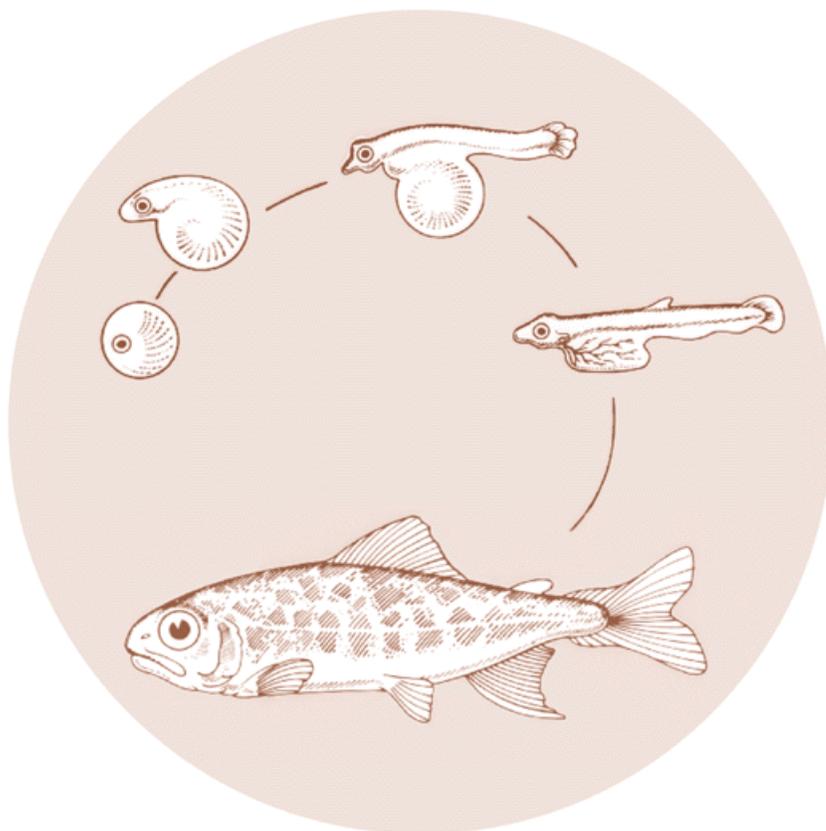


September 1991

RESEARCH TO IDENTIFY EFFECTIVE ANTIFUNGAL AGENTS

Annual Report



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RESEARCH TO IDENTIFY EFFECTIVE ANTIFUNGAL AGENTS

Annual Report

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Abstract

Selected chemicals were tested for antifungal activity on cultures of *Saprolegnia hypogyna* and on eyed rainbow trout eggs infected with *S. ferax*. Minimum inhibitory concentrations were less than 100 mg/L for amorolfine in 60-min exposures, iodine in 15- and 60-min exposures, and malachite green in both exposures. Compounds effective on cultured fungus at concentrations between 100 and 500 mg/L were acetic acid, fenpropidin, fenpropimorph, formalin, Herbisan, and melaleuca. Concentrations of sodium chloride required for effective fungus control were the highest at levels up to 50,000 mg/L. Amorolfine inhibited fungus growth on trout eggs and improved hatch rate at 50 mg/L in 60-min exposures and at 100 mg/L in 15- and 60-min exposures. Iodine inhibited fungus growth on eggs and increased hatch rate at 50 and 100 mg/L in 15- and 60-min exposures. Sodium chloride inhibited fungus growth on eggs at 3% in 60-min exposures and at 5% in both exposures. Malachite green and formalin, tested as references, were effective at use-pattern concentrations and exposures. Acetic acid and Herbisan did not show efficacy in this test system and will not be evaluated further.

Adult salmon were tested with iodine at 0.1 and a combination of 0.02 and 0.15 mg/L. Although mortalities in these groups were not initially as high as controls (untreated), surviving animals treated with iodine still showed frequent occurrence of fungal infection. At the conclusion of the iodine treatments, the percentage of total mortalities that were accompanied by fungal infection ranged from 12.5 to 33.3 compared to 70.6 in controls and 0 to 4.0 in malachite green-treated groups. Many of the survivors at this point had fungal infections and died within 2 weeks. At the conclusion of the study, between 57.9 and 69.2% of all mortalities in iodine-treated fish were accompanied by fungal infections compared to 84.8% in control fish and 0 to 8.3% in malachite green-treated fish.

Introduction

Aquatic fungi (Saprolegniales) are ubiquitous in natural water supplies of fish hatcheries and often cause serious disease problems. Malachite green is effective in control of fungus on fish and fish eggs, but due to suspected teratogenicity (Meyer and Jorgenson 1984) its use is limited to the treatment of non-food fish (i.e., eggs or adult salmon held for spawning) under an Investigational New Animal Drug Application held by the U.S. Fish and Wildlife Service. The reregistration of malachite green for treatment of fungus on food fish is highly unlikely. Presently, there is one registered aquatic fungicide, formalin, but it is not completely effective in control of fungus on fish or their eggs. Consequently, the search for safe and effective aquatic fungicides must continue.

Bailey (1984) and Bailey and Jeffrey (1989) reported results of tests with over 200 compounds that were chosen for fungicidal activity. More than half were found to be unsuitable as aquatic fungicides in preliminary tests because of their lack of activity against fungi, toxicity to fish or their eggs, insolubility in water, or potential carcinogenicity. However, several of the better candidates showed potential for control of fungus on fish eggs and were chosen for further evaluation.

