United States Department of the Interior, J. A. Krug, Secretary Fish and Wildlife Service, Albert M. Day, Director

Fishery Leaflet 222

Washington 25, D. C.

March 1947

DISEASE CONTROL IN HATCHERY FISH

By Frederic F. Fish Aquatic Biologist, Division of Fishery Biology

Introduction

A direct method for controlling diseases among hatchery fish has long been sought as an alternative to the earlier technique of hand dipping. A simple, practical, and effective method of direct pond treatment not only obviates all need for handling fish weakened by disease, but it would materially reduce the time and effort required by hatcherymen in keeping diseases under control. Furthermore, a direct pond treatment could be used for routine prophylaxis to eradicate diseases during their incipient stages--long before their presence becomes apparent.

Several methods for applying direct pond treatments in coldwater-fish culture have been proposed. Kingsbury and Embody (1932) developed a technique for applying disinfectants directly to the inflowing water of a pond. Later, Fish (1939) advocated a method involving the recirculation of a disinfecting solution through a fish pond. For various reasons, neither of these methods proved to be an effective answer to the problem of controlling hatchery diseases and neither became widely adopted.

The method described herein has been extensively tested, both in the laboratory and at the producing hatchery, over a period of several years. Once familiarity with the details of application have been mastered, the reduction in effort required to treat fish is amazing. For example, two men have treated 20 large ponds containing several million fish, in one morning with no significant increase in mortality of the fish, whereas a crew of eight men required a full day to treat a single similar pond by hand dipping the fish with a subsequent loss approximating 50 percent of the stock.

RECOMMENDED TECHNIQUE OF APPLICATIONS

1. Basic Calculations

If the weight of water contained in a pond or trough is known, any desired concentration of disinfectant can be obtained and held for a period of time merely by shutting off the inflowing water and adding the required quantity of disinfectant. This, in essence, constitutes the method recommended for applying prolonged treatments. Two disadvantages immediately become apparent. A uniform distribution of the disinfectant would be required to avoid the formation of localized areas of hyperconcentration, or "hot spots" that might prove toxic to the fish. Furthermore, coldwater fishes would quickly deplete the store of essential oxygen contained in the water and suffocate. By exercising suitable precautions, however, both of these difficulties can be overcome.

Certain basic data are required before the time of treatment to assure a safe and effective application. The volume of water contained in the pond or trough at regular intervals of depth is a necessary basis for calculating the quantity of disinfectant required. As disinfectant-concentrations are expressed in terms of weight for both disinfectant and water, conversion of the pond volume to weight is required. Once determined, however, the tasic data may be filed for ready reference whenever a treatment is needed. For convenience, use of the metric system is recommended. Not only are cubic centimeters and grams much smaller units than cubic inches and prunds, but they possess the convenient relationship of one cubic centimeter of water weighing one gram. Thus, by calculating the volume of water contained in a pond in terms of cubic centimeters, one also calculates the weight of that water in grams. This is not true of cubic inches and pounds.

Determination of the Weight of Water Present at Progressive Depths

The weight of water contained in a pond or trough of regular outline may be quickly and easily calculated by standard measuration formulae. The volume of water in terms of cubic inches contained in a rectangular pond at a one-inch center depth may be calculated by multiplying the length by the width-both measurements being taken in inches. The volume of water present at any desired depth then may be obtained by multiplying the volume at the one-inch depth by the number of inches of desired depth. The volumes then may be converted into cubic centimeters (and thus to weight expressed in grams) merely by multiplying the volume in cubic inches by 16.4.

In the case of irregularly shaped ponds, or ponds such as the Foster-Lucas type, calculation of the weight of water present at progressive depths by the use of formulae becomes too involved. It is simpler merely to plug the drain water-tight and then add known weights of water by any convenient method. The depths attained by known weights of water then can be marked on some permanent vertical structure in the pond, such as the tailscreen guides, for future reference. A water meter often may be borrowed for a sufficient period of time to calibrate all ponds at a hatchery. As these instruments usually are read in terms of cubic feet, the values can be converted into grams by multiplying the volume in cubic feet by 28,339.

The determination of the weight of water present at progressive intervals of depth does involve some initial effort, but once obtained, the record is of permanent application.

Usually, a table is prepared for reference when treatments are needed. The table should show the weight of water present in a pond at progressive intervals of depth and the quantity of disinfectant required to produce the concentration desired for treatment. At the present time, the only disinfectants known to be efficacious in prolonged treatments are formalin, which

is used at a concentration of one gram in each 4,000 grams of water (usually written as 1:4,000), and Roccal used at a 1:50,000 concentration. The weight of disinfectant(in grams) required to produce a desired concentration is obtained by dividing the weight of water present by the disinfectant-concentration desired.

