimensions of the experimental area were 24 feet 10 inches by 14 feet by 16 inches. The depth of water was approximately 11 inches during the period of the study, and the water was not in motion.

The electrodes used in the array were of 1/2 inch outside diameter aluminum tubing, 12 inches long. Two holes were drilled at one end of each electrode; a T-shaped copper wire with a copper slim-nosed alligator clip soldered onto the base of the T was inserted into the holes. The electrodes were suspended in the water, to an approximate depth of 10 1/2 inches, from ten rows of parallel copper wires secured to insulators. The wires were strung at right angles across the center of the experimental area. The distance between the rows was 18 inches, and the electrodes were spaced approximately 17 inches apart in each row in a staggered array (figs. 1 and 2).

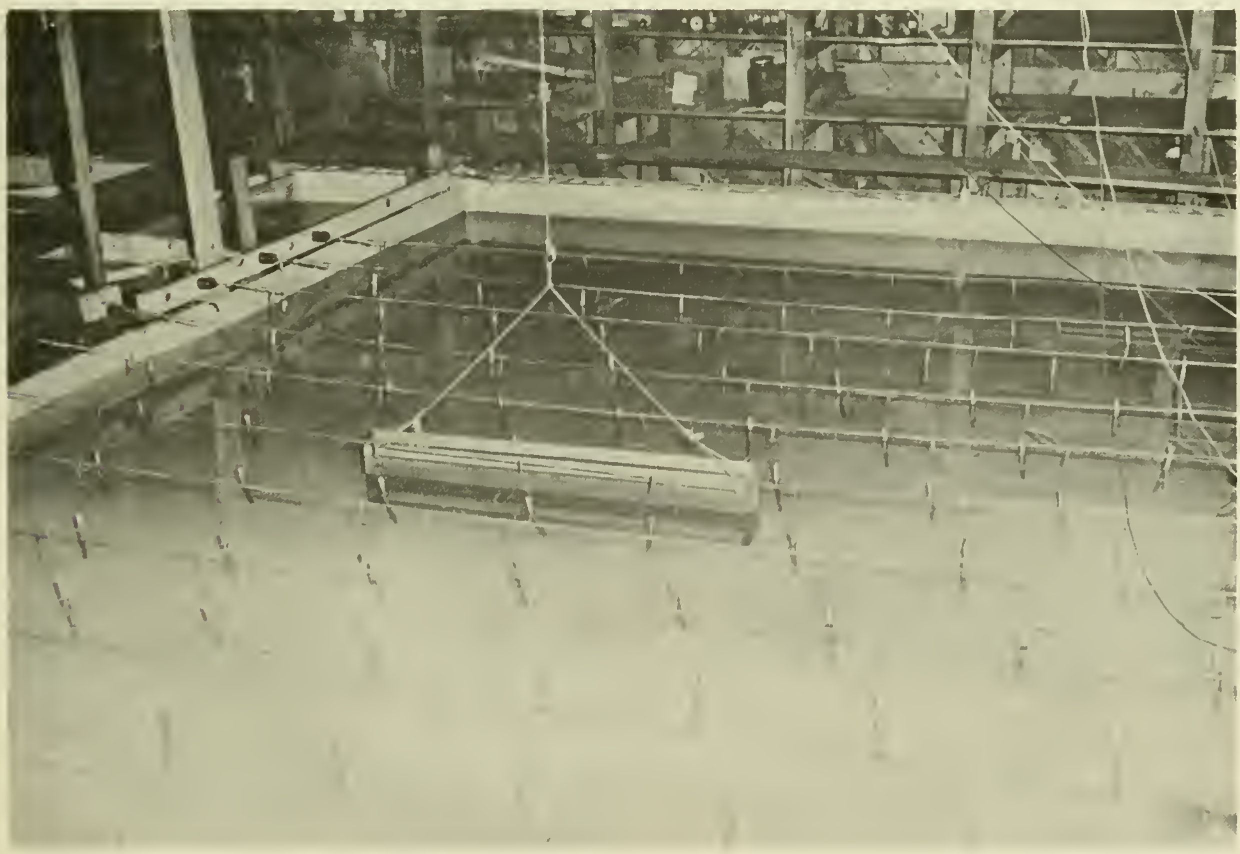

Figure 2.--Staggered electrode array used in laboratory.