This study is a continuation of "Research to Identify Effective Antifungal Agents" sponsored by Bonneville Power Administration (Schreck et al. 1990). The objective of the present study was to evaluate up to 10 candidate fungicides. Evaluations involve laboratory studies on efficacy of candidate compounds on cultured Saprolegniales, and on eggs of rainbow trout and chinook salmon that were previously infected with the fungus. Candidates that are demonstrated to be effective for control of fungus on eggs will be tested further on adult spring chinook salmon. During this reporting period, iodine was tested for controlling fungus in adults.

Materials and Methods

Goal I. Eggs and Juveniles

In Vitro Tests

Pure strains of aquatic fungi were obtained from the American Type Culture Collection (ATCC). *Saprolegnia hypogyna* (ATCC 28275) was used for both range finding and minimum inhibitory concentration tests. Test procedures used were those developed by Bailey (1983a,b). The method involved an *in vitro* screening technique modified from that of Golden and Oster (1947) and a minimum inhibitory concentration determination based on the percent inhibition of growth in diameter of colonies (Bailey and Jeffrey 1989).

Range Finding

Standard petri dishes were filled with 20 mL of corn meal agar and inoculated with agar plugs augmented with fungi (5 mm in diameter). The fungi was allowed to incubate at 20°C for approximately 96 hours, Agar plugs were removed from the edge of the colonies with a #1 cork borer. Stock solutions of chemicals were prepared to achieve final concentrations of 1, 10, and 100 mg/L. The depressions of Coors porcelain spot plates were filled with three replicates of each of the candidate chemicals and the solvent, positive, and negative controls, Agar plugs were then added to the depressions of the spot plates for exposures of 15 and 60 min. Agar plugs were removed from the spot plates, rinsed three times with sterile distilled water, and placed on tri-petri dishes containing 30 mL of corn meal agar. Cultures were incubated in continuous light inside an environmental control chamber maintained at 20°C (+ 2°C). The plates were examined for mycelial growth after 48, 96, and 168 hours of incubation.

Minimum Inhibitory Concentration

Inoculations, incubation, and stock solution preparation were as stated above. Agar plugs were removed from the edge of the fungal colonies as previously described and exposed in triplicate to five delineative concentrations between 0.1 and 1.0 mg/L, 1.0 and 10.0 mg/L, or 10.0 and 100.0 mg/L, or at higher levels, depending on the activity observed in the range-finding test.

The agar plugs were exposed to the test chemicals in triplicate for 15 or 60 min. They were rinsed with sterile distilled water and placed on standard petri dishes containing 10 mL of corn meal agar. Cultures were incubated in a lighted environmental control chamber at 20°C for 48 hours, and the colony diameters were measured with a vernier caliper.

In Vivo Tests

Green eggs from Trout Lodge (Sumner, Washington) were placed in Heath incubating trays (500 eggs per tray) with the use of a modified egg counting board. Characteristics of the well water used for incubation was a total hardness of 138 mg/L as CaCO₃, alkalinity of 105 mg/L as CaCO₃, pH of 8.0, and temperature of 12°C. Concentrations of dissolved oxygen remained at 9.0 mg/L or above during the exposures. Water flow was about 1 L/min during incubation and treatments to conserve test chemical quantities. The eggs were confined within a 6-inch diameter acrylic ring that was 1 inch in height and attached with silicone to the screen of each incubator tray. Two trays were used as replicates for each treatment level. Eggs were inoculated with *S. ferax* on hemp seeds suspended by a tea ball in the upper tray of each replicate treatment for exposures of approximately seven days or until the initial infection rate was about 20% or greater. Eggs were exposed to the fungicides for 15 or 60 min.

The chemicals were delivered to the water inflow of a separate mixing tray

above the egg hatching trays with the use of a peristaltic pump to achieve specific desired concentrations. The mixing tray contained a maze of baffles to ensure complete mixing. Concentrations were calculated on the basis of amount of material added to a specific volume of water flow. Treatments were administered three times weekly for a period of 2 weeks or until eggs began hatching. Mortality and fungal infection were assessed prior to the first treatment (pretreatment), after the last treatment (post-treatment), and after the eggs hatched (post-hatch). Infection rates (% increase) were calculated by subtracting pretreatment infection rates from post-treatment rates. The percent hatch was corrected for initial mortality by subtracting the pretreatment mortality from the total number hatched according to the following formula:

$$\text{Percent hatch} = \frac{\text{number hatched}}{\text{total} - \text{initial morts}} \times 100.$$

Toxicity Testing

Toxicity of the candidate fungicides was performed in the egg incubators simultaneously with the efficacy treatments. The set-up was the same as stated above for the in vivo testing; however, the eggs were uninfected. The dilution series was generally based on a use pattern of 1X, 3X, and 5X. The 1X concentration was the concentration we felt would be effective for control of fungus. Mortality observations were taken daily and egg hatching success was recorded at the end of each test. Margins of safety for each chemical were established by dividing the toxic concentration by the effective concentration for respective exposure times.

Goal II. Adults

Based on the Goal I results, iodine was selected as the candidate compound for testing on adult spring chinook salmon. Elemental iodine was purchased from Sigma Chemical Co. (St. Louis, MO). Toxicity tests were initially performed on

juvenile spring chinook salmon to narrow the choices of exposure concentrations for testing on adults. Groups (n=5) of yearling spring chinook salmon were placed in 3-foot circular tanks with flows adjusted to 2.1 L/min to simulate water turnover time in 10-foot circular tanks that were used for holding adults. An initial screening revealed that iodine treatment at 50 mg/L resulted in immediate mortality. Therefore, in test 1, concentrations of 5.0, 0.50, and 0.05 mg/L of iodine were used. In test 2, concentrations of 0.25 and 0.10 mg/L of iodine were used. In both tests, exposures were duplicated in separate tanks. Treatments consisted of three exposures administered every other day.

