TR 73-5

MICHIGAN DEPARTMENT OF NATURAL RESOURCES

Fisheries Division

STEROID INJECTIONS OF RAINBOW TROUT IN AN ATTEMPT TO DEVELOP A BETTER UNDERSTANDING OF THE FACTORS CONTRIBUTING TO THE DEVELOPMENT OF FUNGUS INFECTION IN FRESHWATER FISH

John G. Hnath
Fisheries Pathologist
And

Vern Bennett
Fisheries Biologist

SUMMARY

Duplicate lots of 50 fish each (RBT 70 HA) were injected intramuscularly with cortisone, estradiol, or saline control solutions and observed closely over the next 30 days with an uninjected control lot. The incidence of fungus increased in all injected lots during the study, while in the uninjected controls the incidence of fungus actually dropped. It appeared that the stresses of handling could induce increased fungus, but that the steroid injections induced even higher levels of fungus.

Technical Report: 73-5
Submitted for publication August, 1973.

STEROID INJECTIONS OF RAINBOW TROUT IN AN ATTEMPT TO DEVELOP A BETTER UNDERSTANDING OF THE FACTORS CONTRIBUTING TO THE DEVELOPMENT OF FUNGUS INFECTION IN FRESHWATER FISH

John G. Hnath, Fisheries Pathologist
Vern Bennett, Fisheries Biologist

INTRODUCTION

External infections of fish caused by fungus of the family Saprolegniaceae have been one of the most serious infectious diseases of cultured fishes since the inception of fish husbandry. Problems occur both in the wild, and in hatchery situations. The predominant predisposing factor in many (if not most) cases seems to be some form of stress. In hatchery situations this stress may be produced by handling and/or spawning; often similar stresses apply to wild fish which subsequently develop fungal infestations.

Dr. R. J. Roberts, Director, Unit of Aquatic Patholbiology, University of Stirling (personal communication) feels that many cases of fungal problems of fish occur as secondary infections of epithelial lesions of the skin. These lesions, he feels, are "...similar in some ways to pubertal acne in man where (again) high steroid levels result in increased susceptibility to infections, or thrush of the female genital tract, which is a mormone related fungal infection of a mucus membrane." The postulation is, then, that the high steroid levels (whether caused by hormonal changes at spawning or merely as a response to stress) predispose fish to development of epithelial "lesions" which are highly susceptible to secondary fungal invasion.

In an attempt to gain a better understanding of the fungus problems often noted in Michigan, study was set up to see if artificially induced high steroid levels would in fact, increase the incidence of fungus in experimental fish. The Harrietta State Fish Hatchery was chosen as a site because it has been plagued with fungus problems from as early as 1946 (the earliest records at Grayling).

These problems recurr with unerring frequency despite all attempts to reduce their occurrence. In other words, we could be sure of having fungus problems at Harrietta if we handled the fish.

METHODS AND MATERIALS

Duplicate lots of 50 fish each (RBT 70 HA) were set up on May 9, 1973 for each injection as below. Average weight per fish was 0.5 kilogram.

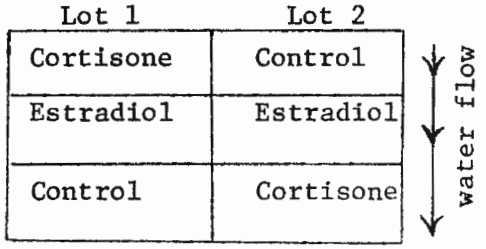
Two lots served as controls and received a 0.45 - 0.50 ml injection per fish of a stock solution of 2:1 balanced salt solution: propylene glycol.

Two lots received a 0.45-0.50 ml injection per fish of the stock solution containing 1.0 mg cortisone per ml for a dose rate of 1.0 mg cortisone per fish.

Two lots received a 0.45-0.50 ml injection per fish of the stock kilogram containing 1.0 mg estradiol per ml for a dose rate of 1.0 mg estradiol per fish.