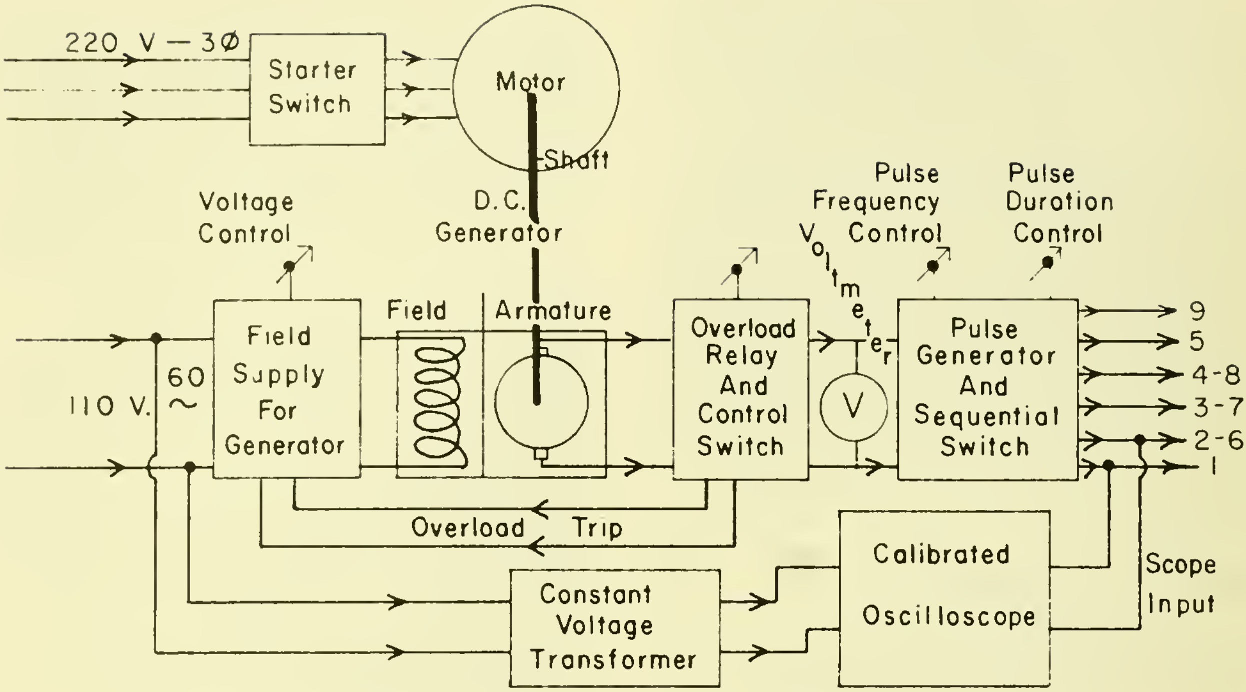

Figure 3.--Block diagram of electrical equipment.

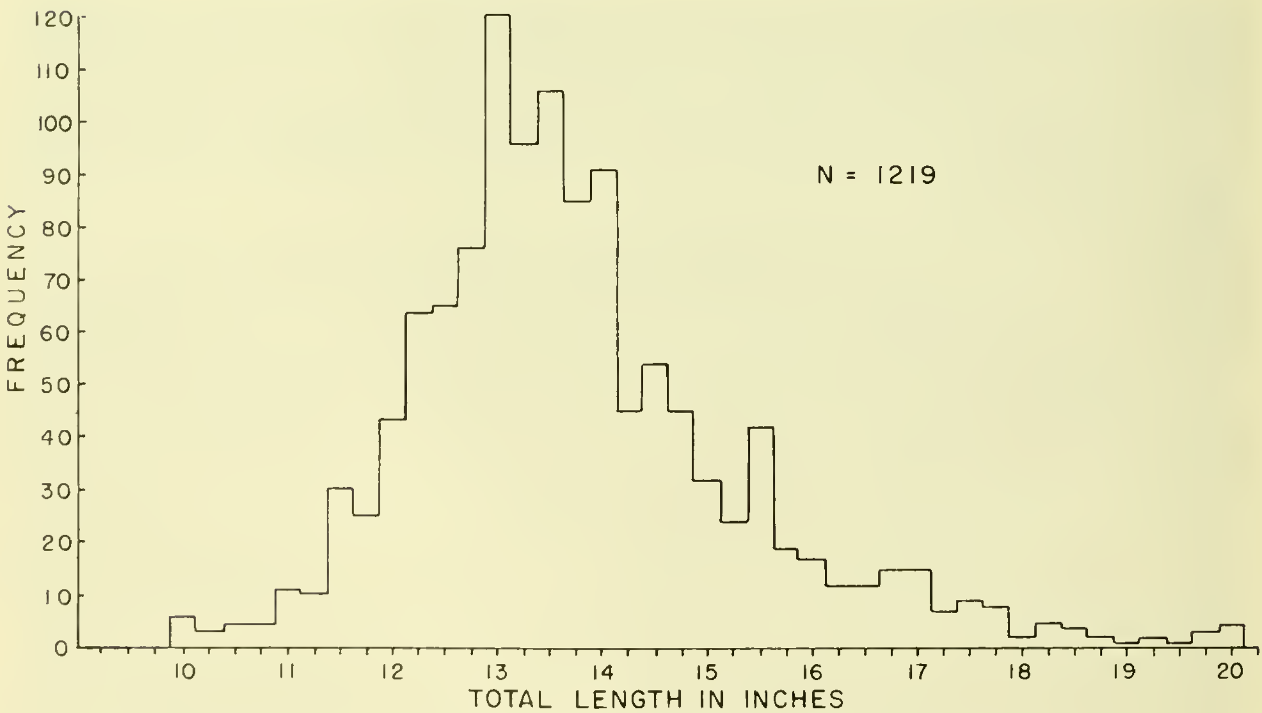

Figure 4.--Length frequency distribution of adult squawfish.