Based on the results of these toxicity tests, concentrations of 0.25 and 0.10 mg/L of iodine were selected for toxicity testing on adults. On 14 June 1991, 25 adult spring chinook salmon were delivered to Smith Farm Experimental Hatchery (Corvallis, Oregon) from Oregon Department of Fish and Wildlife's Dexter Holding Facility (Willamette River) and distributed among four 10-foot circular tanks (flow = 45 L/min; water temperature = 12-13°C). On 18 June, replicate tanks were treated with 0.25 or 0.10 mg/L iodine. Treatment occurred every other day for one week. On 3 July, 101 adult spring chinook salmon were delivered and distributed among four tanks. On 9 July, 99 adults were delivered and distributed among four other tanks. Replicate tanks were treated in the following manner: controls (no treatment), malachite green (0.50 mg/L), iodine (0.10 mg/L) and iodine (0.02 or 0.15 mg/L). All adults received erythromycin and oxytetracycline just before transport to the facility and again on 7 August (at which time all fish were examined for the presence of fungus on gills or body surfaces). Treatment consisted of adding the appropriate volume of stock chemical to a bucket, diluting to 20 L with water, and then adding the contents to the tank at the inflow over 1 min.

Results

Goal I. Eggs and Juveniles

Ten chemicals (Table 1) were chosen to test for antifungal activity on cultured fungus (*S. hypogyna*). Minimum inhibitory concentrations were less than 100 mg/L for amorolfine in 60-min exposures, iodine in 15- and 60-min exposures, and malachite green in both exposures. Compounds effective on cultured fungus at concentrations between 100 and 500 mg/L were acetic acid, fenpropidin, fenpropimorph, formalin, Herbisan, and melaleuca. Concentrations of sodium chloride required for effective fungus control were the highest at levels up to 50,000 mg/L. Eight of these compounds were tested further on infected eggs of rainbow trout to assess the minimum effective concentrations, toxicity, and safety indices.

The infection rate of healthy rainbow trout eggs varied between each group of eggs tested, but was fairly consistent in each group (Tables 2-9). The initial infection rate was allowed to reach approximately 20% before treatments were started. Infection rates for these studies were enhanced by the use of about 12 fully infected hemp seeds rather than 4, as had been used previously. The efficacy of candidate chemicals was easier to evaluate when the initial infection was 20% or greater.

Candidate chemicals chosen for *in vivo* tests were selected on their performance on cultured fungus. Malachite green and formalin were tested as reference compounds to compare efficacy and to verify the accuracy of the test system and procedure. Efficacy and toxicity values in Tables 2-9 were used for the following evaluations.

Acetic Acid

Acetic acid has been reported to be an effective fungicide in egg and fish culture. In our tests, acetic acid demonstrated little effectiveness towards suppressing the spread of fungus on the eggs (Table 2). Concentrations as high as 400

mg/L did not suppress the infection rate or increase the hatching rate. This candidate fungicide was not toxic to the eggs at concentrations up to 1000 mg/L. At that concentration the exposure solution had a pH of 4.0 which may cause undetectable stress to the eggs even for short exposures. Acetic acid will not be pursued as a candidate fungicide.

Amorolfine

Amorolfine, a morpholine derivative, possesses remarkable antifungal activity against a broad spectrum of fungus that are pathogenic to plants, animals, and humans. Preliminary tests of amorolfine at low treatment concentrations (Table 3a) indicated that this chemical at 40 mg/L for the 60-min exposure slowed the fungal growth and improved the hatch percentage. Considering these promising results, this chemical was tested again at higher concentrations (Table 3b). Amorolfine at 100 mg/L for the longer exposure exhibited a strong inhibitory response on the initial fungal infection and improved the hatch percentage. This chemical at concentrations greater than 500 mg/L showed no toxic effects on the eggs. Amorolfine demonstrated promising results and will be investigated further as a antifungal agent. Information will also be obtained on registration and marketing prospects.

Formalin

Formalin was tested as a reference chemical to validate our treatment method and confirm the antifungal activity. The suggested antifungal treatment concentration of 1,667 mg/L (Table 4) proved to be effective; 77% of the eggs hatched in the 60-min exposure, whereas only 48% hatched in the infected control. Formalin produced some toxicity at 1,667 mg/L in the 60-min exposure and in both exposures at higher concentrations. Formalin exhibited a positive antifungal

activity and its role as an alternative fungicide should be continued.

Herbisan

Initial tests with Herbisan were inconclusive because the liquid preparations were insoluble in water at fungicidal concentrations that were reported by the supplier. The manufacturer reformulated the chemical into a solid formulation. Our tests demonstrated no fungicidal activity at concentrations greater than 2.5 mg/L for the 15- or 60-min exposure times on the infected eggs (Table 5). This candidate fungicide did not control fungal infection or increase percent hatch at any treatment level. Also, at concentrations greater than 25 mg/L for the 60-min exposures was toxic to the eggs. Herbisan will not be pursued as a potential antifungal agent.

Iodine

Iodine has been reported to be an effective fungicide in egg and fish culture. Tests with iodine at low treatment concentrations showed that the chemical at 25 mg/L or less did not hinder the growth of fungus or improve the hatch percentage (Table 6a). However, in subsequent tests, a concentration of 50 mg/L for both exposure times suppressed the spread of fungus on the eggs and improved the hatch percentage (Table 6b). Higher levels of 100 and 200 mg/L were equally or more effective. Toxicity was apparent in 60-min exposures at 300 and 500 mg/L and in 15-min exposures at only the higher level. Iodine exhibits potential effectiveness as a replacement fungicide and should be considered for further testing and development.