Liquid disinfectants, for the sake of convenience, may be considered as weighing one gram per cubic centimeter. If 99.2 grams of a liquid disinfectant are required, for example, it will be sufficiently accurate to measure 99 cubic centimeters in a graduated glass cylinder.

As an example of the basic calculations required, assume that a hatchery is equipped with a series of rectangular ponds having uniformly sloping bottoms. The internal length of the ponds is 30 feet and the internal width is 6 feet. At a one-inch depth (measured at the center of the pond), the pond will contain: $(30 \times 12) \times (6 \times 12)$, or 25,920 cubic inches. Converting to grams: 25,920 x 16.4 yields 425,088 grams of water present at the one-inch depth. For each one-inch of depth, therefore, 106 grams of formalin will be required to produce a ::4,000 concentration (425,088 - 4,000). Likewise, 8.5 grams of Roccal will be required to produce a 1:50,000 concentration (425,088 -50,000). The hatcheryman then would prepare a reference table somewhat as follows:

at center	Weight of water present (in grams)	Weight of formalin required (in grams) (1:4,000)	Weight of Roccal required (in grams) (1:50,000)
; 1	425,088	106	8.5
6	2,550,528	638	51.0
7	2,975,616	744	59•5
8	3,400 ,7 04	850	68.0
9	3,825,792	956	76.5
10	4,250,880	1,063	85.0
11	4,675,968	1,169	.93.5
12	5,101,056	1,275	102.0

2. Calculations Required at the Time of Treatment

3

Treatments administered to ponds will require an adjustment of the weight of water present during the treatment to a definite relationship with the total weight of the fish being treated. This precaution lessens the risk of suffocation during treatment; it greatly facilitates thorough mixing of the disinfectant with the pond water; and it reduces the quantity of disinfect ant required. In the case of troughs, adjustment of the weight of water to the weight of fish present is not necessary as, in most instances, the quantity of disinfectant saved is insignificant, thorough mixing is easily accomplished, and the weight of fish present usually permits no reduction in the quantity of water present.

Obviously, determination of the weight of fish present in a pond cannot be made long in advance of treatment. The total weight of fish confined in a pond is periodically determined, or estimated, at hatcheries practicing feeding on the basis of body weight. Except under conditions of maximum growth rates, weight records obtained within a week or two of the time of treatment are sufficiently accurate for this purpose. At hatcheries where no record of the weight of stock is maintained, the average length and the number of fish in each pond is, or should be, known. The relationship between total length and body weight may be grossly approximated by reference to Figure 1. The approximate body weight multiplied by the number of fish present yields the approximate total weight of the fish in the pond.

Under conditions wherein the fish have been starved for a period of 24 hours prior to treatment, the water temperature lies between 50° and 60° F., and the water contains a normal complement of dissolved oxygen, a relationship of 75 grams of water for each gram of fish present is ample to maintain the fish safely, without additional water, for 90 minutes which represents an adequate factor of safety for a 60-minute treatment.

In instances wherein the fish cannot be starved for 24 hours prior to treatment, or the incoming water is significantly under-saturated with dissolved oxygen, or the water temperature is in excess of 60° F., or the fish are suffering from a gill disorder or anemia, the relationship between the weight of the fish present and the weight of water present should be reduced, possibly to 1:100 or even less, according to circumstances. Conversely, if the water termperature is below 50° F., a relationship between the weight of the fish present and the weight of l:50 can be employed with safety.

The effect of two dominant variables, namely the recency of feeding and water temperature upon the rate of oxygen consumption, is of sufficient importance to warrant further consideration. Table 1 summarizes data obtained from laboratory experiments in which each of these factors was considered as a single variable. The marked increase in oxygen required by the fish as a result of the higher water temperature, and the decrease in oxygen-demand as time elapses following feeding emphasize the need for revision of the relationship between the weight of the fish and the weight of water present during treatment according to the importance of the variables at the time of treatment

Table 1.--The effect of water temperature and recency of feeding upon the oxygen consumption of a 2-inch chinook-salmon fingerlings.