The fish were released in the center of the array (between rows 5 and 6) from a rectangular enclosure made from small-mesh wire screen fitted with wooden laths for support, the inside dimensions being length, 4 feet; width, 4 inches; and height, 15 1/2 inches. The enclosure was raised out of the water by means of a bridle and a line running through pulleys on the ceiling. After the array was energized, the operator stationed at the electronic switching unit pulled the line to release the fish within the moving electrical fields.

By means of an electronic switching unit developed in the Seattle laboratory, pairs of the rows of the electrodes were energized successively to establish a sequence of pulsating, direct-current fields that moved in the direction of positive polarity. In the north block, the positive row of a pair of energized rows was always to the north. Figure 1 shows for the north block the start of the sequence when the first set of electrical fields is established by energizing the second and third, and the sixth and seventh rows of electrodes simultaneously. The progress of the entire sequence through the electrode rows is indicated in table 1. As the table shows, the

Table 1.--Sequence for energizing electrode rows to set up moving electrical fields in laboratory array.

Change in polarity	Polarity									
	Row 1	Row 2	Row 3	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9	Row 10
First	0	(-)	(+)	0	0	(-)	(+)	0	0	0
Second	0	0	(-)	(+)	0	0	(-)	(+)	0	0
Third	0	0	0	(-)	(+)	0	0	(-)	(+)	0
Fourth	0	0	0	0	(-)	(+)	0	0	(-)	(+)
Sequence begins again	0	(-)	(+)	0	0	(-)	(+)	0	0	0

sequence is automatically repeated after the tenth row of electrodes has been energized. The frequency and duration determine the rate and length of time each pair of rows of electrodes is energized. For example, at a setting of 30 milliseconds pulse duration and frequency of 2 pulses per second, a pair of rows is energized 30 milliseconds with a lapse of 470 milliseconds before the next pair is energized. The total time to energize the four sets of electrical fields in the desired sequence (from rows 2 and 3 through row 10) at the above conditions of frequency and duration is 2 seconds. With pulse frequencies of 5 and 8 pulses per second, at a pulse duration of 30 milliseconds, it required .8 and .5 seconds, respectively.

The direction of movement of the electrical fields shown in figure 1 was reversed for the south block of tests. Upon reversal, row 10 in figure 1 became row 1 (the electrode row not energized). In the south block, the positive row of a pair of energized rows was always to the south.

Although the end zones of the experimental area were not electrically charged except for fringe effects from the electrical fields, it was convenient in conducting our tests to name the zones "positive" and "negative". The "positive" end zone was beyond row 10 (the last positive charge row) of the array, and the "negative" end zone was before row 1 (which was uncharged).

A schematic description of the switching unit designed to perform this operation is shown in the block diagram (fig. 3). A detailed description of the electrical circuits controlling the square-wave pulses throughout this experiment and determining the sequence of firing will be published later.4/

The direct current was provided by a 10-kilowatt motor-generator set, with a maximum output of 500 volts at 20 amperes. A calibrated oscilloscope operated from a voltage-regulating transformer was used for setting potential and observing wave form, and a vacuum-tube volt meter for plotting the field with a voltage gradient probe. As a safety device, an overload relay with push-button reset was installed to prevent serious damage to the electrical apparatus.

Areas to hold the fish prior to and following each test were constructed from plywood at the north end of the tank. A plywood wall separated these holding areas from the experimental area of the tank to eliminate any possible visual effect that

4/ Dale, Harry P., and C. D. Volz. A pulse generation and distribution system for electrical fish guiding. United States Fish and Wildlife Service.

might attract the fish during a test. These holding areas were not within the influence of the electrical field.

The tests were conducted under a constant light intensity provided by two 500-watt lamps suspended 10 feet above the water at each end of the large tank.

Potential was measured by a calibrated oscilloscope, specific resistance of the water by an industrial conductivity bridge, and temperature of water by a standard mercury thermometer. The desired pulse frequency and pulse duration for each test were set by dials on the calibrated switching unit.

The fish were measured for total length (from the tip of the snout to the extreme end of the tail) to the nearest quarter-inch. Each new lot of fish was sorted and counted into two size groups: Group 1--small fish, 10 to 15 inches; Group 2--large fish, 15 through 20 inches. Fish shorter than 10 and longer than 20 inches were eliminated. Figure 4 (page 6) shows the length frequency of the adult squawfish used in the tests. These measurements were taken of fish which died after one exposure and of fish twice exposed to the electrical field.

The number of fish available was not sufficient to permit testing with one specific size group, or to make a comparison between two or more size groups. Therefore, a ratio of small to large fish was determined for each lot of new fish, and this ratio was used to make up the 10 fish in each test. This size ratio varied as the weeks of testing progressed, but the average ratio was 8 small fish to 2 large fish.

Experimental Procedures

We conducted 216 tests (8 series of tests, 27 tests per series) from August 1 through October 31, 1956, exposing 1,080 fish in 10-fish groups as unshocked and once-shocked fish. Actually a total of 1,219 fish was required to perform the tests because of mortalities of some of the once-shocked fish.