Malachite Green

Malachite green was tested as a reference chemical for comparison with

candidate fungicides. It was effective for decreasing the infection rate on eggs at 1.0 mg/L for 60 min of exposure and at 3.0 mg/L for both exposure periods (Table 7). The 60-min exposures were more effective for increasing the hatch rates. No toxic effects were noted at concentrations up to 5 mg/L. Since malachite green is a suspected teratogen and can only be used under an INAD permit for use on restoration species only, it cannot be considered for further development or registration.

Melaleuca

Melaleuca, an oily tea plant extract, has been reported to be an effective fungicide and bactericide. This candidate fungicide did suppress the infection rate on eggs at a concentration of 300 mg/L for 60 min of exposure; even though the hatch rate was unexpectedly lower (46.2%) in that exposure (Table 8). The most improved percent hatch rate was demonstrated in the treatment level of 500 mg/L for both 15- and 60-min exposures. This chemical at concentrations up to 1,500 mg/L showed no toxic effects on the eggs. Further testing is required to assess the efficacy of this candidate material.

Sodium Chloride

Sodium chloride has been reported to be an effective fungicide in egg and fish culture. Due to sodium chloride's economics and safety, it was tested even though the inhibitory concentration was very high (~ 50,000 ppm). This antifungal agent actually decreased the amount of initial fungal growth at the treatment level of 50,000 mg/L. for the 15- and 60-min exposure times (Table 9). The hatch percentage was improved and it was not toxic at concentrations of 50,000 mg/L or less for both exposure times. Sodium chloride demonstrated promising results and will be investigated further as a replacement fungicide for malachite green.

Goal II. Adults

The toxicity of iodine was first established on juvenile chinook salmon and then on adult salmon. Juvenile chinook salmon exposed to 5.0 mg/L iodine all died within 1 hr, 20% of those exposed to 0.50 mg/L iodine died within 24 hrs, and 10% of those exposed to 0.05 mg/L iodine died within 96 hrs. No mortalities of juveniles occurred at either 0.25 or 0.10 mg/L iodine. In toxicity tests with adult salmon, 8 animals (75%) exposed to 0.25 mg/L iodine died within 24 hrs (treatment was discontinued at this point), whereas no mortalities were suffered in animals treated with 0.10 mg/L iodine every other day for one week.

Mortalities of adult spring chinook salmon began soon after transport to our holding facility, although most of these mortalities had no signs of fungal infection. Fish in both control tanks suffered the highest overall mortality and the mortality accompanied by fungal infection (Fig. 1; Table 10). The malachite green exposed fish had less overall mortality than controls and rare incidences of fungal infection (Fig. 1; Table 10). All fish exposed to iodine (either concentration) had mortalities associated with or without fungal infection between those in the control and malachite green tanks through mid-July (Fig. 2). However, surviving individuals in the iodine treatments had such frequent occurrence of fungal infection on their bodies that beginning on 18 July, all iodine-exposed fish received five malachite green treatments over 6 days and then iodine treatments were resumed (0.02 mg/L treatment was increased to 0.15 mg/L, whereas 0.10 mg/L was maintained). Subsequently, the incidence of mortality in iodine-exposed fish varied between replicates with higher mortalities associated with fish from the last delivery. The overall mortality in one iodine-treated tank at each concentration was comparable or even better than that in the malachite groups through 31 August (Table 10); however, the percentage of mortality accompanied by fungal infections was higher

in each of these iodine treatments when compared to that of the malachite treatment. The surviving individuals in the iodine treatments again showed considerable external fungal infections such that on 31 August these tanks were switched to malachite green treatments (every other day). By 13 September, the cumulative percentage of mortality accompanied by fungal infections in every iodine-treated group was much higher than that in the malachite-treated groups.

On August 7, the surviving fish received a booster injection of erythromycin and oxytetracycline. Each fish was examined for the presence of fungus on the gills or body. On this date, only the surviving control fish and one of the iodine-treated groups demonstrated considerable fungal infection (Table 11).

Discussion

Three compounds emerged from the previous study on antifungal agents (Schreck *et al.* 1990). They were Abbott Laboratories A-73336, diquat, and potassium permanganate. Following that evaluation, Abbott Laboratories determined that candidate A-73336 posed some risk of oncogenicity, the market potential was unfavorable, and they would not support the development of that compound as a malachite green replacement. The U.S. Environmental Protection Agency (EPA) has proposed a new regulation under the Safe Drinking Water Act that would restrict residue levels of diquat to 0.02 mg/L. Since the effective level for control of fungus with diquat was 100 mg/L, the EPA would most likely not permit the use of such high concentrations. Consideration of further testing and development of diquat will depend on the approval of the new regulations. Potassium permanganate also showed fungicidal activity, but effective levels were high at ,100 mg/L. Regulatory agents should be consulted regarding the high treatment levels before commitment of additional funding and effort for development of permanganate as a replacement fungicide.

In the present study amorolfine, iodine, and sodium chloride offer some potential for control of fungus on incubating trout eggs. Amorolfine was shown to have broad spectrum antifungal activity with minimum inhibitory concentrations of 0.1 mg/L against dermatophytic and dematiaceous fungi and most yeasts (Polak and Zaug 1990). The molds were more resistant and greater than 10 mg/L were required to inhibit many of them. Amorolfine compared favorably with other antimycotics already available. No information was generated on the aquatic fungi, but apparently they are as resistant as some molds.

Salt treatments have also been reported useful for control of fungus as well as other pathogens and parasites. Although treatment levels are high (up to 5%) they seem to be safe in up to 60-min exposures. Combinations of sodium and calcium chloride have also been suggested.

Iodine has been reported to be effective for control of fungus on eggs and our data suggest that 50 to 100 mg/L may be required for treatment. Those levels were not toxic and seemed to be effective for decreasing infection rate and increasing hatch rate. In the interest of development of a registration, the U.S. Food and Drug Administration has indicated that use of iodophor water solutions is consistent with public health requirements when used as a surface disinfectant during salmon spawn-taking and water hardening of fertilized eggs. Treatment levels, however, were not specified.

