AN ABSTRACT OF THE THESIS OF

Howard Franklin Horton for the Ph. D. in Fisheries
(Name) (Degree) (Major)

Date thesis is presented May 14, 1963

Title THE FACTORS LEADING TO POST-LIBERATION MORTALITIES OF TROUT TRANSPORTED IN TANK TRUCKS

Abstract approved Signature redacted for privacy.
(Major professor)

The results of experiments conducted in 1957 and 1958 to isolate the cause of post-transportation mortalities among hatchery-reared rainbow trout, Salmo gairdneri Richardson, in Oregon are reported. Although death rate varies greatly, the average loss occurring from one to seven days following liberation has been estimated to be ten percent of the fish transported. Studies conducted over the past 15 years on problems arising from the live shipment of fishes are reviewed.

General experimental procedure was to place fish in aquaria and subject them to certain environmental conditions perhaps associated with delayed planting mortalities. After a specified interval, animals were removed from test containers and placed in observation troughs. Subsequent losses were recorded and compared to
the measured experimental conditions in an effort to identify causal relationships.

Simulated in-transit motion, density of fish transported, and pre-hauling starvation period had no apparent influence on the magnitude of delayed trout deaths. Zinc from zinc chloride and from galvanized iron plating was toxic to trout, and, at low concentrations, produced delayed mortalities characteristic of those that occurred following tank truck transportation in Oregon.

The toxic effect of zinc increased with increasing zinc concentration, and with increasing length of exposure to the metal. Of the trout exposed for six hours to an initial zinc concentration of 0.10 mg/l, an average of 8.2 percent suffered a delayed mortality. When the concentration was increased to 1.00 mg/l for the same time period, the delayed loss increased to an average of 63.2 percent. When length of trout exposure to various zinc concentrations was increased from two to eight hours, the mean delayed mortality increased from 39 to 68 percent.

The toxicity of zinc increased with decreasing total water hardness. At 0.75 mg/l zinc, mean trout mortalities increased from five to 28 percent when total water hardness was decreased from 40 to 20 p.p.m. When hardness was decreased from 20 to zero p.p.m., mean mortalities increased from 28 to 88 percent. On the basis of research previously conducted in Oregon, it was
shown that delayed trout losses were inversely correlated with
total alkalinity of the transporting water. Assuming that calcium
was the principal cation involved, the relationship was attributed
to the effects of increased water hardness.

Samples of transportation water from 32 inter-hatchery
transfers of fish were taken at the completion of the station to sta-
tion trips and analyzed for zinc. In every instance where delayed
mortalities greater than one percent occurred, appreciable quan-
tities of zinc were detected. When delayed losses of trout were
much less than one percent, no zinc was detected in the water used
during the transfer.

Based on the results of the field and laboratory tests reported,
it was concluded that zinc from galvanized iron surfaces was re-
sponsible for the post-transportation mortalities of trout in Oregon.
Fish exposed to low zinc concentrations in liberating tanks were
subjected to threshold toxic conditions in which a varying portion
of the animals died a delayed death. Relatively small changes in
the natural water hardness or zinc concentration could either in-
crease or eliminate subsequent mortalities.
THE FACTORS LEADING TO POST-LIBERATION MORTALITIES OF TROUT TRANSPORTED IN TANK TRUCKS

by

HOWARD FRANKLIN HORTON

A THESIS

submitted to

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in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

June 1963
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Date thesis is presented ______________ May 14, 1963

Typed by Muriel Davis
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inter-hatchery transfers of fish. Mr. Homer J. Campbell, Fishery Research Biologist, offered useful suggestions on the design of the laboratory apparatus.

Research Assistants Richard Wallace and James Meehan, and Assistant Hatchery Superintendent William Wingfield, helped me record data at crucial times. Mrs. Marge Jackson and Miss Marcia Wright typed several revisions of the manuscript. My wife, Jeannine, drew the thesis figures, and endured my inattentiveness in silence.
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THE FACTORS LEADING TO POST-LIBERATION MORTALITIES OF TROUT TRANSPORTED IN TANK TRUCKS

INTRODUCTION

The results of experiments conducted in 1957 and 1958 to isolate the cause of post-transportation mortalities among hatchery-reared rainbow trout, *Salmo gairdneri* Richardson, in Oregon are reported in this thesis. Delayed mortalities\(^1\) of trout following live transportation have been recognized in Oregon for at least 35 years. Although death rate varies greatly from time to time and place to place, the average loss has been estimated to be ten percent of the fish transported (25, p. 122). Considering that in recent years the Oregon State Game Commission has planted annually approximately 500,000 pounds of rainbow trout (29, p. 285) valued in excess of one dollar per pound, the monetary loss has approached and probably exceeded 50,000 dollars per year.

Initial sporadic investigations into the problem were conducted in Oregon during the late 1930's, but failed to establish any consistent pattern of delayed loss occurrence. Isolated efforts by Game

\(^1\) In this report, the term "delayed mortality" and synonyms will be used to denote trout deaths which occur from one to seven days following liberation. Apart from immediate hauling loss, delayed mortalities generally commence about 24 hours subsequent to release and cease entirely within seven days.
Commission biologists to solve the problem persisted until 1950, when it was assigned to the Oregon Cooperative Wildlife Research Unit. Preliminary studies were conducted the next year, and intensive field investigations commenced in 1952. The early Research Unit studies had the primary objective of developing a technique for controlling such losses. The experimental procedure was to simulate normal methods of transporting fish by truck so that certain variables could be controlled and measured. Resulting delayed mortalities were then compared with measured conditions in an attempt to identify causal relations.

The initial objective was achieved in 1955, when research findings demonstrated that delayed losses could be controlled by maintaining transportation water temperatures from about 4.4°C to 7.2°C. While this technique afforded a means by which trout could be transported safely, little information was gained regarding the cause of the problem.

In June, 1955, a second general program was inaugurated by the Oregon Cooperative Wildlife Research Unit. This program was directed toward identifying the factor or factors responsible for post-transportation deaths. All laboratory experiments were

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2 Oregon State Game Commission, United States Fish and Wildlife Service, Oregon State University and Wildlife Management Institute cooperating.
conducted at the Game Commission's Alsea Trout Hatchery, five miles northeast of Alsea, Lincoln County, Oregon. Investigations reported here were carried out during the period from August, 1957, through September, 1958.

A general review of studies conducted over the past 15 years on problems arising from the live shipment of fishes revealed that most occurrences of transportation mortalities were associated with one or more of the following factors: (1) Insufficient dissolved oxygen (13, p. 175-183); (2) excessive carbon dioxide concentrations (23, p. 291-310); (3) high levels of ammonia nitrogen (13, p. 175-183; 37, p. 429-436); (4) relatively high water temperature (38, p. 27-32); (5) increased blood lactate levels (4, p. 20-27); (6) physical injury (36, p. 1-57); (7) starvation (24, p. 27-45); and (8) zinc poisoning (1, p. 142-169; 3, p. 1-4).

Since conditions responsible for delayed trout mortalities in Oregon were unknown, each of the aforementioned factors was reviewed in relation to the present problem. Saltzman (34, p. 1-59) and Linn (21, p. 1-73) investigated certain chemical and physical aspects of the problem and concluded that dissolved oxygen, carbon dioxide and ammonia nitrogen gases were not implicated in delayed transportation deaths. On the basis of cursory examination, Saltzman (34, p. 1-59) also concluded that zinc poisoning was not a
factor in the delayed losses.

The possibility that blood lactate increase following muscular activity could be the basis for delayed planting mortality of salmonids was theorized by Black (4, p. 21) in 1956. In 1957, Black and Barrett (5, p. 14) made periodic measurements of lactic acid accumulation in the blood of cutthroat (Salmo clarki) and steelhead (Salmo gairdneri) trout following live transportation in British Columbia, and concluded that no unequivocal evidence had been obtained that increase in blood lactate during shipment was the cause of delayed planting mortality.

As a result of 60 sham fish transfers conducted in 1954, I found that post-liberating losses could be controlled by maintenance of low (4.4° to 7.2° C.) transporting water temperature (15, p. 9-11). I theorized that the low temperature may have inhibited the production of noxious substances, but that temperature itself was not the limiting factor. I also autopsied trout that had died following sham transportation in Oregon. The only abnormality found was a dark spot on the wall of the intestinal cavity in about 15 percent of the cases (15, p. 4). No other physical injuries were noticed.