After we released adult squawfish within the sequentially pulsed electrode array, we measured the effectiveness of leading for each combination of potential, pulse frequency, and pulse duration by the percentage of fish that entered the "positive" end zone of the experimental area during the 7-second period of test.

We used equal numbers of fish (10 fish) to test each condition to obtain a reliable comparison of the leading effectiveness of various potentials, pulse frequencies, and pulse durations, the number of tests performed each day being determined by the number of fish available. The squawfish to be tested were placed in holding areas at the north end of the laboratory tank.

If the lot of fish to be tested consisted of unshocked fish (fish not previously exposed to the electrical fields) we sorted it into the two size groups.

To minimize any chance of a learning response, we used a lot of fish no more than twice. The tests in each series were so arranged that the fish were never exposed to the same combination of variable factors as unshocked and once-shocked fish.

The experimental tank was drained and filled with fresh water once a week to maintain the desired level of resistance (15,000 ohms/cm^3) of the water. No facilities were available to control the temperature of the water. The temperature measured 62° F. at the beginning of the tests August 1, 1956, and dropped to 50° F. at the end of the tests on October 31, 1956.

At the beginning of each test, after the electronic switching unit was turned on, we visually checked whether the electrode array was pulsing properly by observing a series of argon lamps connected across each pair of electrode rows. After determining that the switching unit was performing as desired, we turned the unit off until we had placed the 10 fish to be tested in the fish release enclosure.

Half the fish for each test were headed toward each end of the fish release enclosure, and aligned across the direction of the sequentially pulsed electrical fields. After turning on the switching unit and releasing the fish, we determined the duration of exposure by a time clock, meanwhile counting the fish during each test according to (1) the number that were led with moving electrical fields into the "positive" end zone, and (2) the number that went against the moving electrical fields into the "negative" end zone. These two counts were made during the test interval because no traps were closed at the end zones to hold the fish at the conclusion of the test. Then we dip-netted the fish from the experimental area, separated them for size, and placed them in the holding areas until the tests for that day were completed. Remarks on the behavior of the fish were recorded for each test.

Two men were required for each test. When the men at the switching unit had turned on the power to energize the electrode array and had received a signal from the second man, he quickly raised the fish release enclosure to liberate the fish.

Following the tests for each day, the two size groups of once-shocked fish were returned to separate holding pens in lake water. These fish were not exposed for the second time until after at least 48 hours. Twice-shocked fish were measured for total length and destroyed to make holding space available for new lots of fish.

RESULTS AND DISCUSSION

We recorded the behavior of groups of squawfish in each test in order to determine the effects of the different levels of potential, pulse frequency, and pulse duration; also counts were made of the number of fish that were effectively led under the different electrical conditions.

Description of Behavior

The squawfish reacted instantaneously to the potential and pulse frequency levels used in these experiments. The pattern of the reactions of the squawfish in the energized fields depended more upon changes in pulse frequency than potential. In fields of pulse frequency of 2 pulses per second, at the three levels of potential: (1) the squawfish appeared to feel the electrical fields, showed no loss of equilibrium, and responded favorably to the direction of the electrical fields; (2) the swimming speed of the individual fish varied from slow to swift; (3) some fish circled in the middle of the array before orienting themselves to the direction of the electrical fields; (4) some fish swam rapidly against the direction of the electrical fields and into the "negative" end zone; and (5) some fish swam rapidly against the direction of the electrical fields, but their swimming speed was slowed to such an extend that (a) they barely reached the end zone, or (b) they suffered electroparalysis and thus never left the electrode array. Many of those fish swimming against the direction of the electrical fields appeared to jerk their heads in response to the pulse, which, however, was not strong enough to reverse their direction.

In fields of pulse frequency of 5 pulses per second, at energy levels of potential of 60 and 75 volts: (1) the squawfish appeared to feel the electrical fields noticeably, revealing loss of equilibrium in from 1 to 8 fish in one-third to one-half of the tests; (2) swimming was sluggish or difficult, the speed slow to moderate; (3) the squawfish generally showed no leading response to the direction of the electrical fields; and (4) most of the fish in half of the tests at the level of potential of 75 volts remained in the middle of the electrode array. At the level of potential of 90 volts: (1) 1 to 10 squawfish, in almost every test, suffered electroparalysis; (2) swimming was sluggish or difficult; (3) the squawfish showed no leading response to the direction of the electrical fields; and (4) most or all of the fish in each test remained in the middle of the electrode array.

In fields of pulse frequency of 8 pulses per second, at levels of potential of 60 and 75 volts: (1) squawfish felt the electrical fields very noticeably, revealing loss of equilibrium in from 2 to all 10 fish in all tests; (2) swimming was sluggish or difficult, with loss of motion in some fish; and (3) most of the fish remained in the middle of the electrode array. At the level of potential of 90 volts: (1) 3 to 10 squawfish in each test suffered immediate or nearly immediate electroparalysis; and (2) when electroparalysis was not immediate, the movements of the fish were generally quite violent, and, in many cases, excellent leading response of short duration was achieved. Typically, an individual squawfish made a violent lunge at the moment of release within the sequentially pulsed electrical fields. This action carried the fish 2 to 3 feet from the release area, or approximately half the distance to the "positive" end zone. This lunge endured usually for the time interval of one sweep of the pulse through the four electrical fields. As the time interval of the pulse to sweep the four fields once is one-half second at 8

Table 2.--Percentage of squawfish that entered "positive" end zone during 7-second test interval.