Immediately prior to inoculation, the fish were simultaneously quieted and treated for fungus with a solution of malachite green at 1:10,000 dilution and MS-222 at 1:0,000 dilution. Only male fish (selected for freedom from fungus) were used. The injection was made IM in the caudal peduncle, in the vicinity of the caudal artery. Care was taken not to handle the fish unduly or to subject them to more than necessary stress. All groups were handled equally.

After injection the fish were released into ponds (see diagram below) and reared as per SOP for 30 days with daily observations made on the appearance of fungus. All mortalities were recorded and the presence or absence of fungus was



noted. At 30 days post inoculum all the fish were anesthetized and examined closely for fungus. Detailed records were kept on this examination indicating the presence or absence of fungus, and if possible, the degree of fungal infestation.

RESULTS

After inoculation, fungus became apparent on some fish the first week, and more obvious the second week. By the fourth week it was felt that the fungus level had peaked, and all fish with fungus had extensive fungal mycelial growth as evidenced by heavy mats of cottony material. Only 8 fish died during the course of the study; all were fungused and are incorporated in the chart below to give the final total incidence of fungus. There are two control lots in this chart; the untreated controls were those production fish from which the experimental fish were selected and which had no injection or excessive handling, the injected controls were those as described in the preceding section and had a saline/propylene glycol only injection.

	Untreated Control	Injected Control	Cortisone	Estradiol	Prior to Study
#with fungus (lot 1)	0	1	5	5	7 of 303
#with fungus (lot 2)	0	3	3	1	"
%with fungus (total)	0	4	8	6	2.3

DISCUSSION

As can be seen in the preceding table, the incidence of fungus increased sharply (2 to 4 fold) in all injected fish, but actually decreased in the untreated controls. It is a normal phenomenon at this time of year for fish at Harrietta which had problems earlier in the spring to self heal, and this is believed to be the case for the untreated controls. The increase in the fungus in the injected controls is assumed to be precipitated by the handling which these fish received. Both the cortisone and estradiol injected groups developed even higher levels of fungus than the injected controls, and this is believed to be due to the enhancement caused by the injected steroids.

The entire study and any conclusions drawn are highly subjective, but it certainly appears that handling did result in higher levels of fungus, and that steroid injected fish had even higher levels of fungus. The speculation is, of course, that handling in itself is sufficient stress to cause the natural release of higher levels of steroids which then predisposed the fish to fungal invasion.

There is evidence for this. It is known that stress causes the release of stress hormones such as cortisone and other corticosteroids from interrenal tissue.[1] And it has further been demonstrated that implantation of cortisol pellets in immature rainbow trout resulted in skin infections and death.[2] It has been shown that various steroids "...have a permissive, facilitating effect upon the occurrence of fungus infection in the white sucker fish." And this "facilitating effect" is presumed to lie in the impaired functions of antibody formation and reactive tissue inflamation.[3]

The most meaningful conclusion that can be drawn from this study is that our work does support the work of others in suggesting that stress leads the way to fungus infections. As a consequence, anytime fish are stressed, their susceptibility to fungus increases and we may expect to see fungus problems. Obviously

conditions will vary, some stresses will be more severe than others, stresses may be combined to further enhance susceptibility to infection, and certain seasons or periods in the life cycles of fish may produce more or less severe infections. But the susceptibility of fish to fungal infestations is a natural phenomenon and the more severe the stress the more likely are the fish to become infected.

LITERATURE CITED

1. Wedemeyer, G. 1970. The role of stress in the disease resistance of fishes. In: A symposium on diseases of fishes and shellfishes, Snieszko, S.F., ed. Fish. Soc. Sp. Pub. No. 5: 30-35.

2. Robertson, O. H., S. Hane, B. C. Wexler, and A. R. Rinfret. 1963. The effect of hydrocortisone on immature rainbow trout. Gen Comp. Endrocrinol. 3: 422-436.

3. Roth, R. E. 1972. Some factors contributing to the development of fungus infection in freshwater fish. J. Wildlife Dis. 8: 24-28.