Both rainbow trout and brook trout (Salvelinus fontinalis) have been shown to be able to fast for ten weeks without excessive mortalities occurring (31, p. 20). In an extreme situation, 50 percent
of a group of hatchery-produced rainbow trout survived a 248-day starvation period (32, p. 14). Since in Oregon, trout were denied food for only two days prior to being transported, there was no reason to suspect the starvation period as a factor contributing to delayed loss.

Since analysis of the literature revealed no particularly promising avenues of inquiry, it was decided to follow certain leads suggested in my earlier work (15, p. 14), and any others that might develop. Large-scale field tests were abandoned in favor of laboratory experimentation, thus facilitating greater control over the many variables encountered during actual liberating procedures, and making possible replication of experiments at greatly reduced costs.

Fish placed in aquaria were subjected to certain environmental conditions perhaps associated with delayed planting mortalities. After a specified interval, animals were removed from test containers and placed in observation troughs. Subsequent losses were recorded and compared to the measured experimental conditions in an effort to identify the cause of post-transportation mortality.

Individual and combined effects of exposure time, fish transportation density, pre-test starvation period, within-transit motion, zinc from two sources, and total water hardness were examined.
The study was terminated when experimental results indicated that acute zinc toxicity was responsible for delayed losses. Subsequent information from inter-hatchery fish transfers supported the conclusion.
METHODS AND APPARATUS

Apparatus

Experimental Aquaria

Tests were performed in five-gallon, widemouth, glass jars. Ten liters of water was the volume used in all of the experiments. Following each use, the aquaria were washed with detergent, thoroughly rinsed and allowed to air dry in an inverted position.

Constant Temperature Tank

The rectangular constant temperature tank was constructed of galvanized-iron sheet metal and measured 62 x 36 x 14 inches in outside dimensions. Peripheral support for the tank was provided by 1- x 10-inch wood planking, and the tank was situated on a table in the center of the laboratory for easy access to the four sides.

The temperature of the water in the bath was maintained at 15° C. by use of a 500-watt immersion heater and a submerged cooling element. Water in the bath was circulated continuously by a small pump. A thermostatic controlled the intermittent operation of the heater, the amount of continuous cooling being adjusted by the amount of coolant passing through the submerged, copper coils. Figure 1 illustrates the temperature control system.
Figure 1. Constant temperature control system, showing (A) circulating pump, (B) red indicator lamp, (C) thermostatic switch, (D) cooling coils, and (E) immersion heater.
Water, Air and Light Sources

Alsea River water was used for experimental purposes. Height of the second-floor laboratory necessitated use of a shallow-well jet pump and pressure tank to lift water from the hatchery pipe lines to the testing area. Water for fish holding and observation troughs was obtained directly from the hatchery supply lines.

A compressor provided air for oxygenating water in the experimental aquaria and, at times, in the observation troughs. Individual air lines led from a manifold to each aquarium. Air volume was controlled by use of a therapeutic regulator and individual manifold valves. A carborundum diffusing stone was attached to the discharge end of each air line.

Illumination was provided by two 40-watt fluorescent lamps centrally located above the constant temperature tank. The observation troughs were illuminated by natural light only, except during the short intervals required for mortality counts at night.

Motion Device

In order to simulate the effect of in-transit motion, a device was constructed to oscillate aquaria within the constant temperature tank. Four aquaria were held in each of two compartments of an
angle-iron frame measuring 42 x 21 x 13 inches. An axis about which the device could rock was attached to the base of the frame and supported by three bearings. A 1/4-inch thick piece of marine plywood covered the bottom of the frame. This was perforated with 1 1/2-inch holes to reduce the resistance of the water to oscillations of the device.

The frame was moved by a 1/4-horsepower, electric motor coupled to a 49:1 Winsmith Speed Reducer (Model No. 3CT). A four-stage (3-, 4-, 5- and 6-inch diameter), V-belt pulley was secured to the output shaft of the speed reducer and matched to an identical, but opposed, sliding, four-stage pulley. One end of the upper shaft projected over the constant temperature tank and supported a 3-inch eccentric (Figure 2). The eccentric was attached to one corner of the transporting frame by a 14-inch actuating rod.

When the apparatus was in operation, a choice of 13 different oscillating speeds could be made by sliding the movable pulley into the desired position over the other pulley. The motion device is illustrated in Figure 3.

Observation Troughs

Following a simulated transportation experience, fish were transferred for observation to hatchery troughs which measured 18
Figure 2. Power source for motion device, showing (A) 1/4-h.p. motor, (B) 49:1 speed reducer, (C) stationary pulley, (D) movable pulley, and (E) eccentric.
Figure 3. Motion device ready for operation in constant temperature tank.
feet long by 17 inches wide by 8 inches deep. In the earlier experiments, individual test groups were held in fish egg hatching baskets set into the troughs (Figure 4). A four-inch depth of running water was maintained in the baskets, and water temperature was not controlled. Later, due to variations in results attributed to the observation technique, fish were held in compartments formed by 1/4-inch-mesh barrier screens in the troughs (Figure 4). A depth of five inches of flowing water was maintained in each unit.

A later improvement in the observation technique was temperature control of the flowing water at 15°C. A 2000-watt immersion heater, situated at the head of each trough, was controlled by a thermo-regulator acting through a supersensitive mercury relay. Limited capacity of the immersion heaters necessitated recirculation of 8.7 of the 13.0 l/min total flow of water during winter months when stream temperatures were low. The thermo-regulator was located four feet from the mixing chamber situated at the head of each trough (Figure 5). The water was aerated at intervals along the observation troughs to assure dissolved oxygen concentrations near the saturation level. Fish were prevented from jumping out of the troughs by inverted egg baskets used as covers.
Figure 4. Two types of observation compartments: (A) egg baskets, and (B) barrier screened units.
Figure 5. Observation trough temperature control mixing chamber, showing (A) cold water inlet (B) immersion heater, and (C) recirculated water inlet.
Biological Methods

Experimental Animals

Rainbow trout, _Salmo gairdneri_ Richardson, used in all experiments, were from fall-spawning stock and were reared at the Roaring River Trout Hatchery, near Scio, Oregon. Identified as hatchery lot numbers 54-03 and 54-05, these fish were transported without loss to the Alsea Trout Hatchery, where they were held in outdoor ponds and fed the hatchery production diet twice daily.

Test animals destined for use in an experimental series were graded to an average size of 60 grams and then were brought into the laboratory for the recommended minimal acclimation period of seven days (10, p. 1383). The fish were held in the laboratory in 1000 gallon concrete tanks, which were supplied with water from the same source as were the outdoor and experimental facilities.

The experimental animals were starved for 48 hours prior to testing in all experiments but the one exploring the effects of various starvation periods. Purpose of this procedure was to reduce the production of metabolic wastes during experiments, as explained by Phillips, _et al._ (31, p. 13). In no case was the same specimen used twice for experimental purposes.
Blood Cell Counts

A Spencer Bright-Line Haemacytometer was used to make the red and white cell counts. Blood was drawn from the caudal artery into a pipette, diluted 200 times with Toisson's Fluid, and placed on the counting chamber. The number of erythrocytes or leucocytes per cubic millimeter was determined as follows:

\[
\text{No.} / \text{mm}^3 = \frac{\text{cells} \times \text{dilution} \times 4000}{\text{no. 1 mm. squares counted}}
\]

Since 80 small squares were counted and the dilution was 200:1, the formula reduced to a factor of 10,000, and the estimate of blood cells was obtained by adding four ciphers to the number of cells counted.

Autopsy Procedure

After making blood cell counts, the fish were immediately autopsied for presence of parasites and diseases. Microscopic examinations of mucus smears, the alimentary canal and internal and external structures were made. The relative abundance of identified organisms was noted.

Death Criterion

The criterion used to determine death of a test animal was
similar to that described by Doudoroff (8, p. 40) in which a fish was considered dead if at the time of observation no movement, either spontaneous or induced by mechanical stimulation, could be detected. Otherwise, the specimen was recorded as having survived to that particular observation time. Dead fish were removed from post-treatment containers as losses were enumerated.

Chemical Methods

Dissolved oxygen and pH determinations of experimental solutions were always made at the completion of each test, and sometimes at the beginning. Total water hardness was determined and adjusted to desired levels in most experiments concerned with the toxic effects of zinc. Zinc and cadmium analyses were contracted to competent laboratories otherwise not associated with the research program.