Adult salmon appear to be more sensitive to the toxic effects of iodine than either eggs or juveniles. Iodine at concentrations of 0.25 mg/L have proven to be effective in the treatment of rainbow trout fry against IHN virus (Batts *et al.* 1991); however, such a concentration proved highly toxic to adults in our study. Although iodine treatment of adults at lower concentrations resulted in fewer mortalities accompanied by fungal infections during early phases of the study, surviving animals in these treatments had frequent occurrences of fungal infections on body

surfaces that were growing in size and distribution. Therefore, iodine at the concentration and frequency tested may only be effective in controlling fungal growth once initial infections have been minimized. The species of fungus that infected the adult salmon has been tentatively identified as *S. parasitica* (H. Whistler, U. of Washington, pers. comm.) and seems to have a parasite associated with it. Dr. Whistler's group also observed that in fungi samples taken from adults on August 7, the fungi from iodine-treated fish were not producing zoospores in contrast to the fungi from untreated control fish. It is not clear if the limitation of zoospore production was a result of the intermittent treatment with malachite green or the iodine treatments.

In conclusion, the use of amorolfine, iodine, and sodium chloride have potential for control of fungus on eggs, The use of iodine to control fungus on adults is problematic because of the limited effectiveness at low doses as tested and of toxicity at higher doses. Iodine treatment in combination with other chemicals may have more potential as a control treatment for fungus on adults.

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Table 1. Effectiveness (mg/L) of candidate fungicides on cultured fungus (*Saprolegnia hypogyna*) and on eyed eggs of rainbow trout infected with fungus (*S. ferax*) in terms of MIC (minimum inhibitory concentration), MEC (minimum effective concentration), MSC (minimum safe concentration), and safety index.

Chemical name	Exposure time (min)	<i>In vitro</i>		In vivo MEC	Toxicity (MSC) eyed eggs	Safety index eyed eggs
		Range	MIC			
Acetic acid	15	>100	>100<300	400	>1,000	<2.5
	60	>10<100	>100<300	400	>1,000	<2.5
Amorolfine	15	>100	<300	300	>500	>1.7
	60	>10<100	<50	100	>500	>5
Fenpropidin	15	>100	>300<500	--	--	--
	60	>100	>200<300	--	--	--
Fenpropimorph	15	>100	2500	--	--	--
	60	>100	1100	--	--	--
Formalin	15	>100	<300	1,667	5,000	3
	60	>100	<300	1,000	1,667	1.7
Herbisan	15	>100	--	2.5	<100	<40
	60	>100	--	2.5	<25	<10
Iodine	15	>10<100	>70<100	50	<500	<10
	60	>10<100	>50<70	50	<300	<6
Malachite green	15	>1<10	>1<3	1.0	25	25
	60	>1<10	>1<3	1.0	25	25
Melaleuca	15	>100	>200<400	--	--	--
	60	>10<100	>200<400	--	--	--
Sodium chloride	15	>1,000	>50,000	50,000	>50,000	>1
	60	>1,000	>30,000	30,000	>50,000	>1.7

Table 2. Effectiveness of acetic acid on infected eggs of rainbow trout and toxicity of 1,3, and 5X concentrations to uninfected eggs at 12°C.

Treatment (mg/L)	Exposure (min)	Infection (%)		Hatch (%)
		Initial	Increase	
<u>Efficacy</u>				
(-) Control	--	1.6	36.7	60.2
(+) Control	--	10.1	66.1	41.7
100.0	15	19.8	74.8	27.3
100.0	60	24.7	67.6	31.9
200.0	15	20.4	59.1	27.1
200.0	60	19.7	71.8	31.8
400.0	15	22.2	69.6	41.3
400.0	60	16.8	65.6	36.3
<u>Toxicity</u>				
(-) Control	--	--	--	67.5
(-) Control	--	--	--	65.7
200.0	15	--	--	70.7
200.0	60	-	--	69.2
600.0	15	--	--	71.5
600.0	60	--	--	73.2
1,000.0	15	-	--	76.0
1,000.0	60			73.4

Table 3a. Effectiveness of amorolfine on infected eggs of rainbow trout and toxicity of 1,3, and 5X concentrations to uninfected eggs at 12°C.

Treatment (mg/L)	Exposure (min)	Infection (%)		Hatch (%)
		Initial	Increase	
<u>Efficacy</u>				
(-) Control	--	2.3	28.1	86.9
(+) Control	--	20.4	25.8	81.5
10.0	15	26.8	23.2	77.8
10.0	60	25.8	14.6	78.3
25.0	15	14.9	20.2	88.9
25.0	60	21.5	17.1	84.7
40.0	15	21.9	21.1	85.5
40.0	60	23.0	10.3	88.5
<u>Toxicity</u>				
(-) Control	--	--	--	95.0
(-) Control	--	--	--	95.2
25.0	15	--	--	94.5
25.0	60	--	--	94.6
75.0	15	--	--	93.6
75.0	60	--	--	95.8
125.0	15	--	--	94.5
125.0	60	--	--	93.5

Table 3b. Effectiveness of amorolfine on infected eggs of rainbow trout and toxicity of 1,3, and 5X concentrations to uninfected eggs at 12°C.