Temperature	Elapsed time since feeding (in hours)	Approximate oxygen consumption (expressed as milligrams of oxygen consumed per hour)
50	1 24 48 72 96	23.0 19.8 15.0 12.6 12.6
55	1 24 48 72 96	27.8 24.6 20.6 17.7 17.1
60	1 24 48 72 96	34.6 31.0 22.9 17.5 17.6

The effect of two other variables, not so easily measured, nevertheless should be considered. Any physiological handicap to the normal respiratory processes of the fish obviously would directly affect the safe duration of treatment. Gill disorders and anemia are two very important and common handicaps to normal respiration. Anemic fish will have equilibrium and exhibit every indication of sufficient when the water still contains six parts per million of dissolved oxygen whereas normal fish will tolerate a reduction to less than three parts per million with no apparent ill effect. Advanced anemia may be detected easily by the pink to white coloration of the gill filaments in contrast to the cherry-red gill filaments of the healthy fish. Gill disease offers a similar handicap to the respiration of the fish although its presence can be detected only with a microscope. If the presence of either anemia or advanced gill disease is suspected, it is advisable to use a low ratio between the weight of the fish and the weight of water present.

As an example of the calculations required at the time of treatment, assume that one of the ponds previously quoted carried a normal operating water depth of 14 inches. At the time a 1:50,000 Roccal treatment was needed, the pond contained 94 pounds of fish, the water temperature was 57° F., the fish had not been starved, and the gill disease present had not advanced to the stage where respiration was materially handicapped. The pounds of fish present should be converted to grams, merely by multiplying by 453. The answer of 42,582 is obtained. All factors considered, the hatcheryman probably would choose a 1:75 relationship between the weight of the fish and the weight of the water that should be present in the pond during treatment. To determine the weight of water required to form the 1:75 ratio, the weight of the fish present (in grams) would be multiplied by 75, yielding an answer of 3,193,650. By consulting the permanent pond calibrations, it is found that the nearest point over the desired value is the 8-inch level at which the pond contains 3,400,704 grams of water. The table also shows that 68.0 grams of Roccal will be required to form a 1:50,000 concentration at the 8-inch depth. The excess water present in the pond over the 3,193,650 cubic centimeters actually needed tends to compensate for the fact that the fish had not been starved. At the time of treatment, therefore, the hatcheryman would drain the pond from the normal operating level of 14 inches to the treatment level of eight inches before adding the disinfectant.

3. Applying the Treatment

After the quantity of water to be present during treatment has been determined, the next steps are to shut off the inflowing water and to add the proper quantity of disinfectant to form the desired concentration in the quantity of water present.

It is essential that the disinfectant be so applied as to form a homogeneous concentration throughout the pond or trough in the shortest possible time, thus avoiding the formation of "hot spots" that would be toxic to the fish.

The most effective means of applying the disinfectant depends upon the particular type of rearing equipment being treated. In all cases, however, the disinfectant should be diluted with at least 100 times its weight of water--the more dilute the disinfectant at the time of application, the less consequential are the unavoidable irregularities in distribution. In no instance should the time required to distribute the diluted disinfectant exceed ten minutes.

Trough treatments best can be applied with an ordinary garden sprinkling can. It is advisable to fill the can with water taken from the trough <u>after</u> the inflowing water is stopped. The full strength disinfectant may be rapidly mixed with the water in the sprinkling can and the mixture then spread evenly over the water surface. Following the addition of the diluted disinfectant, it is advisable to facilitate thorough mixing by two or three trips over the length of the trough with a wide flat paddle.

Small ponds of the raceway type can be treated in exactly the same manne as troughs. Large ponds, however, require too great a volume of 1:100 diluted disinfectant to be added within the required period of ten minutes by means of sprinkling cans. In this event, it is necessary to resort to some type of power sprayer that is capable of handling large quantities of liquids.

- 6

A portable spraying outfit that has proven satisfactory consists of a motor-driven, one-inch, herring-bone gear pump with an injector mounted on the intake arm which introduces the prediluted disinfectant into the circulating water. A pump of this type will deliver about 12 gallons per minute against a backpressure of 20 pounds when turning at 900 r.p.m. A one-third horsepower motor will produce ample power for the pump, yet requires suffice a ciently little current to permit its attachment to the usual electrical circuit. The injector on the inlet arm of the pump consists of a "T" bearing a one-eighth-inch needle valve inserted in the hose line from the disinfectant reservoir. The rate at which the prediluted disinfectant is added to the pond water through the pump may be controlled accurately by properly setting the needle valve. Prior to its initial use, the apparatus should be calibrated to determine how much of the disinfectant is removed from the reservoir at different settings of the needle valve. In use, the intake hose is coupled to the intake pipe and the free end dropped behind the tail screen of the pond. The required quantity of prediluted disinfectant is added to the bottle and the needle valve set to empty the bottle in approximately ten minutes. An outlet hose, connected to the opposite side of the pump, is used to spray the greatly diluted disinfectant over the pond surface.