Dissolved Oxygen

The Alsterberg (azide) modification of the basic Winkler method was used to determine dissolved oxygen concentrations. This modification reduces interference due to nitrogenous materials. Bi-iodate was used to standardize the thiosulfate solutions at weekly intervals (2, p. 253, 255).
pH Determination

The pH levels were determined with a Beckman, Model N-2, pH meter. In some experimental series, normal solutions of sodium hydroxide and sulfuric acid were used to adjust the pH of the experimental solutions to neutrality.

Water Hardness

Total calcium carbonate hardness was determined with a Braun-Knecht-Heimann water hardness testing set. When necessary, water hardness was increased by adding calcium chloride, or decreased by adding river water which had been demineralized by passing it through a LaMotte Filtr-ion.

Zinc Determination

Zinc concentration of the experimental solutions was measured colorimetrically. A dithizone extraction of zinc, copper and cobalt, followed by a hydrochloric acid separation of zinc was made according to the method of Parks, et al. (30, p. 527-529). The zinc extract was turned blue by the addition of Zincon. Light transmittance was then measured at 620 μ against a reagent blank.

3 The compound 2-carboxy-2'-hydroxy-5' sulfoformazylbenzene.
with a Coleman spectrophotometer (33, p. 1345-1346). Transmittance values were then converted to parts per million zinc.

Stock solutions containing zinc ion were prepared in two ways during the research. Reagent grade zinc chloride was used to prepare a normal stock solution. Another stock solution of zinc was prepared from galvanized iron by bubbling carbon dioxide gas over sheet metal strips and plumbing pipe for five days. This produced a stock solution having a zinc concentration of 170 p.p.m.

Because of a tendency for zinc to precipitate onto the side of glass and metal containers, samples were collected in plastic bottles and refrigerated pending analysis. Most analyses were made within two days of the sample collection date. When field personnel collected samples at locations a considerable distance from the laboratory, as much as five days elapsed before zinc determinations could be made.

**Cadmium Determination**

A simple qualitative analysis for the presence of cadmium was used. The technique required saturation of an acidified sample with hydrogen sulfide; precipitation of cadmium sulfide indicated the presence of cadmium ions in the sample solution (14, p. 364-365).
RESULTS

Examination of Test Fish

**Blood Cell Counts**

At the start of each experimental series, red and white blood cell counts were made for ten fish selected at random from the test population. Red cell counts were used to evaluate the nutritional condition of the animals, since the number of erythrocytes responds quickly to dietary deficiencies (17, p. 184). The mean count for the 70 fish sampled was 1,296,000 red cells per cubic millimeter (Table 1). Compared to the mean erythrocyte count of 1,143,000 for 98 rainbows previously observed in Oregon (Table 2), the test fish averaged over 150,000 more red cells per cubic millimeter of blood. Counts from the experimental animals also compared favorably to the mean red cell counts for rainbow of 1,100,000 found by Schlicher (35, p. 121-200) and 1,220,000 found by Tunison (39, p. 9-10), according to Katz (17, p. 185). Based on red blood cell counts, the experimental fish appeared to be in a normal nutritional condition.
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### Table 2. Mean red blood cell counts for Oregon rainbow trout

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<th>Date</th>
<th>Number of fish</th>
<th>Mean erythrocyte count/mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaburg Trout Hatchery</td>
<td>Aug., 1954</td>
<td>27</td>
<td>1,387,000</td>
</tr>
<tr>
<td>Klamath Trout Hatchery</td>
<td>Aug.-Sept., 1954</td>
<td>25</td>
<td>1,095,600</td>
</tr>
<tr>
<td>Roaring River Trout Hatchery</td>
<td>July-Aug., 1954</td>
<td>32</td>
<td>953,300</td>
</tr>
<tr>
<td>Wizard Falls Trout Hatchery</td>
<td>July, 1954</td>
<td>9</td>
<td>1,255,000</td>
</tr>
<tr>
<td>Metolius River (wild fish)</td>
<td>July, 1954</td>
<td>5</td>
<td>1,068,000</td>
</tr>
<tr>
<td><strong>Column mean</strong></td>
<td></td>
<td></td>
<td><strong>1,152,000</strong></td>
</tr>
<tr>
<td><strong>Weighted mean</strong></td>
<td></td>
<td></td>
<td><strong>1,143,000</strong></td>
</tr>
</tbody>
</table>
Autopsies

Following determination of erythrocyte abundance, a detailed autopsy was performed on each fish. The stalked ciliate, *Epistylis* spp., was a protozoan commonly found in limited numbers on the surface tissues. *Trichodina* was found on the body surfaces of two specimens. To prevent an outbreak of these ciliated protozoans, the entire lot of trout was treated with a 1:500 acetic acid dip for one minute. No further occurrences of *Trichodina* were noted.

The internal organisms identified were *Hexamitus salmonis* (octomitus); metacercariae of the salmon poisoning fluke, *TrogloTrema salmincola*; and a larval cestode, probably *Diphyllobothrium* spp.

None of the identified organisms occurred in epizootic abundance; treatment for *Trichodina* was purely a precautionary measure. Salmon poisoning fluke metacercariae were present in about 70 percent of the autopsied fish. Except in cases of extreme concentrations, the metacercariae are not recognized as a serious hazard to trout (27, p. 197-199; 28, p. 321). Four spleen hematomas were noted in one specimen.

Post-liberation losses were evidently not sex linked. Of the 287 fish which had died a delayed death that were examined, 143 were males and 144 were females.
Dissolved Oxygen and pH Determinations

All of the voluminous data on dissolved oxygen and pH determinations are omitted. Dissolved oxygen concentrations rarely dropped below 6.0 mg/l and never appeared to be important in the interpretation of test results. The pH levels were seldom outside the range of 6.5 to 7.5, and were not found to be directly associated with mortalities of the trout.

Effects of Motion and Time

An experimental series was designed to test simultaneously (1) whether post-transportation mortalities could be produced in the laboratory, and (2) if so, would the sequence of mortalities follow a delayed pattern, and (3) would the mortalities increase with prolonged periods of sham hauling.

Into each of eight aquaria held by the previously described motion device were placed ten liters of water and ten trout. A control lot of ten fish was placed in a covered egg basket partially submerged in water in the unheated observation trough. The apparatus was adjusted to 18 revolutions, or 36 oscillations, per minute and started.

At the end of one hour, fish from two aquaria were placed in
baskets in the observation trough. Fish from two more aquaria were removed each time at the end of two, four and six hours, and were placed under observation. Four replications of this procedure resulted in a total of 80 fish in eight containers being tested for each exposure duration. Forty trout served as controls. Mortality data accumulated over the five days of observation after experimental exposure are presented in Table 3.

Delayed mortalities were produced which began the first day after testing, peaked on the second day and declined thereafter. The mortality pattern was similar to that encountered following live transportation of trout during earlier experiments in Oregon (15, p. 3; 21, p. 1).

More fish died after six hours in an oscillating test container than after four, two or one hours confinement under like conditions. That losses increased with duration of the experiment was obvious, but whether higher mortalities were due to water turbulence, stress of confinement, the concomitant accumulation of metabolic products, or other factors was not clear. None of the control fish died.
Table 3. Mortality following sham transportation for four time periods.

<table>
<thead>
<tr>
<th>Days after test</th>
<th>Mortality following motion at 36 oscillations per minute for:</th>
<th>Total daily mortality</th>
<th>Control mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hour</td>
<td>2 hours</td>
<td>4 hours</td>
</tr>
<tr>
<td>1st day</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2nd day</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3rd day</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4th day</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5th day</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total mortality</strong></td>
<td><strong>6</strong></td>
<td><strong>6</strong></td>
<td><strong>7</strong></td>
</tr>
</tbody>
</table>

| Number tested | 80 | 80 | 80 | 80 | 320 | 40 |
| Percent mortality | 7.5 | 7.5 | 8.8 | 37.5 | 15.3 | 0 |
Effects of Motion, Time and Density

A second series of tests was designed to explore further the effects of motion. Additional treatments were incorporated into the series to probe the influence of fish density and length of experimental exposure on delayed trout mortalities.

