EFFICACY OF FEEDING NITROFURAZONE FOR CONTROL OF THE MICROSPORIDIAN PARASITE *PLEISTOPHORA OVARIAE*

By

MARY LOUISE NAGEL Leida

Bachelor of Arts

Texas Lutheran College

Seguin, Texas

1973

Submitted to the Faculty of the Graduate College
of Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1975

EFFICACY OF FEEDING NITROFURAZONE FOR CONTROL OF THE MICROSPORIDIAN PARASITE PLEISTOPHORA OVARIAE

Thesis Approved:

Robert C. Summerfelt
Thesis Adviser

Milton R. Curd

Ronald W. McNew

N. N. Durham
Dean of the Graduate College

ACKNOWLEDGMENTS

Equipment and materials for this study were provided by the Oklahoma Cooperative Fishery Research Unit. Cooperators are the Oklahoma Department of Wildlife Conservation, the Oklahoma State University and the United States Fish and Wildlife Service.

I want to express appreciation to my major advisor, Dr. Robert C. Summerfelt, for his assistance, encouragement, and guidance throughout this study. The other members of my committee were Dr. Milton R. Curd and Dr. Ronald W. McNew. They also provided valuable advise and editorial assistance.

In addition I would like to thank the several students who helped me in feeding the shiners and obtaining pond samples.

TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	5
	Description of *Pleistophora ovariae*	5
	Drugs Used to Control Microsporidians	7
	Selected Drugs for Microbial Fish Diseases	9
	Description of Nitrofurazone	12
	Effects of Hormones on Ovary Maturation	14
III.	METHODS AND MATERIALS	16
	Description of Experimental Groups	16
	Drug Treatment	17
	Hormone Injection	19
	Sampling	19
	Histology	19
	Evaluation	21
IV.	RESULTS	23
	Laboratory Experiments	23
	Influence of Hormone on Gonadal Development	23
	Effects of Nitrofurazone on *P. ovariae*	34
	Pond Experiments	36
	Effects of Nitrofurazone on *P. ovariae*	36
	Additional Observations	48
	Occurrence of *Myxobolus argenteus*	48
	Occurrence of *Lernaea cyprinaceae*	51
V.	DISCUSSION	54
	Efficacy of Nitrofurazone	54
	Effects of Hormone Injection	58
VI.	SUMMARY	59
	SELECTED BIBLIOGRAPHY	60
	APPENDIX	66

LIST OF TABLES

Table		Page
1.	Various expressions of dosage levels of nitrofurazone used in efficacy experiments.	18
2.	Mean gonadal somatic index of female golden shiners with and without hormone for fish given nitrofurazone (treated) and controls	24
3.	Computation of analysis of variance of group means of gonadal somatic index of laboratory fish 7 and 21 March. .	25
4.	Mean frequency of occurrence of individual egg stages	28
5.	Mean percents of total ovary area for individual egg stages .	31
6.	Mean squares computed for analysis of variance of frequency of occurrence of five oocyte stages observed in laboratory females following hormone injections.	33
7.	Incidence (%)[a] of *Pleistophora ovariae* infection in female golden shiners held in the laboratory.	35
8.	Mortality of laboratory fish from causes other than sampling during the nitrofurazone experiment.	37
9.	Incidence of *Pleistophora ovariae* in pond-reared shiners. . .	38
10.	Average intensity of *Pleistophora ovariae* in pond-reared shiners.	44
11.	Average gonadal somatic index of female golden shiners held in ponds	46
12.	Incidence and intensity of *Myxobolus argenteus* in shiners held in the laboratory	49
13.	Incidence and intensity of *Myxobolus argenteus* in shiners held in ponds.	50
14.	Incidence and intensity of *Lernaea cyprinaceae* in shiners held in ponds.	52

Table Page

15. Average total length (mm) of female golden shiners held in the laboratory 67

16. Average body weight (g) of female golden shiners held in the laboratory 68

17. Average gonadal weight (g) of female golden shiners held in the laboratory 69

18. Average total length (mm) of female golden shiners held in ponds 70

19. Average body weight (g) of female golden shiners held in ponds 71

20. Average gonadal weight (g) of female golden shiners held in ponds 72

21. Computation of X^2 of incidence of *P. ovariae* in pond-reared shiners (composite data) 73

22. Computation of X^2 of incidence of *P. ovariae* in pond-reared shiners (post-spawning data) 74

23. Computation of X^2 of incidence of *P. ovariae* in controls compared to individual treatment groups[a] (composite data) . . 75

24. Computation of X^2 of incidence of *P. ovariae* in controls compared to individual treatment groups[a] (post-spawning data) 76

25. Computation of X^2 of intensity classes of *P. ovariae* in pond-reared shiners (composite data) 77

26. Computation of X^2 of intensity classes of *P. ovariae* in pond-reared shiners (post-spawning data) 78

27. Computation of X^2 of intensity classes of *P. ovariae* in controls compared to individual treatment groups[a] (composite data) 79

28. Computation of X^2 of intensity classes of *P. ovariae* in controls compared to individual treatment groups[a] (post-spawning data) 80

29. Computation of X^2 of incidence of *Lernaea cyprinaceae* in pond-reared shiners 81

30. Analysis of variance of mean gonadal somatic index from post-spawning fish from 28 May sample 82

LIST OF FIGURES

Figure — Page

1. Change in mean gonadal somatic index of shiners held in the laboratory related to time and mean water temperature . . . 27

2. Change in mean gonadal somatic index of pond-reared shiners, divided by treatment groups, related to time and mean water temperatures. 41

3. Relationship of incidence of P. ovariae to nitrofurazone dosage. 42

CHAPTER I

INTRODUCTION

The golden shiner, Notemigonus crysoleucas, is the most widely cultured bait minnow in the United States, extensively utilized by fishermen for both Micropterus and Pomoxis species. In 1969 it made up 84% of all United States acreage in bait minnow production (Summerfelt and Warner 1970a). In Arkansas in 1972, 71.9% of the 40,064 acres devoted to intensive fish culture was in bait fish production, and 91.2% of this area was in production of golden shiners (Bailey et al. 1974).