	Percentage of unshocked fish						Percentage of once-shocked fish					
	To north zone at frequency [1/] of			To south zone at frequency [1/] of			To north zone at frequency [1/] of			To south zone at frequency [1/] of		
	2	5	8	2	5	8	2	5	8	2	5	8
Tests at 60 volts potential:												
Duration:												
10 milliseconds: 1st test	90	30	30	50	50	30	60	80	50	60	0	10
2nd test	50	80	0	30	30	10	60	40	10	20	10	0
20 milliseconds: 1st test	50	60	20	60	10	10	60	60	60	40	30	10
2nd test	80	0	0	60	30	0	40	40	0	60	10	0
30 milliseconds: 1st test	40	50	0	40	10	20	100	50	10	50	10	0
2nd test	70	40	20	40	40	0	80	40	0	80	20	0
Tests at 75 volts potential:												
Duration:												
10 milliseconds: 1st test	90	70	30	60	0	10	80	50	0	30	0	0
2nd test	80	50	0	30	10	0	60	0	0	10	30	0
20 milliseconds: 1st test	50	20	30	60	20	0	60	60	10	20	0	0
2nd test	50	20	0	40	10	0	90	30	0	20	0	0
30 milliseconds: 1st test	60	30	50	40	20	0	40	70	10	70	10	0
2nd test	80	20	0	40	0	10	80	40	0	80	0	0
Tests at 90 volts potential:												
Duration:												
10 milliseconds: 1st test	70	40	0	20	0	0	50	50	30	70	10	0
2nd test	50	0	0	40	0	0	60	0	0	70	0	0
20 milliseconds: 1st test	60	0	20	60	0	0	70	60	0	10	0	0
2nd test	40	10	0	50	0	0	70	10	0	20	10	0
30 milliseconds: 1st test	60	0	20	40	0	0	80	60	10	60	0	0
2nd test	70	10	0	50	0	0	40	0	0	40	0	0

1/ Frequency in pulses per second.

pulses per second, the fish reached an electroparalytic state in about one-quarter of a second. Nearly all the fish remained in the middle of the electrode array.

Analysis of Results

For each test, as outlined in "Experimental Procedures," the percentage of adult squawfish that entered the "positive" end zone of the laboratory experimental area is recorded in table 2. The mean percentage of the adult squawfish that entered the "positive" end zone for each value of the three variable factors of potential, pulse frequency, and pulse duration is recorded in table 3 for north and south blocks and unshocked and once-shocked fish separately.

Table 3.--Mean percentage of squawfish that entered the "positive" end zone in north and south blocks for each value of the variable factors of potential, pulse frequency, and pulse duration and for once-shocked and unshocked fish separately. Each mean percentage in this table is based on 18 observations.

	Unshocked		Once-shocked	
	North "positive"	South "positive"	North "positive"	South "positive"
Potential (volts)				
60	39.44	28.89	46.67	22.78
75	40.56	19.44	37.78	15.00
90	25.00	14.44	32.78	16.11
Frequency (pulse-second)				
2	63.33	45.00	65.56	45.00
5	29.44	12.78	41.11	7.78
8	12.22	5.00	10.56	1.11
Duration (milliseconds)				
10	42.22	20.56	37.78	17.78
20	28.33	22.78	40.00	12.78
30	34.44	19.44	39.44	23.33

Data were transformed from percentages (table 2) to arc sin $\sqrt{\text{percentage}}$, and all analyses were run on such transformed observations. Preliminary analyses showed, for both unshocked and once-shocked fish, negligible average difference in effects between the two times the test conditions were repeated. It was impossible to test both unshocked and once-shocked fish at the same time with each combination of variable factors, because of the limited supply of fish for testing. Therefore, we analyzed separately the data from the tests with unshocked and once-shocked fish as well as the data on the two groups combined. The completed analyses are shown in table 4 for unshocked fish and in table 5 for once-shocked fish, and the analysis for the two groups combined in table 6.

Shocking condition.--As may be seen from table 6, there was no difference between shocking conditions (unshocked vs once-shocked), nor was the interaction of shocking condition with any other variable significant.

Effect of blocks.--All three analyses (tables 4, 5, and 6) showed highly significant differences between "blocks" for both unshocked and once-shocked fish.