Two levels of each factor (motion, density and duration) were arranged into a randomized block design. Coding of the factors was:

\[
\begin{align*}
M_0 &= \text{No motion} \\
M_1 &= \text{Motion at 56 oscillations per minute} \\
D_0 &= \text{Density of one fish per liter (60 g/l)} \\
D_1 &= \text{Density of three fish per liter (180 g/l)} \\
T_0 &= \text{Two hour test duration} \\
T_1 &= \text{Eight hour test duration}
\end{align*}
\]

Possible treatment combinations were:

\[
\begin{array}{ccc}
M_0 & T_0 & D_0 \\
0 & 0 & 1 \\
0 & 1 & 0 \\
0 & 1 & 1 \\
1 & 0 & 0 \\
1 & 0 & 1 \\
1 & 1 & 0 \\
1 & 1 & 1
\end{array}
\]

Four replications of the eight possible treatment combinations were tested. Each replication required the use of 160 animals in addition to 30 fish placed directly into the observation trough as controls. Percent mortality data for the 32 individual tests are presented in Table 4. The fish were observed in egg baskets placed in
Table 4. Percent mortality following motion, duration, and density treatments

<table>
<thead>
<tr>
<th>Motion (oscillations per minute)</th>
<th>2-hr. test, 1 fish/liter ($T_O$ $D_O$)</th>
<th>2-hr. test, 3 fish/liter ($T_O$ $D_1$)</th>
<th>8-hr. test, 1 fish/liter ($T_1$ $D_O$)</th>
<th>8-hr. test, 3 fish/liter ($T_1$ $D_1$)</th>
<th>Mean treatment mortality</th>
<th>Control mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ($M_O$)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>6.67</td>
<td>9.17</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>3.33</td>
<td>10.00</td>
<td>23.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.00</td>
<td>3.33</td>
<td>20.00</td>
<td>23.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56 ($M_1$)</td>
<td>10.00</td>
<td>0.00</td>
<td>30.00</td>
<td>20.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>3.33</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>6.67</td>
<td>40.00</td>
<td>13.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean treatment mortality</td>
<td>3.75</td>
<td>2.50</td>
<td>16.25</td>
<td>15.83</td>
<td>9.58</td>
<td>0.00</td>
</tr>
</tbody>
</table>
water controlled to 15° C.

Motion, as produced by oscillating aquaria (Figure 6), apparently had little influence on the magnitude of the experimentally induced mortality. Mean mortality among oscillated fish was 10.00 percent, while those in stationary aquaria suffered a 9.17 percent delayed loss. The difference between these two treatment means was not significant.

There was no significant difference in survival between fish confined at a density of one fish per liter and those tested at three fish per liter. Mean delayed mortality was 10.00 percent at one fish per liter and 9.16 percent at three fish per liter. Other investigators have increased fish concentrations to the extent that their metabolites limited carrying capacity of liberating units (13, p. 182). Evidence that the fish densities tested influenced post-release deaths in the current study was lacking.

Length of experimental exposure had the greatest influence on subsequent trout losses. Delayed mortality averaged 16.04 percent following eight hours of confinement in the test aquaria, while only 3.13 percent of the rainbow trout died after the two-hour exposure periods. Difference between the two mortality rates was significant, indicating mortality rate to be related to exposure time. All control fish survived.
Figure 6. Aquaria being oscillated by experimental apparatus to simulate in-transit motion.
Mortalities following sham transportation occurred after fish were removed from the experimental aquaria and placed in the observation troughs. The possibility existed that conditions in these troughs could have influenced the experimentally induced mortality rates. To test this, four more replications of the motion treatments were conducted. Instead of transferring the fish to water controlled to 15°C., they were transferred to unheated river water having a temperature of 4.5°C. and a much higher exchange rate per trough.

Results of these replications in which fish were transferred to the colder water were compared to the results of the four previously performed replications (Table 5). The mean delayed loss in the troughs with 15°C. water was 10.00 percent. The loss dropped to 0.21 percent in the troughs having the higher water exchange rate and the temperature of 4.5°C. Conditions in the troughs in some way influenced the experimental mortalities. The lower mortalities in the second set of replications may have been due to either the lower water temperature or the higher water exchange rate or due to both of these conditions. Some of the later experiments were planned to make an interpretation of this result possible.
Table 5. Percent mortality comparison between fish observed in cold and warmed water

<table>
<thead>
<tr>
<th>Observation conditions</th>
<th>Treatment(^1)</th>
<th>Replication 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10(^0) C., 4.3 l/min. exchange rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(M_1T_0D_0)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>10.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(M_1T_0D_1)</td>
<td>3.33</td>
<td>3.33</td>
<td>6.67</td>
<td>0.00</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>(M_1T_1D_0)</td>
<td>10.00</td>
<td>10.00</td>
<td>40.00</td>
<td>30.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(M_1T_1D_1)</td>
<td>10.00</td>
<td>3.33</td>
<td>13.33</td>
<td>20.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(M_1T_0D_0)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(M_1T_0D_1)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>(M_1T_1D_0)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(M_1T_1D_1)</td>
<td>3.33</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Treatment code:  
- \(M_1\) = Motion at 56 oscillations per minute  
- \(T_0\) = 2-hour test duration  
- \(T_1\) = 8-hour test duration  
- \(D_0\) = Density of 1 fish/liter  
- \(D_1\) = Density of 3 fish/liter
Effects of Duration of Experimental Exposure and Starvation of Fish

While analysis of the data presented in Table 4 did not suggest that density of fish or metabolic or other waste products were in any way related to the mortality observed, other workers had suggested causal relationships between such products and delayed mortality. Other than by varying density of fish, various levels of metabolic and other waste products could be experimentally obtained either by increasing exposure time or by holding fish for longer periods of time after feeding before introducing them into the experimental aquaria. To determine whether mortalities might be related to various levels of metabolic and other wastes, an experiment was designed in which every possible combination of five levels of pre-testing starvation period (0, 24, 48, 72 and 96 hours) and four levels of experimental exposure time (2, 8, 16 and 24 hours) were arranged into a statistical design with three replications. In this experiment, the aquaria were not rocked and the density was two fish per liter. Following treatment, the fish were held in egg baskets placed in the 15°C observation trough. Coding of the factors and treatment combinations comprising one replication follow:
Analysis of data obtained from these tests (Table 6) revealed no significant correlation between the subsequent delayed mortality and any level of starvation period, experiment duration, or interaction between the two factors. The post-experimental loss averaged 3.08 percent. It was concluded that neither length of exposure (2 to 24 hours) nor starvation period (0 to 96 hours), taken as individual or collective factors, had any pronounced influence on the delayed mortalities.

Toxic Effects of Zinc

While Saltzman (34, p. 40) on the basis of a cursory examination had concluded that zinc ions were not causally involved in delayed mortality, an incident occurring during the present investigation again directed attention to this toxic substance. On June 20,
Table 6. Effects of duration of experiment and pre-test starvation period on percent delayed loss

<table>
<thead>
<tr>
<th>Duration of experiment (hours)</th>
<th>Starvation period (hours)</th>
<th>O(S₁)</th>
<th>24(S₂)</th>
<th>48(S₃)</th>
<th>72(S₄)</th>
<th>96(S₅)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (D₁)</td>
<td></td>
<td>65</td>
<td>55</td>
<td>50</td>
<td>20</td>
<td>35</td>
<td>30.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>30</td>
<td>20</td>
<td>35</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>25</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>8 (D₂)</td>
<td></td>
<td>70</td>
<td>75</td>
<td>35</td>
<td>40</td>
<td>30</td>
<td>37.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>30</td>
<td>25</td>
<td>10</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>20</td>
<td>40</td>
<td>35</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>16 (D₃)</td>
<td></td>
<td>35</td>
<td>10</td>
<td>20</td>
<td>35</td>
<td>45</td>
<td>33.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>50</td>
<td>50</td>
<td>30</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>20</td>
<td>40</td>
<td>30</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>24 (D₄)</td>
<td></td>
<td>15</td>
<td>15</td>
<td>35</td>
<td>20</td>
<td>20</td>
<td>22.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>20</td>
<td>25</td>
<td>20</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>25</td>
<td>15</td>
<td>20</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>32.9</td>
<td>30.8</td>
<td>30.4</td>
<td>26.7</td>
<td>34.6</td>
<td>31.1</td>
</tr>
</tbody>
</table>

1 Nine of 60 control fish died in the observation trough.
1957, 180 pounds of rainbow trout were transferred from the Roaring River Hatchery to the Alsea Trout Hatchery, in one of the Oregon State Game Commission's 175-gallon liberating trucks. The load density was 1.03 pounds of fish per gallon of water (123.5 g/l), and the time enroute was 2.5 hours; other transfer details are provided in Appendix 1. Although low water temperatures (4.4°C to 6.6°C) were maintained during transportation, delayed mortality among the transferred fish was 44.3 percent (Appendix 1). It was believed that zinc poisoning could have caused the mortality, but no water samples were taken for zinc analyses.