The actions of the fish should be carefully watched during treatments. Although the recommended relationships between fish weight and water weight have been established with a considerable margin of safety, they cannot be regarded as infallible under all conditions. Fish gulping at the surface indicate the gradual approach of the suffocation threshold although it is not ordinarily an index of immediate danger. The first evidence of gulping usually appears when the dissolved oxygen has been reduced about halfway from the initial concentration to the suffocation threshold. Periodic dissolved-oxygen determinations made during the treatment are desirable, when they can be obtained, particularly until some experience has been acquired with the water concerned.

At the conclusion of the desired period of exposure--which is calculated from the time at which addition of the disinfectant is completed--the water flow to the pond should be resumed immediately and even increased above the normal operating volume to the maximum that will not sweep the weaker fish against the tail screen. To facilitate the complete and rapid removal of the disinfectant, the pond drain should be opened sufficiently to merely maintain the reduced level. After the disinfecting solution has been replaced by fresh water, the normal pond level and volume of inflow should be restored.

It is recommended that the inexperienced hatcherymen first apply treatments to fish in troughs. Pond treatments should not be attempted until complete familiarity with, and understanding of, the details of application have been gained from repeated and successful trough treatments. The mortality subsequent to treatment should be used as an index of the correctness of the technique being employed. Properly applied in the manner described, prolonged treatments will incur no significant increase in the daily mortality rate even among fish somewhat weakened by disease.

7

. .

. .

• 1. ...

RECOMMENDED CONCENTRATIONS OF DISINFECTANTS AND THE PERIOD OF EXPOSURE

1. Parasitic Diseases

A single one-hour exposure to a 1:4,000 concentration of formalin $\frac{1}{}$ will remove the common external parasites of hatchery fish. Formalin treatments are more effective against the common parasites than are the usual hand dips in copper sulphate, acetic acid, or salt.

Formalin treatment may be used effectively, either as a routine prophylactic measure to prevent epidemics, or as a therapeutic after the infections become evident. Formalin treatments have been demonstrated to eradicate infections by <u>Costia</u>, <u>Chilodon</u>, <u>Ichthyophthirius</u>, <u>Gyrodactylus</u> and certain of the stalked protozoans that occasionally attach themselves to fish.

As a prophylactic measure, the routine application of a 1:5,000 concentration of formalin for one hour at two-week intervals is recommended during periods when outbreaks of these parasites are anticipated. Routine prophylaxis with formalin, however, is advisable only during periods when considerable trouble from the external parasites ordinarily is encountered.

As a therapeutic measure, given after evidence of parasitism has been established, a single one-hour exposure to a 1:4,000 concentration of formalin will effectively eradicate all of the external parasites except <u>Ichthyoph-</u> <u>thirius</u>. To combat this parasite, daily applications of 1:5,000 formalin for one hour are required over a period of seven to fourteen days. Chly those stages in the life history of this parasite that are not on the fish can be reached by formalin treatments, hence daily applications are necessary to kill these stages before they can reinfect the fish.

As an alternative thereapeutic measure in instances wherein the usual one-hour exposure is not possible because of exceptionally crowded troughs or abnormally low discolved oxygen concentrations, a 20-minute exposure to 1:4,000 formalin administered on three consecutive days will prove equally effective as a single one-hour treatment.

Formalin 1:6,000 has been recommended in one-hour exposures as an effective measure for eradicating parasitic copepods. A 30-minute exposure to a 1:200 concentration of formalin has been recommended by Watenabe (1940) to combat fungus growing on developing eggs for the so-called "soft-egg". Confirmation of these two suggested uses of formalin treatments is, as yet, lacking.

I/ Formalin is used herein to designate a 40 percent aqueous solution of formaldehyde gas to which has been added a small quantity of methyl (wood) alcohol to prevent polymerization. In trade, this solution is commonly referred to as "formaldehyde" although strictly speaking, formaldehyde exists only as a gas at ordinary temperature. The U. S. P. grade of formalin should be used for treatment of fish.