Differences between blocks cannot be attributed to a single cause (see page 4); therefore, we tested for the significance of the relationship between temperature difference and percentage difference in numbers of squawfish that entered the "positive" end zone. Separate tests were made for the north and south blocks, and for the unshocked and once-shocked fish in each block. The four "t" values obtained were

(1) t = 0.598 for unshocked fish in north block
(2) t = 0.466 for once-shocked fish in north block
(3) t = 0.219 for unshocked fish in south block
(4) t = 1.188 for once-shocked fish in south block

The 5-percent significant level for "t" with 25 degrees of freedom is 2.06. There is, therefore, no evidence of a relationship between temperature and percentage of squawfish led. For illustration we include a graph (fig. 5) showing the difference in percentage of fish that entered the

Table 4.--Analysis of variance of the effects of potential, pulse frequency, and pulse duration on the movement of unshocked squawfish.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Blocks	2,990.310	1	2,990.310	15.68**
Potential	3,857.553	2	1,928.776	10.11**
Frequency	25,760.850	2	12,880.425	67.53**
Duration	334.526	2	167.263	.88
BxF	244.255	2	122.128	.64
BxD	411.247	2	205.624	1.08
BxP	484.821	2	242.410	1.27
FxD	353.210	4	88.302	.46
FxP	2,151.541	4	537.886	2.82*
DxP	428.383	4	107.096	.56
BxFxD	1,034.338	4	258.584	1.36
BxFxP	145.278	4	36.320	.19
BxDxP	184.293	4	46.073	.24
FxDxP	575.853	8	71.982	.38
BxFxDxP	329.230	8	41.154	.22
Error	10,299.116	54	190.724	
Total	49,584.804	107		

* $.01 < P < .05$

** $P < .01$

Table 5.--Analysis of variance of the effects of potential, pulse frequency, and pulse duration, on the movement of once-shocked squawfish.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Blocks	7,455.400	1	7,455.400	36.13**
Potential	2,187.477	2	1,093.738	5.30**
Frequency	31,036.691	2	15,518.346	75.19**
Duration	214.771	2	107.386	.52
BxF	1,217.141	2	608.570	2.95*
BxD	163.244	2	81.622	.82
BxP	130.666	2	115.330	.56
FxD	1,254.778	4	313.694	1.52
FxP	461.759	4	115.440	.56
DxP	533.727	4	133.432	.65
BxFxD	427.380	4	106.845	.52
BxFxP	250.762	4	62.690	.30
BxDxP	878.586	4	219.646	1.06
FxDxP	1,107.749	8	138.469	.67
BxFxDxP	2,443.621	8	305.453	1.48
Error	11,144.405	54	206.377	
Total	60,908.151	107		

* $.01 < P < .05$

** $P < .01$

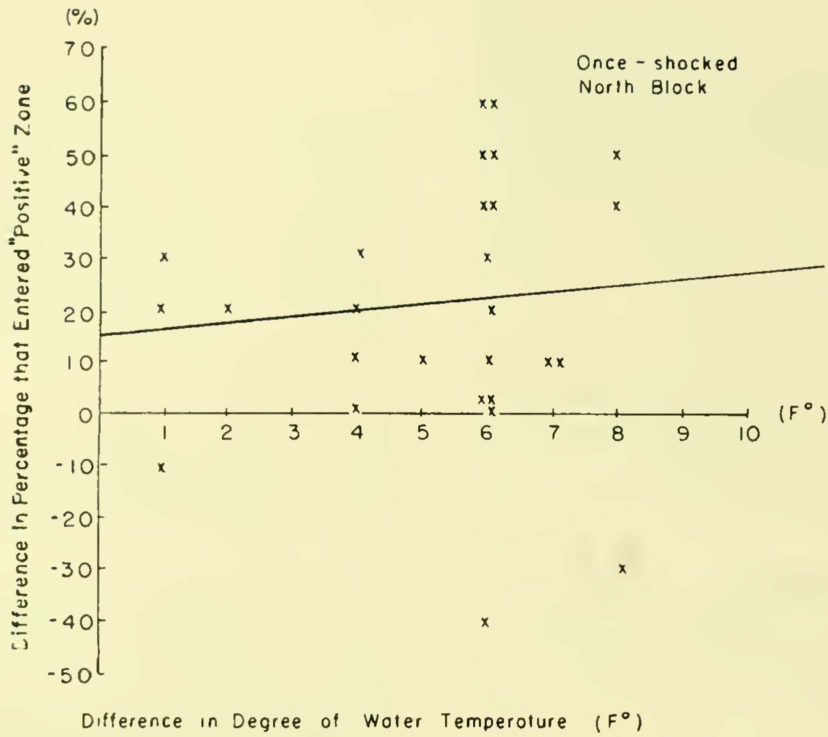

Figure 5.--Difference in percentage of squawfish that entered the "positive" zone against the difference in water temperature between times a combination was run.