Treatment (mg/L)	Exposure (min)	Infection (%)		Hatch (0/0)
		Initial	Increase	
<u>Efficacy</u>				
(-) Control	--	0.7	5.5	96.0
(+) Control	--	18.7	21.4	83.2
50.0	15	26.2	18.3	80.7
50.0	60	19.6	12.1	90.2
100.0	15	22.5	10.9	88.0
100.0	60	22.6	5.3	93.6
300.0	15	18.3	7.2	94.5
300.0	60	19.7	5.2	96.3
<u>Toxicity</u>				
(-) Control	--	--	--	98.4
(-) Control	--	--	--	98.5
100.0	15	--	--	98.4
100.0	60	--	--	98.0
300.0	15	--	--	98.0
300.0	60	--	--	98.2
500.0	15	--	--	98.5
500.0	60	--	--	98.9

Table 4. Effectiveness of formalin on infected eggs of rainbow trout and toxicity of 1,3, and 5X concentrations to uninfected eggs at 12°C.

Treatment (mg/L)	Exposure (min)	Infection (%)		Hatch (%)
		Initial	Increase	
<u>Efficacy</u>				
(-) Control	--	14.9	60.0	54.7
(+) Control	--	35.2	38.6	48.4
1,000	15	24.0	45.5	53.2
1,000	60	10.8	29.5	62.3
1,667	15	38.3	20.5	63.5
1,667	60	-10.5	0.5	77.0
2,000	15	38.5	7.3	69.4
2,000	60	38.5	10.7	74.9
<u>Toxicity</u>				
(-) Control	--	--	--	80.8
(-) Control	--	--	--	75.7
1,667	15	--	--	67.3
1,667	60	--	--	38.3
5,001	15	--	--	39.4
5,001	60	--	--	39.9
8,335	15	--	--	38.0
8,335	60	--	--	47.2

Table 5. Effectiveness of Herbisan on infected eggs of rainbow trout and toxicity to uninfected eggs at 12°C.

Treatment (mg/L)	Exposure (min)	Infection (%)		Hatch (0/0)
		Initial	Increase	
<u>Efficacy</u>				
(-) Control	--	2.2	74.6	38.9
(+) Control		14.9	58.6	33.2
2.5	15	25.7	51.4	27.3
2.5	60	14.9	61.3	19.9
25.0	15	14.2	57.3	19.4
25.0	60	22.3	47.4	1.5
100.0	15	14.3	62.9	6.7
100.0	60	23.7	49.5	0.0
<u>Toxicity</u>				
(-) Control		--		71.5
(-) Control		--		69.7
2.5	15	--	--	71.3
2.5	60	--	--	69.3
25.0	15	--	--	67.4
25.0	60	--	--	51.0
100.0	15	--		61.6
100.0	60	--		23.4

Table 6a. Effectiveness of iodine on infected eggs of rainbow trout and toxicity of 1,3, and 5X concentrations to uninfected eggs at 12°C.

Treatment (mg/L)	Exposure (min)	Infection (%)		Hatch (%)
		Initial	Increase	
<u>Efficacy</u>				
(-) Control	--	0.2	13.6	76.0
(+) Control	--	7.6	42.4	63.3
5.0	15	8.3	48.1	53.0
5.0	60	6.8	50.0	50.6
10.0	15	11.6	54.1	56.5
10.0	60	6.7	50.0	57.1
25.0	15	11.8	52.1	53.2
25.0	60	13.4	48.5	54.2
<u>Toxicity</u>				
(-) Control	--	--	--	82.5
(-) Control	--	-	--	82.2
10.0	15	-	--	79.9
10.0	60	-	--	80.8
30.0	15	--	--	80.8
30.0	60	-	--	80.1
50.0	15	--	--	83.4
50.0	60	--	--	78.2

Table 6b. Effectiveness of iodine on infected eggs of rainbow trout and toxicity of 1,3, and 5X concentrations to uninfected eggs at 12°C.

Treatment (mg/L)	Exposure (min)	Infection (%)		Hatch (%)
		Initial	Increase	
<u>Efficacy</u>				
(-) Control	--	0.9	51.9	76.8
(+) Control	--	30.2	32.6	54.1
50.0	15	33.3	19.1	73.4
50.0	60	23.2	18.4	69.3
100.0	15	34.3	24.2	71.5
100.0	60	28.1	12.2	70.2
200.0	15	26.2	21.5	73.6
200.0	60	35.5	16.3	46.9
<u>Toxicity</u>				
(-) Control	--	--	--	90.1
(-) Control	--	--	--	90.0
100.0	15	--	--	90.5
100.0	60	--	--	82.9
300.0	15	--	--	83.5
300.0	60	--	--	4.4
500.0	15	--	--	76.4
500.0	60	--	--	0.0

Table 7. Effectiveness of malachite green on infected eggs of rainbow trout and toxicity of 1,3, and 5X concentrations to uninfected eggs at 12°C.

Treatment (mg/L)	Exposure (min)	Infection (%)		Hatch (%)
		Initial	Increase	
<u>Efficacy</u>				
(-) Control	--	1.6	42.8	75.2
(+) Control	--	43.0	35.2	42.5
0.5	15	43.2	33.8	41.3
0.5	60	44.9	26.6	49.9
1.0	15	38.6	32.5	53.0
1.0	60	36.4	18.5	60.8
3.0	15	38.3	28.7	57.3
3.0	60	30.3	10.4	75.5
<u>Toxicity</u>				
(-) Control	--	--	--	94.7
(-) Control	--	--	--	95.1
1.0	15	--	--	95.3
1.0	60	--	--	94.5
3.0	15	--	--	94.5
3.0	60	--	--	94.8
5.0	15	--	--	93.8
5.0	60	--	--	95.0

Table 8. Effectiveness of melaleuca on infected eggs of rainbow trout and toxicity of 1,3, and 5X concentrations to uninfected eggs at 12°C.