Certain of the stalked Ciliata occasionally encountered attached to hatchery fish appear quite refractory to formalin treatments. Although all available evidence points to the fact that these organisms are quite harmless, they may be removed, if desired, by a single, one-hour exposure to a 1:10,000 concentration of glacial acetic acid administered in exactly the same manner as formalin. The effect of the acetic acid upon these organisms appears to be through the action of the hydrogen ions rather than through any specific toxicity of the acetate ion. Presumably, a similar pH produced by other acids would work equally well. Caution is definitely recommended in the use of acetic acid for this purpose as the margin of safety between an effective concentration and one lethal to the fish is very narrow, much narrower than that of formalin.

2. Bacterial Diseases

Formalin has no effect upon the bacterial diseases. For the control of bacterial gill disease, the application of a 1:50,000 concentration of Roccal $\frac{2}{2}$ for one hour is particularly effective.

Roccal treatments demand more care than formalin treatments for the margin of safety between an effective concentration and one toxic to the fish is very narrow. When used as recommended, however, Roccal treatments are safe and appear to actually stimulate the fish in much the same manner as do saltings.

As a curative measure, applied when bacterial gill disease is known to be present, one-hour Roccal treatments at a 1:50,000 concentration are recommended on two or three consecutive days.

As a prophylactic measure, a one-hour Roccal treatment at a 1:50,000 concentration at weekly intervals is efficacious. Controlled experiments have demonstrated that, at least under certain conditions, weekly prophylactic treatments with 1:50,000 Roccal applied as soon as the fish are free-swimming will eliminate all danger of epidemics of bacterial gill disease. The bene-. fits derived from routine weekly Roccal treatments at hatcheries where bacterial gill disease frequently appears are well worth the low cost and slight additional effort required for their application. Weekly prophylactic treatments with Roccal appear to eliminate infections by most of the external parasites as well. Single treatments, however, appear less effective than formalin as a therapeutic measure. The potential value of Roccal, and other compounds of this nature, in the control of fish diseases have not been fully explored.

2/ "Roccal" is the trade name of a 10 percent solution of the high molecular group of alkyl-dimethyl-benzyl-ammonium chlorides. It is manufactured by the Winthrop Chemical Company, 17 Varick Street, New York 13, and by various licensees. When ordering Roccal, the fact that it is to be used for fish-cultural purposes should be emphasized and shipments showing any trace of a brown coloration or an unpleasant odor should be rejected. Although Roccal is considerably more expensive than formalin, 1 the greater dilution used reduces the cost per treatment below that of formalin.

CONCLUSION

A technique for the direct application of certain disinfectants to hatch ery ponds and troughs is described. The cost of, and the effort involved in controlled common diseases at fish hatcheries may be greatly reduced by prolonged treatments in comparison with the usual method of hand dipping. The particular advantage of prolonged treatments applied as described lies in their value as routine prophylactics to eliminate infections by the commoner parasitic and bacterial diseases of hatchery fishes in the early stages before their presence can be detected.

Promptly and properly applied, prolonged treatments will incur no significant mortality, even among fishes somewhat weakened by disease. In fact, the mortality subsequent to treatment should be used as an index of the procedure employed-any significant increase in mortality being indicative of an incorrect application or of failure to compensate adequately for pertinent variables affecting this type of treatment.

Approximate Conversions

To convert:

- 1. Cubic inches to cubic centimeters, multiply by 16.4
- 2. Cubic feet to cubic inches, multiply by 28,339
- 3. Gallons to cubic centimeters, multiply by 3,785
- 4. Pounds to grams, multiply by 453
- 5. Ounces (avoirdupois) to grais, multiply by 28.3

LITERATURE CITED

FISH, FREDERIC F.

- 1939 Simplified methods for the prolonged treatment of fish diseases. Trans. Am. Fish. Soc. Vol. 68, 1938, pp. 178-187.
- 1940 Formalin treatments pass new tests. The Progressive Fish-Culturist, No. 49, March-April 1940, pp. 31-32. Bureau of Fisheries, now Fish and Wildlife Service, Dept. of the Interior.
- FISH, FREDERIC F., and BURROUS, ROGER E.
 - 1940 Experiments upon the control of Trichodoniasis of salmonid fishes by the prolonged recirculation of formalin solutions. Trans.Am. Fish. Soc., 1939, Vol. pp. 94-100.

LINGSBURY, OLIVER R., and EMBODY GEORGE C.

1932 The prevention and control of hatchery diseases by treating the water supply. Special Report, Conservation Commission. State of New York, 16 pp.

MATENABE, M.

1940 Salmon culture in Japan. The Progressive Fish-Culturist, Mo. 48, pp. 14-18. Bureau of Fisheries, now "ish and "ildlife Serwice, Dept. of the Interior.

10