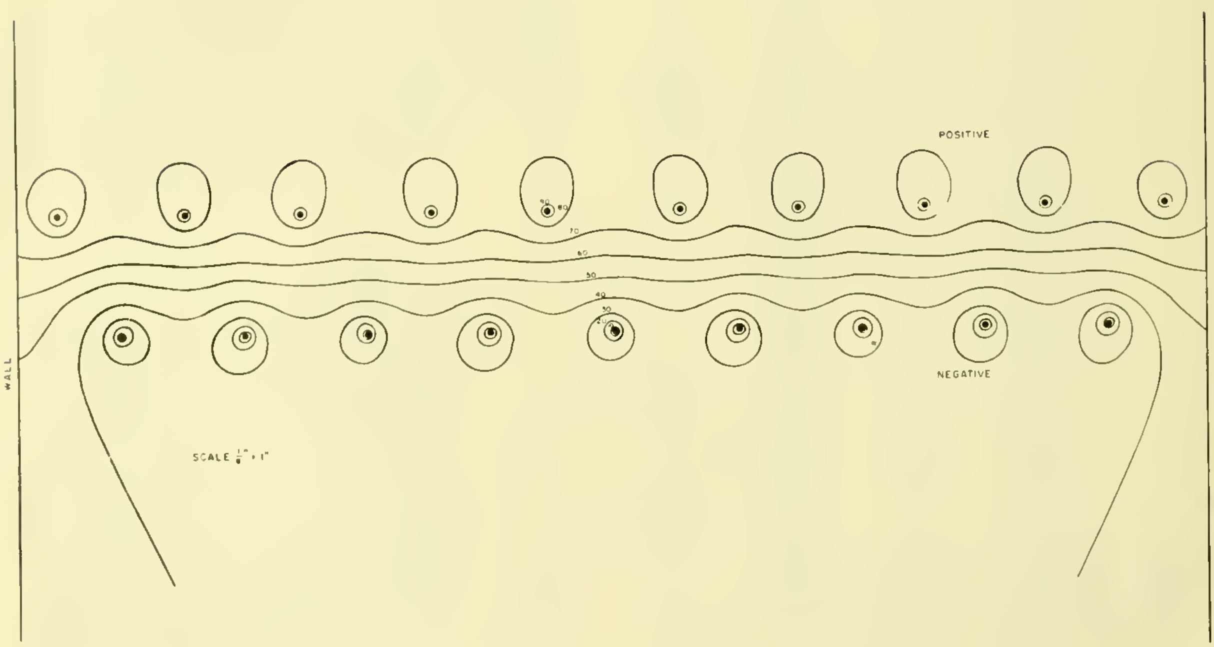

Figure 6.--Diagram of potential contours for sequentially pulsed field.

"positive" end zone (first result minus second result) plotted against the difference in water temperature (first measurement minus second measurement) between the two times a combination was tested on once-shocked fish in the north block.

Assuming that the electrical field in the water (fig. 6) was not changed by reversing the direction of the electrical fields, the difference between north and south blocks of tests probably represented a preference by the fish for the north end (fig. 7).

Effect of potential.--There was a highly significant difference between the different levels of potential as shown by the three analyses of variance (tables 4, 5, and 6).

For the unshocked fish, the overall potential effect was obscured by the significant interaction of potential with pulse frequency (table 4). In particular, the rate of decrease in effect, with increasing potential, is much less at 2 pulses per second than at the higher frequencies of 5 and 8 pulses per second.

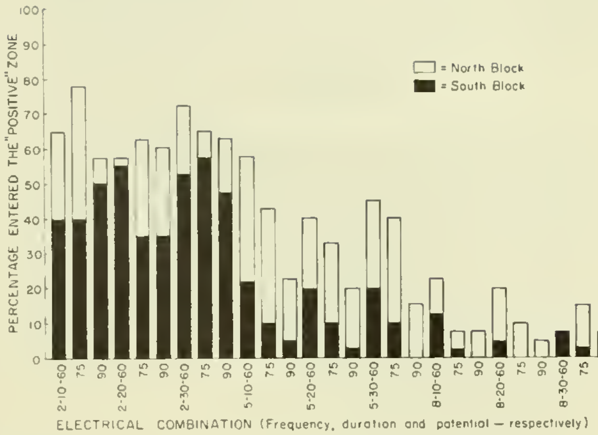

Figure 7.--Comparison between blocks of the mean percentage of squawfish that entered the "positive" end zone.

For the once-shocked fish, a rapid rate of decrease in effect with increasing potential occurred only at 8 pulses per second (table 5).

A more detailed examination of the potential effect may be made using Tukey's Least Significant Difference (L.S.D.) test.[5/] The L.S.D. is found by multiplying the standard error by the Q value (tabulated in table 10.6.1, p. 252, of Snedecor) and dividing the result by the square root of the number of observations in the means to be compared. The standard error is

$$\sqrt{\frac{10,299.166 + 11,144.405}{108}} = 14.09;\ \text{this}$$

is determined by pooling the error sum of

Table 6.—Analysis of variance of tests of unshocked and once-shocked squawfish combined.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Shocking condition (Unshocked vs Once-shocked)	3.20	1	3.20	.02
Blocks	9,944.50	1	9,944.50	57.23**
Potential	5,127.35	2	2,563.68	14.75**
Frequency	56,497.84	2	28,248.92	162.57**
Duration	262.82	2	131.41	.76
SCxB	501.21	1	501.21	2.88
SCxF	299.71	2	149.86	.86
SCxD	286.48	2	143.24	.82
SCxP	917.68	2	458.84	2.64
BxF	1,211.61	2	605.80	3.49*
BxD	43.11	2	21.56	.12
BxP	572.43	2	286.22	1.65
FxD	290.93	4	72.73	.42
FxP	2,473.41	4	618.35	3.56**
DxP	440.16	4	110.04	.63
SCxBxF	249.79	2	124.90	
SCxBxD	531.38	2	265.69	
SCxBxP	43.05	2	21.52	
SCxFxD	1,317.06	4	329.26	
SCxFxP	139.89	4	34.97	
SCxDxP	521.95	4	130.49	
Third & fourth order interactions	7,377.07	56	131.73	
Error	21,443.52	108	198.55	
Pooled Error ***	31,623.71	182	173.76	

* .01 < P < .05
** P < .01
*** Pooled error is found by combining all sums of squares below the double line since none of these are significant even at the 10% level.