Treatment (mg/L)	Exposure (min)	Infection (%)		Hatch (%)
		Initial	Increase	
<u>Efficacy</u>				
(-) Control	--	0.7	38.0	76.8
(+) Control	--	27.9	31.1	56.7
100	15	14.9	30.1	67.0
100	60	29.4	29.0	59.0
300	15	25.8	24.2	68.7
300	60	36.3	5.6	46.2
500	15	29.4	22.7	72.1
500	60	40.4	13.2	71.3
<u>Toxicity</u>				
(-) Control	--	--	--	91.8
(-) Control	--	--	--	89.8
300	15	--	--	93.1
300	60	--	--	90.3
900	15	--	--	93.1
900	60	--	--	91.2
1,500	15	--	--	91.7
1,500	60	--	--	88.0

Table 9. Effectiveness of sodium chloride on infected eggs of rainbow trout and toxicity of 1,3, and 5X concentrations to uninfected eggs at 12°C.

Treatment (mg/L)	Exposure (min)	Infection (%)		Hatch (%)
		Initial	Increase	
<u>Efficacy</u>				
(-) Control	--	1.4	64.1	71.4
(+) Control	--	49.0	21.7	47.3
10,000	15	51.6	23.9	40.6
10,000	60	43.9	20.5	63.2
30,000	15	46.7	17.5	64.2
30,000	60	45.2	1.5'	80.2
50,000	15	42.8	-0.7	83.1
50,000	60	46.4	-12.9	83.7
<u>Toxicity</u>				
(-) Control	--	-	--	91.3
(-) Control	--	-	--	91.3
10,000	15		--	90.3
10,000	60	-	--	92.7
30,000	15		--	92.1
30,000	60	-	--	93.0
50,000	15	-	--	90.5
50,000	60		--	91.2

Table 10. Mortality and incidence of fungal growth in adult spring chinook salmon held in Corvallis, Oregon. Numbers indicate the cumulative percentage of total population that suffered mortality and percentage of mortalities that had fungus, as determined on three specified dates (treatment of animals with iodine stopped on 30 August and was replaced with malachite green).

Treatment	N	7 August Total w/fungus		31 August Total w/fungus		13 September Total w/fungus	
Control A	27	66.7	51.9				
Control B	24	70.8	66.7				
Combined Controls				84.3	70.6	90.2	84.8
Malachite Green C	25	24.0	4.0	44.0	4.0	48.0	8.3
Malachite Green D	27	11.1	0.0	33.3	0.0	33.3	0.0
Iodine E (0.10)	25	36.0	20.0	40.0	24.0	52.0	69.2
Iodine F (0.10)	25	44.0	16.0	60.0	28.0	88.0	63.6
Iodine G (0.02 + 0.15)	24	25.0	8.3	29.2	12.5	42.0	60.0
Iodine H (0.02 + 0.15)	30	30.0	23.3	60.0	33.3	63.0	57.9

Table 11. Incidence of fungal infection in adult spring chinook salmon on 7 August 1991. On that date, the specified number of surviving individuals from each treatment were examined for the presence of fungus on the gills and body surface.

Treatment	N	Gill Fungus (%)	Body Fungus (%)
Control A	7	100.0	42.9
Control B	10	100.0	12.5
Malachite Green C	19	0.0	0.0
Malachite Green D	24	0.0	0.0
Iodine E (0.10)	25	0.0	0.0
Iodine F (0.10)	25	6.7	0.0
Iodine G (0.02 + 0.15)	18	5.6	0.0
Iodine H (0.02 + 0.15)	22	22.7	0.0