A second transfer of fish between these two stations was made on July 29, 1957. The liberating tank used and the density of fish in the load were the same as on the June 20 trip, and 2.25 hours elapsed enroute; additional data on the transfer are presented in Appendix 2. Even though the temperature of the water during transportation was between 2.2°C to 3.3°C, a delayed loss of 22.8 percent of the transferred fish occurred. Water samples for zinc and copper ion analyses were collected from the liberating unit at the beginning and end of the haul. Data from analyses performed by the Charlton Laboratories, Portland, are presented in Table 7.
Table 7. Zinc and copper ion concentrations and pH of water used for transferring fish on July 27, 1957

<table>
<thead>
<tr>
<th>Ion</th>
<th>Start of haul</th>
<th>End of haul</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (Cu++)</td>
<td>&lt;0.02 p.p.m.</td>
<td>&lt;0.02 p.p.m.</td>
</tr>
<tr>
<td>Zinc (Zn++)</td>
<td>0.36 p.p.m.</td>
<td>1.15 p.p.m.</td>
</tr>
<tr>
<td>pH value</td>
<td>6.60</td>
<td>6.48</td>
</tr>
</tbody>
</table>

Data for copper and pH did not indicate these were responsible for the mortality (9, p. 411; 10, p. 823). The increase in the concentration of zinc ion from 0.36 p.p.m. to 1.15 p.p.m. could have caused the loss of fish (26, p. 8-10).

There has been some disagreement among various investigators concerning toxic concentrations of zinc for trout. The toxicity of zinc to fish has been shown to be affected by water hardness and temperature. In general, toxicity of zinc increases with decreasing hardness as well as with rising temperature (7, p. 7).

Bioassays were conducted at the Alsea Hatchery to determine lethal zinc concentrations for six hours exposure for rainbow trout at water hardmesses ranging from 26 to 30 p.p.m. and at a temperature of 15° C. The trout were removed from the experimental aquaria after the six-hour exposure period and placed in the troughs for observation. No mortalities occurred during the exposure period. Percent mortalities occurring in the observation troughs for
fish previously exposed to various concentrations of zinc ion are presented in Figure 7.

Accumulated data were tested to determine if the population regression coefficient \( \beta \) was equal to zero (20, p. 264). The hypothesis that \( \beta = 0 \) was rejected at the 99 percent level, and the conclusion was reached that mortality varied with zinc concentration. Six hours of exposure to 0.32 mg/1 of zinc was sufficient to cause a 20 percent mortality of the experimental fish. After 2.25 hours of transportation in water having zinc concentrations varying from 0.36 to 1.15 mg/1, 22.8 percent of the trucked fish died. The probable source of zinc in the transportation tank was a galvanized iron "chiller" device.

The loss of fish after exposure in aquaria to concentrations of zinc ranging from 0.1 to 1.0 mg/1 followed a time pattern resembling that of the losses of fish after truck transportation. The mean percentages of the total delayed mortalities which occurred on particular days after transportation by truck are graphed in Figure 8 (the data coming from 32 trips in 1954 in which delayed loss was greater than one percent). The data from the 32 aquarium tests are also presented in the same manner in Figure 8.

Further substantiation was given the hypothesis that zinc poisoning was the specific cause of fish transportation mortalities by a
Figure 7. Mortality of rainbow trout following six hours exposure to known concentrations of zinc.
Figure 8. Sequential patterns of trout mortality following truck transportation or laboratory exposure to zinc.
subsequent transfer of fish from the same lot between the same two stations on July 31, 1957. The transportation tank which was used for this transfer had no zinc surfaces exposed to the water. Hauling density was 0.5 pounds of fish per gallon of water, and time enroute was 2.0 hours; other hauling details are given in Appendix 3. There were no delayed mortalities of fish as a result of this transfer.

Observation Trough Toxicity and Length of Experimental Exposure

Data presented in Table 5 indicated delayed mortalities were much higher in an observation trough having a water temperature of 15°C and a low exchange flow than in a trough having a water temperature of 4.5°C and a high exchange flow. In the experiment on the effects of the duration of exposure and the starvation of fish on delayed mortality (Table 6), nine out of 60 control fish placed directly into the observation trough died. The mortalities in these two experiments could best be explained on the basis of a build-up in the troughs of some toxic substance like zinc, which would reach higher levels at the low exchange flow. Water samples were collected at various locations in the hatchery and analyses showed the zinc concentrations to be 0.000 mg/l, 0.004 mg/l, and 0.028 mg/l at the hatchery source, at the entrance to an observation trough and
at the outflow of a trough having a low exchange flow, respectively. A possible source of zinc ion in the troughs was the hatching baskets in which the fish were confined. The hatching baskets were constructed of 1/4- x 1/8-inch mesh galvanized hardware cloth coated with white, non-toxic paint. In numerous places, the painted surfaces were cracked and chipped.

To test whether zinc ion from the baskets was causing the observed mortalities, a system of newly painted barrier screens was devised to divide one of the troughs into separate compartments, egg baskets being retained in a second trough (Figure 4). Water temperature was maintained at 15° C. in both troughs.

Since results of five-day observation periods were to be compared, an opportunity existed to check further the effects of duration of experimental exposure. Confinement intervals of 0, 15, 30, 60, 120, 240 and 480 minutes in experimental aquaria were selected and tested with seven replications to explore the influence of short-term test exposures.

Groups of 20 fish were placed into each aquarium for a designated exposure period. At the end of the period, they were discharged into one or the other observation trough. Twenty fish were placed directly into an observation compartment in each trough to serve as a control.
Analysis of results failed to reveal any correlation between duration of exposure and delayed loss. On the other hand, fish held in egg baskets suffered a ten percent loss, while fish held in compartments separated with barrier screens suffered only a 0.47 percent loss. None of the control fish held in the barrier screened trough died, while ten percent succumbed in the trough having egg baskets (Table 8). Water analyses at the end of the five day observation period revealed 0.003 mg/l zinc at the outlet of the barrier screened trough and 0.040 mg/l zinc at the outlet of the egg basket channel. Exposure to a zinc concentration of 0.040 mg/l for five days might bring about a ten percent mortality, since 0.100 mg/l zinc caused an eight percent delayed trout loss after but six hours exposure (Figure 7).

Water Hardness and the Toxicity of Solutions Containing Zinc Ion

While zinc ion from zinc chloride solutions was employed in the toxicity studies which have been reported, the source of zinc ion in hatcheries and liberating trucks would usually be galvanized iron. Some comparison of the toxicity of solutions having the same zinc ion concentrations but prepared from the two different materials seemed desirable. An experimental series was planned to compare the toxicity of solutions of zinc ion prepared from zinc
Table 8. Percent mortality in experiment testing effect of reduced duration of exposure and two sets of observation conditions

<table>
<thead>
<tr>
<th>Observation conditions</th>
<th>Time in minutes</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>60</td>
<td>120</td>
<td>240</td>
<td>480</td>
<td>Control</td>
</tr>
<tr>
<td><strong>Barrier screens</strong></td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>10.00</td>
<td>5.00</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Egg baskets</strong></td>
<td>5.00</td>
<td>0.00</td>
<td>5.00</td>
<td>0.00</td>
<td>5.00</td>
<td>0.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>0.47</td>
<td>0.00</td>
<td>0.00</td>
<td>2.50</td>
<td>1.25</td>
<td>0.00</td>
<td>10.00</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Column mean</strong></td>
<td>6.43</td>
<td>4.29</td>
<td>3.57</td>
<td>5.00</td>
<td>2.86</td>
<td>5.00</td>
<td>5.00</td>
<td>4.29</td>
</tr>
</tbody>
</table>
chloride and from galvanized iron at concentrations of 0.05, 0.35, 0.65 and 0.95 mg/l of zinc for two and eight hour exposure periods. Two replications of the 16 possible treatment combinations were performed utilizing 20 trout in each test and each control aquarium. Post-treatment observations of test fish were made in barrier-screened compartments at 15° C. While the results of this experiment are quite variable, regarding both the toxicity of the solutions of the two materials and the exposure-mortality relationship, there is some indication that the solutions prepared from galvanized iron were more toxic than the solutions prepared from zinc chloride (Table 9).

Total hardness of the water used for the tests increased from 22 to 30 p.p.m. as the test sequence progressed, and some of the variability shown in Table 9 was due to this -- the toxic effects of zinc decreasing with increasing water hardness. Thus, a given quantity of zinc was more toxic to the fish at the beginning than at the end of the experimental series. The relationship between total fish losses and water hardness for both two and eight hour exposures is illustrated in Figure 9.