The F values listed in the table are found by comparing each mean square (M.S.) with the pooled error mean square.

5/ Snedecor, George Waddel. 1956. Statistical methods applied to experiments in agriculture and biology. 5th edition. Down State College Press, Ames, Iowa.

squares for the unshocked and once-shocked fish. The tabulated Q value for three "treatments" and an error mean square based on 108 d.f. is 3.37. Since each potential mean is calculated from 72 observations.

$$\text{L.S.D.} = \frac{(14.09)\ (3.37)}{\sqrt{72}} = 5.60$$

Any difference of means exceeding 5.60 is significant at the 5 percent level.

The three potential means are:

60 : 34.44

75 : 28.20

90 : 22.08

and the differences are:

60-75 : 6.24

60-90 : 12.36

75-90 : 6.12

Since these three differences all exceed 5.60 the three potential means are all significantly different at the 5 percent level.

Effect of pulse frequency.--The differences between pulse frequencies were highly significant for both unshocked and once-shocked fish--F values were highest in all three tables (tables 4, 5, and 6). Examination of the data, as well as qualitative observations, leads to the conclusion that optimum pulse frequency is below 5 pulses per second. For example, see figure 8(b) and table 3 (page 10).

Effect of pulse duration.--This variable factor for the values tested (10, 20, 30 milliseconds) was not significant in the three analyses (tables 4, 5, and 6), nor was any significant interaction found.

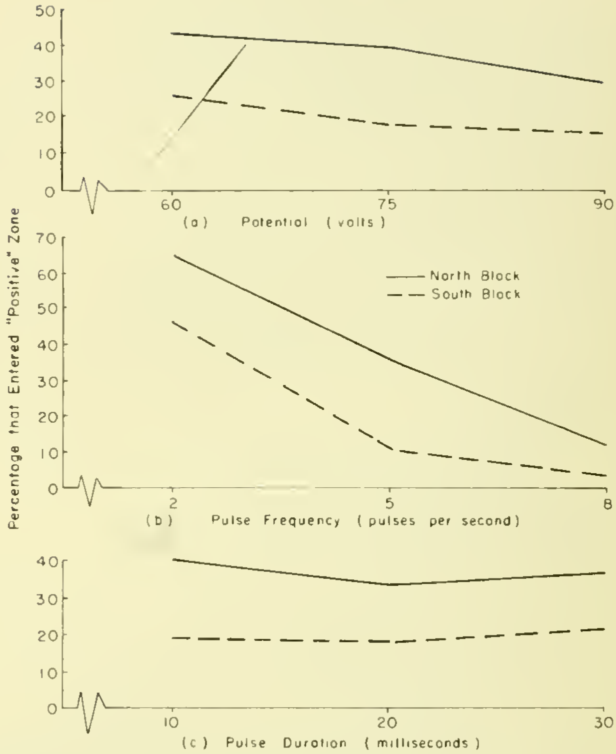

Figure 8.--Mean percentage of squawfish that entered the "positive" end zone in north and south blocks for each value of the variable factors of potential, pulse frequency, and pulse duration, unshocked and once-shocked fish combined. Each mean percentage based on 36 observations.

CONCLUSIONS

A. Optimum electrical conditions: Pulse frequency was the most critical variable. Potential also was significant. Substantiating the preliminary observations, pulse duration was not significant, at least in the range tested. The optimum values of the electrical variables were as follows:

1. Potential--Of the three potentials, 60, 75, and 90 volts, 60 volts was the optimum;

2. Pulse frequency--Of the three pulse frequencies, 2, 5, and 8 pulses per second, 2 pulses per second was the optimum;

3. Pulse duration --With the three pulse durations, 10, 20, and 30 milliseconds, no optimum was observed.

B. Direction of the electrical fields: The direction of the electrical fields was a highly significant variable. The fish showed a greater response when the electrical fields moved toward the north end of the laboratory tank than toward the south. The reason for this reaction was not determined.

RECOMMENDATIONS

The following recommendations are made for future laboratory experiments in leading adult squawfish within a direct-current sequentially pulsed electrod array using electrodes of 1/2-inch diameter with 18 inches between rows and 17 inches between electrodes:

1. Future experiments should be limited to the optimum values of potential and pulse frequency: 50 volts, and 2 pulses per second, respectively, found in these experiments.

2. The possibility of an effect from pulse duration should not be overlooked. In future experiments, a range of values of shorter duration than 10 milliseconds and longer duration than 30 milliseconds should be systematically investigated in combination with the optimum potential and pulse frequency values.

3. The cause of the significant difference between directions of the electrical fields should be explored. If this phenomenon is peculiar to the laboratory, it is of little general interest. If it is due to a variable which might also operate in a field control installation, the effects of this source of variation should be estimated.

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