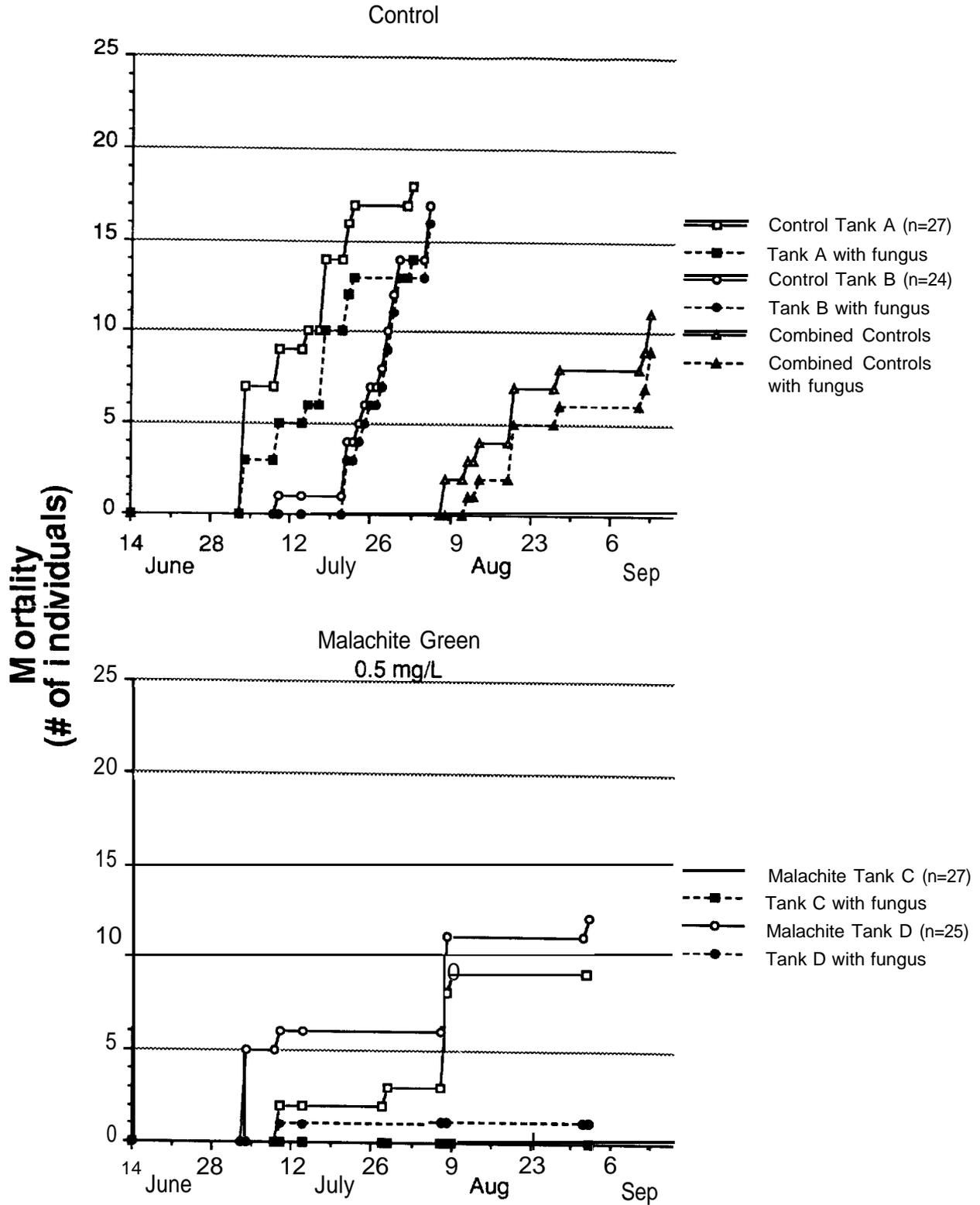


Figure 1. Cumulative total mortalities (-) and mortalities accompanied by fungal infection (----) in adult spring chinook salmon that received either no treatment (Control) or malachite green. On 7 August, the 17 surviving control animals were combined into a single tank ("Combined Controls").

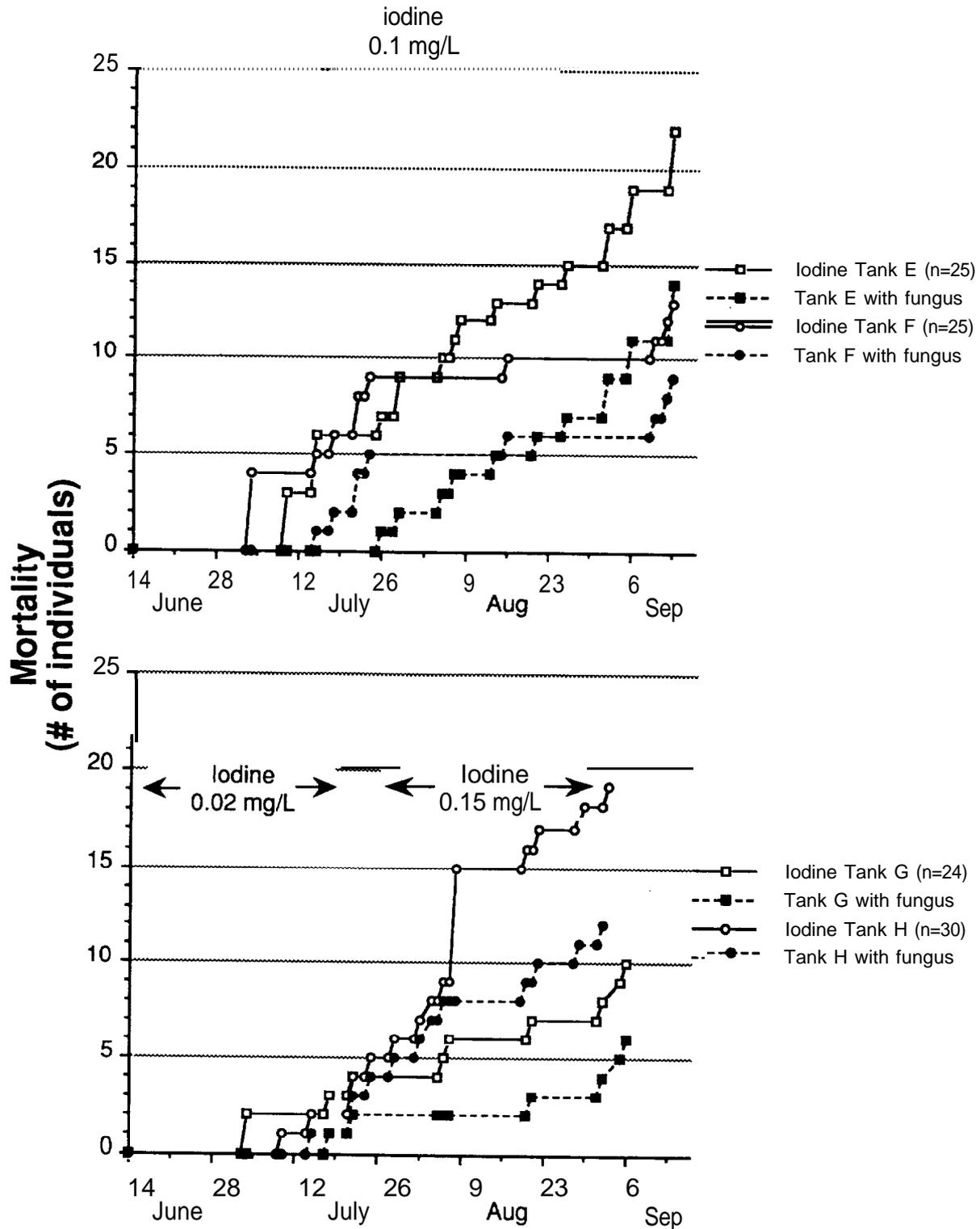


Figure 2. Cumulative total mortalities (-) and mortalities accompanied by fungal infection (----) in adult spring chinook salmon that were treated with iodine. Shaded areas represent intermittent periods of malachite green treatment at 0.5 mg/L (see text for details).