A final experimental series was planned to test the effects of water hardness on the relative toxicity of solutions prepared from zinc chloride and from galvanized iron to rainbow trout for
Table 9. Percent mortality of trout following tests comparing the toxic effects of ZnCl$_2$ and Zn-gal

<table>
<thead>
<tr>
<th>Zinc source and exposure duration</th>
<th>Zinc concentration (mg/l)</th>
<th>Mean</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05</td>
<td>0.35</td>
<td>0.65</td>
</tr>
<tr>
<td>ZnCl$_2$ 2 hour</td>
<td>00</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>Zn-gal 2 hour</td>
<td>05</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>00</td>
<td>00</td>
<td>05</td>
</tr>
<tr>
<td>ZnCl$_2$ 8 hour</td>
<td>05</td>
<td>00</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>00</td>
<td>00</td>
<td>10</td>
</tr>
<tr>
<td>Zn-gal 8 hour</td>
<td>05</td>
<td>05</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>Mean</td>
<td>1.88</td>
<td>5.00</td>
<td>11.25</td>
</tr>
</tbody>
</table>
Figure 9. Total trout losses in relation to total water hardness for two- and for eight-hour durations of exposure.
two time periods. The factors of water hardness (0, 20 and 40 p.p.m.), zinc from zinc chloride (0, 0.75 and 1.50 mg/l zinc), zinc from galvanized iron (0, 0.75 and 1.50 mg/l zinc), and experimental time (2 and 8 hours) were arranged into a split-block statistical design. Three replications each of the 30 possible treatment combinations were tested.

In this study, experimental procedure differed in that ten fish were used per aquarium, and pH was adjusted to 7.0 at the start of each test. In addition, observation conditions were controlled to match test conditions, except for the presence of zinc. Post-treatment fish were retained for observation in aquaria containing 15 liters of water having a temperature of 15°C. The observation water was adjusted to pH 7.0 and to the total hardness of the particular treatment tested. Observation water was changed every 24 hours to avoid excessive contamination by waste products.

High concentrations of water hardness effectively reduced the toxicity of solutions containing zinc from both zinc chloride and galvanized iron (Table 10). At 0.75 mg/l zinc, the mean mortality of trout resulting from both kinds of solutions and from both exposure durations decreased from 88.5 percent at zero p.p.m. total hardness to five percent at 40 p.p.m. The trend was the same at 1.50 mg/l zinc, where mean mortalities decreased from 88.5 percent at zero p.p.m. hardness to 40.8 percent at 40 p.p.m. (detailed
results are given in Appendix 4. Figure 10 presents these means graphically.

Table 10. Mean percent mortality of fish subjected to certain concentrations of zinc ion (from solutions prepared from zinc chloride and galvanized iron) for different exposure durations at different water hardnesses

<table>
<thead>
<tr>
<th>Duration of exposure (hours)</th>
<th>Water hardness (p.p.m.)</th>
<th>Zn-Cl₂ (mg/l)</th>
<th>Zn-gal (mg/l)</th>
<th>Mean Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.75 1.50</td>
<td>0.75 1.50</td>
<td></td>
</tr>
<tr>
<td>2 hours</td>
<td>0</td>
<td>67 67</td>
<td>87 87</td>
<td>77 0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3 37</td>
<td>13 60</td>
<td>28 0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0 10</td>
<td>0 33</td>
<td>11 0</td>
</tr>
<tr>
<td>8 hours</td>
<td>0</td>
<td>100 100</td>
<td>100 100</td>
<td>100 10</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>40 83</td>
<td>57 100</td>
<td>70 3</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>13 47</td>
<td>7 73</td>
<td>35 0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>37 57</td>
<td>44 76</td>
<td>2</td>
</tr>
</tbody>
</table>

Mortalities increased with increases in experimental exposure time. An average of 30 percent more fish succumbed after eight hours exposure to zinc solutions than died following two hours exposure to identical test media. Zinc toxicity decreased with increased water hardness in both the two and eight hour tests (Figure 11).

From the results presented in Tables 9 and 10 and in Figure 12, there appeared to be a difference in the toxicity of zinc in
Figure 10. Influence of water hardness on the toxicity of zinc to rainbow trout. Each plotted observation represents the mean percent mortality of 120 fish.
Figure 11. Toxicity of zinc to test animals in relation to duration of exposure and to water hardness.
Figure 12. Comparative toxicity of two levels of Zn-Gal and ZnCl$_2$ to rainbow trout.
solutions prepared from zinc chloride and galvanized iron, solutions prepared from the latter being apparently more toxic at the same zinc ion concentrations. Based on the experience of Hublou, et al. (16, p. 13), who considered both zinc and cadmium used in plating metals to be involved in the mortality of chinook salmon, a cadmium analysis was made of the stock solution prepared from galvanized iron. The results were negative, no trace of cadmium being found.

Throughout the two experiments comparing the toxicity of the solutions prepared from the two materials, samples for zinc analyses were periodically taken at the beginning and end of test periods. The results of the analyses suggested that zinc ion dilutions prepared from the galvanized iron stock solution were three percent higher than intended, while zinc ion dilutions prepared from zinc chloride solutions were 22 percent lower than intended. The actual concentrations of zinc ion in dilutions prepared from zinc chloride probably averaged 25 percent lower than the corresponding actual concentrations in dilutions prepared from the galvanized iron stock solution. When this is taken into account, there is little reason to believe that dilutions having similar concentrations of zinc ion prepared from the two materials were different in their toxicity. This should be borne in mind when data presented in Tables 9 and 10 and
Inter-Hatchery Transfer Data

Following the accumulation of evidence that delayed transportation mortalities were caused by small quantities of zinc, confirmation of the thesis was sought by study of losses which occurred under field conditions. Cooperation of the Oregon State Game Commission was enlisted, and detailed information was obtained on all inter-hatchery transfers of fish made during the spring and summer of 1958. Samples of transportation water were taken at the completion of the station to station trips and placed under refrigeration. If mortalities occurred after a transfer, the sample was analyzed for zinc (Appendix 5). Samples from three transfers after which no delayed losses occurred were also analyzed for zinc.

In all, reports on 32 trips which conveyed 581,266 fish were received. A delayed loss greater than one percent occurred on seven occasions, and six water samples for analysis were received. In every instance where delayed transportation mortalities occurred, appreciable quantities of zinc were detected (Table 11). The concentration determined at the end of a transportation period does not necessarily represent the highest concentration of zinc occurring during the trip. From zinc analyses made at the beginning and end
Table 11. Zinc concentration and delayed mortalities following inter-hatchery transfers

<table>
<thead>
<tr>
<th>Date</th>
<th>Species</th>
<th>Number transported</th>
<th>Delayed loss</th>
<th>Percent delayed loss</th>
<th>Concentration of zinc (mg/1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/19/58</td>
<td>Rainbow</td>
<td>1,122</td>
<td>17</td>
<td>1.52</td>
<td>0.021</td>
</tr>
<tr>
<td>6/4/58</td>
<td>Rainbow</td>
<td>1,780</td>
<td>29</td>
<td>1.63</td>
<td>0.035</td>
</tr>
<tr>
<td>6/16/58</td>
<td>Rainbow</td>
<td>3,500</td>
<td>171</td>
<td>4.90</td>
<td>no sample</td>
</tr>
<tr>
<td>6/24/58</td>
<td>Chinook</td>
<td>6,413</td>
<td>259</td>
<td>4.14</td>
<td>0.020</td>
</tr>
<tr>
<td>8/7/58</td>
<td>Steelhead</td>
<td>65,240</td>
<td>7,071</td>
<td>10.84</td>
<td>0.040</td>
</tr>
<tr>
<td>8/8/58</td>
<td>Rainbow</td>
<td>15,600</td>
<td>210</td>
<td>1.35</td>
<td>0.026</td>
</tr>
<tr>
<td>4/13/59</td>
<td>Rainbow</td>
<td>79,916</td>
<td>1,957</td>
<td>2.45</td>
<td>0.039</td>
</tr>
<tr>
<td>5/8/58</td>
<td>Rainbow</td>
<td>21,375</td>
<td>6</td>
<td>0.00</td>
<td>0.000</td>
</tr>
<tr>
<td>6/23/58</td>
<td>Rainbow</td>
<td>21,150</td>
<td>4</td>
<td>0.00</td>
<td>0.000</td>
</tr>
<tr>
<td>7/21/58</td>
<td>Rainbow</td>
<td>11,310</td>
<td>2</td>
<td>0.00</td>
<td>0.000</td>
</tr>
</tbody>
</table>
of experiments, it was observed that a decline of 88 percent of the initial zinc concentration sometimes took place. This was probably due to the presence of the fish. When delayed losses of trout were much less than one percent, no zinc was detected in the water used during the transfer.
DISCUSSION

In soft waters, characteristic of western Oregon streams, zinc is very toxic to rainbow trout. Of the trout exposed for six hours to an initial zinc concentration of 0.10 mg/l, an average of 8.2 percent suffered a delayed mortality (Figure 7). Appreciable mortalities of trout (four to ten percent) occurred following transportation in liberating tanks in which the final concentration of zinc in the water ranged from 0.02 to 0.04 mg/l (Table 11).

Depending upon the concentration of zinc in the water, trout mortalities may be immediate or delayed. At concentrations of 1.50 mg/l zinc and above, most trout deaths in the laboratory occurred within eight hours; below this concentration, time to death increased. As the zinc concentration was decreased to threshold levels, mortalities in the laboratory were delayed and occurred in a sequential pattern similar to that observed following truck transportation (Figure 8). Death syndromes of fish succumbing to the toxic effects of zinc (12, p. 194) were similar to those described in Oregon for trout suffering delayed mortality (15, p. 4).

The toxicity of zinc decreases with increases in total water hardness. This effect was observed both in waters having relatively high natural hardness (Figure 9), and in waters in which hardness
was adjusted to specified levels (Figure 10). At 0.75 mg/l zinc, mean trout mortalities increased from five to 28 percent when total hardness decreased from 40 to 20 p.p.m. When hardness decreased from 20 to zero p.p.m., mean mortalities increased from 28 to 88 percent. Similar relationships between water hardness and zinc toxicity were reported by Lloyd (22, p. 92) for rainbow trout, and by Cairns and Scheier (7, p. 6) for bluegills (*Lepomis macrochirus*).

The toxic effect of zinc generally increased with increasing length of exposure to the metal ions (Figure 11). In experiments testing the effects of starvation period and observation trough toxicity, however, trout losses did not appear to be correlated with duration of experimental exposure. It was determined in the experiment that the toxic concentrations of zinc were present in the observation troughs and not in the experimental aquaria.

Simulated in-transit motion had no apparent influence on the magnitude of delayed trout deaths. Water motion produced by rocking the aquaria at 56 oscillations per minute seemed to disturb the fish initially, but they apparently became somewhat accustomed to the regular movements. When trout were first placed in the test aquaria, their mean frequency of opercular movements was 147 per minute, and this gradually decreased to 125 per minute, at the end of eight hours. Normal respiratory frequency was 76 mean
opercular beats per minute determined for resting fish in the outdoor rearing ponds at comparable temperatures.

No correlation was found to exist between experimental density of fish and subsequent mortalities. The highest density tested was 180 g/l (1.5 lbs/gal), which approximated maximum densities occurring under actual fish transportation conditions. The densities tested were somewhat less than the limiting load capacity of 2.5 lbs/gal in liberating tanks obtained by Haskell (13, p. 178) in New York.

Starving the fish previous to testing for periods up to 96 hours had no effect on delayed mortality. However, water in aquaria containing fish starved for periods of zero to 24 hours became very turbid with feces, regurgitated food particles, and other materials. The turbidity increase was noticeable in two hours, and became more apparent as time progressed, until at the end of 24 hours the water was dirty-brown in color (Figure 13). The larger suspended particles became lodged on the gill rakers of the trout, necessitating increased opercular cleaning motions.

Zinc from galvanized iron sources has previously been associated with trout deaths in fish cultural operations (12, p. 191-194; 16, p. 8-14; 18, p. 1-25). In some instances, trout deaths occurred 12 to 48 hours after exposure to low zinc concentrations (1, p. 168;
Figure 13. Aquaria containing fish starved for 96, 72, 48, 24 and 0 hours, from left to right.
Saltzman (34, p. 37-40) found no correlation between 0.06 to 0.29 mg/l zinc concentrations, occurring during fish transfers in Oregon, and ensuing trout losses and concluded that zinc poisoning was not a contributing factor in delayed loss.

Alkalinity of waters in which trout were transported was measured at the beginning and end of six hour truck transportation trips by Saltzman (34, p. 19-21) and Linn (21, p. 29-33). Since total alkalinity and hardness can be expected to vary together where calcium is a principal cation, an inverse correlation between alkalinity and delayed mortality would be expected if the fish deaths were caused by zinc toxicity. Saltzman found no correlation between total alkalinity and delayed losses, but variation in his experimental procedure makes comparison of the results of his 19 tests of questionable value. Linn (21, p. 30-31), using more refined procedures, found changes in total alkalinity and delayed mortality correlated in 18 tests performed at the Roaring River Hatchery (Table 12). The ice used to control water temperature in these tests had a total alkalinity concentration of 245 p.p.m. The ice was made from well water having a much higher alkalinity than that of the water from Roaring River. Linn (21, p. 29) reasoned that alkalinity concentration changes were primarily due to the melting ice. From the data of Linn (21, p. 62), it can be shown
that delayed losses at the Roaring River Hatchery were inversely correlated with the total alkalinity at the end of the six hour tests (Figure 14).

Table 12. Total alkalinity change in relation to amount of ice and percent delayed mortality at three temperatures

<table>
<thead>
<tr>
<th>Water temperature in °F.</th>
<th>Mean amount of ice used in pounds</th>
<th>Mean total alkalinity change in p. p. m.</th>
<th>Mean percent delayed mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>380</td>
<td>26.9</td>
<td>0.28</td>
</tr>
<tr>
<td>47</td>
<td>210</td>
<td>25.5</td>
<td>1.85</td>
</tr>
<tr>
<td>55</td>
<td>70</td>
<td>15.2</td>
<td>9.21</td>
</tr>
</tbody>
</table>

Linn had not analyzed the water for zinc, and he was therefore unable to understand the relationship between total alkalinity and delayed mortality. Both Linn and Saltzman performed sham transportation tests at the Wizard Falls Trout Hatchery and reported negligible delayed mortalities. Significantly, both investigators reported that the hatchery waters had relatively high concentrations of total alkalinity, ranging from 52 to 57 p. p. m. (21, p. 32; 34, p. 21). It appears that water hardness may have had an antagonistic effect on the toxicity of small quantities of zinc.

It is now possible to explain how I was able to control delayed trout mortalities by reducing the transporting water temperature in the 18 sham hauls from the Roaring River Trout Hatchery.
Figure 14. Methyl orange alkalinity at the end of six hours sham transportation in relation to percent delayed mortality at Roaring River Hatchery, July, 1954. (Data from Linn [30, p. 62]).
Temperature influences the toxicity of zinc to fish. Lloyd (22, p. 89, 93) found that an increase in temperature from 12° to 22° C. caused a decrease in the median periods of survival of rainbow trout by a factor of 2.35. Cairns and Scheier (7, p. 6) also reported small differences in the toxicity of zinc ions to bluegills at 18° and 30° C. The reduction in temperature from 12.8° to 4.4° C. probably reduced the toxicity of zinc to the trout in my studies. However, the probably increase in hardness brought about by the use of ice to lower temperatures may have been responsible for most reductions in mortalities.

Personnel investigating the delayed trout mortality problem in Oregon had noticed that losses following transportation were usually greater in the spring of the year than in summer or fall. This phenomenon was reported by Saltzman (34, p. 8), and is illustrated on the basis of his data (34, p. 9) in Figure 15. Seasonal changes in mortality may perhaps be explained on the basis of seasonal changes in water hardness. Since natural water hardness comes from the minerals in the watershed, hardness can usually be expected to be highest in summer and fall, when stream flows are low, and lowest in winter and spring, when maximum dilution occurs. Data presented in Figure 16 illustrate this seasonal phenomenon for the North Fork Alsea River.
Figure 15. Seasonal pattern of delayed trout mortalities occurring after three and six hours sham transportation, May-August, 1952.
Figure 16. Mean monthly total water hardness concentrations and stream flow volumes for the North Fork Alsea River, 1958-1959.
Based on the results of the field and laboratory tests reported, it was concluded that zinc from galvanized iron surfaces was responsible for the post-transportation mortalities of trout in Oregon. Fish exposed to low zinc concentrations in liberating tanks were subjected to threshold toxic conditions in which a varying portion of the animals died a delayed death. Relatively small changes in the natural water hardness or zinc concentration could either increase or eliminate subsequent mortalities. Results of sham transportation trips were thus made variable and confusing in early studies directed toward the solution of the delayed mortality problem.

Based on the research reported here, an effort has been made to eliminate galvanized iron surfaces from hatchery water supplies and liberating tanks in Oregon; and as a result, post-transportation losses were reduced to less than one-tenth of one percent of almost a million pounds of fish hauled in 1962 (19, p. 1).

It should be emphasized that transportation mortalities of fish cannot be universally attributed to zinc poisoning. From time to time and place to place, mortalities might occur for any of the reasons reviewed in the introduction. The exact causes of such losses can probably best be determined by careful analysis of conditions prevailing before, during and after transfers made under controlled conditions.


34. Saltzman, William O. A preliminary study of certain chemical factors that may be involved in the delayed mortality of rainbow trout following liberation. Master's thesis. Corvallis, Oregon State University, 1953. 59 numb. leaves.


APPENDICES
APPENDIX 1

FISH SURVIVAL OBSERVATIONS

Name of Release Waters: Alsea Trout Hatchery  Date: June 20, 1957
Species: Rainbow Trout  No./lb.: 14.7  Ave. Lgth.: 5-6 in.
Origin: Roaring River Trout Hatchery  Lot No.: 54-03
No. Hauled: 2,646  Load Wt.: 180 lbs.  Miles Hauled: 60
Hour Loaded: 12:00 a.m.  Hour Released: 2:30 p.m.
Hours Hauled: 2.50  Truck Used: 1958 Ford  Driver: Horton
Other Observer: Vroman  How Removed From Truck: Bucket

<table>
<thead>
<tr>
<th>Air Temp.:</th>
<th>Water Temp.:</th>
<th>At Hatchery</th>
<th>Truck at Release</th>
<th>At Release Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.9°C.</td>
<td>9.4°C.</td>
<td>26.7°C.</td>
<td>4.4°C.</td>
<td>13.9°C.</td>
</tr>
</tbody>
</table>

Mortality Table

<table>
<thead>
<tr>
<th>During Haul</th>
<th>1st Day</th>
<th>2nd Day</th>
<th>3rd Day</th>
<th>4th Day</th>
<th>5th Day</th>
<th>Total</th>
<th>Percent Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>279</td>
<td>762</td>
<td>102</td>
<td>24</td>
<td>5</td>
<td>1,172</td>
<td>44.3</td>
</tr>
</tbody>
</table>

Weather: Mild and overcast  Was Truck Water Tempered? Yes
To What Point? 4.4°C.  Was Ice Used? Yes, 200 lbs.
Fish Starved? Yes, approx. 4 hours
Describe Losses: Typical delayed mortality
Remarks: Ice added after fish were loaded. Fish weren't starved the usual 48 hours, and water circulation screens were partially plugged. Mortality possibly caused by zinc poisoning from galvanized chiller plates inside liberating tank. Perhaps ice should have been added before loading fish.
Signed by: Howard F. Horton
APPENDIX 2

FISH SURVIVAL OBSERVATIONS

Name of Release Waters: Alsea Trout Hatchery  Date: July 29, 1957
Species: Rainbow Trout  No./lb.: 10  Av. Lgth.: 6-7 inches
Origin: Roaring River Trout Hatchery  Lot No.: 54-03
No. Hauled: 1,800  Load Wt.: 180 lbs.  Miles Hauled: 60
Hour Loaded: 10:00 a.m.  Hour Released: 12:15 p.m.
Hours Hauled: 2.25  Truck Used: 1958 Ford  Driver: Horton
Other Observer: Jeannine Horton  How Removed From Truck: Bucket

<table>
<thead>
<tr>
<th></th>
<th>At Hatchery</th>
<th>Truck at Release</th>
<th>At Release Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Temp.:</td>
<td>18.3°C.</td>
<td>22.8°C.</td>
<td>22.8°C.</td>
</tr>
<tr>
<td>Water Temp.:</td>
<td>12.2°C.</td>
<td>2.2°C.</td>
<td>15.6°C.</td>
</tr>
</tbody>
</table>

| Mortality Table |
|-----------------|----------------|-----------------|-----------------|
| During Haul     | 1st Day 195    | 2nd Day 182     | 3rd Day 31      | 4th Day 1    | 5th Day 0    | Total 411    | Percent Mortality 22.8 |

Weather: Scattered clouds and warm
Was Truck Water Tempered? Yes
To What Point? 2.2°C.
Was Ice Used? Yes, 275 lbs.
Fish Starved? Yes, 48 hours
Describe Losses: One fish smashed inside liberating unit during haul. Other losses typically delayed mortality.
Remarks: Fish gills appeared bright pink during transport, due perhaps to some irritant. Several fish appeared to be bluish in overall color. All fish appeared in good condition upon release. Water was iced before loading fish. Autopsy was performed on many of the mortalities.
Signed by: Howard F. Horton
APPENDIX 3

FISH SURVIVAL OBSERVATIONS

Name of Release Waters: Alsea Trout Hatchery  Date: July 31, 1957

Species: Rainbow Trout  No./lb. 12  Ave. Lgth.: 6 inches

Origin: Roaring River Trout Hatchery  Lot No.: 54-03

No. Hauled: 4,608  Load Wt.: 384 lbs.  Miles Hauled: 60

Hour Loaded: 1:00 p.m.  Hour Released: 3:00 p.m.

Hours Hauled: 2.00  Truck Used: Oregon State Game Comm.

Driver: Roy DeLosier  Other Observer: Horton

How Removed From Truck: Hose

Air Temp.:  Water Temp.:  

At Hatchery  Truck at Release  At Release Point

\[\begin{array}{cccccc}
\text{Mortality Table} \\
\text{During Haul} & \text{1st Day} & \text{2nd Day} & \text{3rd Day} & \text{4th Day} & \text{5th Day} & \text{Total} & \text{Percent Mortality} \\
1 & 0 & 1 & 0 & 0 & 0 & 2 & 0.00 \\
\end{array}\]

Weather: Fair and warm  Was Truck Water Tempered?  Yes

To What Point?  8.3°C.  Was Ice Used?  Yes, 200 lbs.

Fish Starved?  Yes, 48 hours

Describe Losses: None to speak of

Remarks: None

Signed By: Howard F. Horton
APPENDIX 4

Percent mortality of fish subjected to certain concentrations of zinc ion (from solutions prepared from zinc chloride and galvanized iron) for different exposure durations at different water hardnesses.

<table>
<thead>
<tr>
<th>Test time (p. p. m.)</th>
<th>Water hardness</th>
<th>ZnCl₂ 0.75 (mg/l)</th>
<th>ZnCl₂ 1.50 (mg/l)</th>
<th>Zn-Gal 0.75 (mg/l)</th>
<th>Zn-Gal 1.50 (mg/l)</th>
<th>Mean</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>90</td>
<td>10</td>
<td>30</td>
<td>00</td>
<td>00</td>
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APPENDIX 5

INTER-HATCHERY TRANSFER DATA

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<th>Date</th>
<th>Origin</th>
<th>Release Pt.</th>
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<th>Time Start</th>
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<th>Elapsed</th>
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<th>Species</th>
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<table>
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<tr>
<th>Lbs. Carried</th>
<th>Gallons Water</th>
<th>Lbs. Ice Added</th>
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<th>Truck No.</th>
<th>Aeration</th>
<th>Power Take-off</th>
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<table>
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<tr>
<th>Driver</th>
<th>OVHD Spray</th>
<th>Auxiliary Eng.</th>
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Water Temperature:

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<tr>
<th>Start</th>
<th>Truck Enroute</th>
<th>Finish</th>
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<td>Hatchery</td>
<td>Truck</td>
<td>Hatchery</td>
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Actions of Fish During Haul: Normal _____ Abnormal _____

Explain any Abnormal Behavior:

Water Sample Taken: Yes ____ No ____ Refrigerated: Yes ____ No ____

Mortality:

<table>
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<th>Driver Sign Here</th>
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<table>
<thead>
<tr>
<th>During Haul</th>
<th>1st Day</th>
<th>2nd Day</th>
<th>3rd Day</th>
<th>4th Day</th>
<th>5th Day</th>
<th>6th Day</th>
<th>Per-</th>
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<tbody>
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<td>cent</td>
</tr>
</tbody>
</table>

Describe symptoms of dying fish (initial, mid, and final stages--look for disease, streaming mucus, etc.):

Hatchery Supt. Sign Here: ___________