Pleistophora ovariae (Protozoa, Microsporida) carries out its life cycle within the developing oocytes of the shiner, proliferating to the extent that egg production can be drastically decreased. Many infected oocytes undergo atresia; the spore filled eggs disintegrate (Summerfelt and Warner 1970a). The bulk of the ovary may be filled with coalesced masses of spores. In 1967 half of the spawning failures occurring on 60 fish farms were attributable to infections with P. ovariae (Meyer 1967), and it has been conjectured that the effect of this parasite costs the minnow industry more than any other single problem (Malone 1970).

P. ovariae is nearly ubiquitous in populations of golden shiners from commercial fish farms. It was found in 45 of 49 commercial sources in 12 states with a total incidence of infection of 48% of

2759 fish examined (Summerfelt and Warner 1970b). Infections were not found in four commercial sources, but insufficient sampling from three of these may have prevented detection of the parasite. One source was sampled extensively enough, with histological sections from 161 fish, to be classified as uncontaminated. Subsequent examination of over 100 females from this source also failed to indicate occurrence of P. ovariae.

The effects of P. ovariae within a given population are progressive, intensity of the infection increasing with increasing age. Thus older brood stock bear a heavier parasitic burden and fecundity is substantially reduced. The recommendation to use one-year-old minnows for brood stock is a method to manage around the parasitic sterilization which occurs in older fish (Summerfelt 1964). This recommendation has become accepted practice by commercial producers allowing them to operate successfully without elimination of the parasite from their stock.

Control of parasite problems in domestic animals has traditionally been dealt with by special husbandry, eradication of the infected stock, or chemotherapy. In golden shiner culture, use of young brood fish has been a successful husbandry procedure, but because small fish convert a smaller percentage gain in body weight to gametes than large fish, this practice requires more feed per unit number of eggs produced. Also, young fish do not produce as many eggs per body weight as older fish do, and spawning of young fish is less predictable (Warner 1972). Therefore, use of younger brood stock decreases efficiency because minnow producers need to carry a larger number of fish to meet production levels at increased food and

maintenance costs.

The second alternative to fish farmers with infected stock is destruction of their present stock and replacement with uninfected fish. One commercial source, uninfected by P. ovariae (Summerfelt and Warner 1970b), could be utilized as a replacement source. Introduction of parasite-free stock would be successful, however, only if ponds, equipment, and facilities could be treated to eliminate spores. At present, effective methods for disinfecting hatchery ponds for P. ovariae have not been determined.

Chemotherapy is a potential alternative control or eradication method for P. ovariae. An effective treatment, even one achieving control rather than eradication, offers a rapid and practical solution.

Research efforts at chemotherapeutic control of P. ovariae have been attempted with two drugs, fumagillin and nitrofurazone. The former, a drug which has been proven helpful in controlling other microsporidian parasites, was tested on shiners infected with P. ovariae (Tonguthai 1972). Oral administration of fumagillin did not establish an effective control of P. ovariae, but intraperitoneal injection at a rate of 100 milligrams per pound of fish did reduce the number of microsporidian cysts in the shiner ovaries.

Studies using nitrofurazone suggest it has considerable promise as a chemotherapeutic candidate for controlling Pleistophora. The pathogenicity of a Pleistophora infection of cultured "salmonids" in Japan was attenuated and increased survival noted after fish were fed with nitrofurazone (Putz and McLaughlin 1970). Oral administration of nitrofurazone in 1972 to shiners infected with P. ovariae resulted in a decrease in incidence of infection observed by wet mount

examination of ovaries (personal communication, 1973, Fred P. Meyer, Chief, Fish Control Laboratory, La Crosse, Wisconsin).

The present study was designed to evaluate the efficacy of oral administration of nitrofurazone to golden shiners for treatment of P. ovariae. Pond experiments compared incidence and intensity of P. ovariae in fish receiving nitrofurazone at levels of 0, 50, 75, and 100 grams active ingredient per 45.4 kilograms feed. In the laboratory aquaria, only the 75 gram level was tested, but the experimental and control groups each contained subdivisions with fish injected and not injected with human chorionic gonadotropin (HCG) in an attempt to evaluate the hormone's effect upon ovary development.

CHAPTER II

LITERATURE REVIEW

Description of Pleistophora ovariae

Pleistophora ovariae was described by Summerfelt (1964) who also noted debilitating effects upon fecundity. Although this microsporidian does not kill the adult host, fecundity is significantly reduced by killing many of the developing oocytes. A higher percentage of dead eggs are seen on spawning mats from parents which are heavily infected with P. ovariae than from those which are lightly infected (personal communication, 1974, Frank J. Leteux, Ozark Fisheries, Stoutland, Missouri). In histological sections eggs are seen to contain schizonts, sporonts, and pansporoblasts, each containing 12 to 20 spores. A substantial number of these oocytes become atretic; as ova membranes disintegrate, a dense stroma is produced from coalescence of masses of spores (Summerfelt and Warner 1970a).

Transmission was believed to occur orally and transovarianly (Summerfelt and Warner 1970a). Experimental transmission by per os injections of spores has been demonstrated (Summerfelt, unpublished). Spores have been seen in the yolk of fertilized eggs and blastula stage embryos, providing support for occurrence of transovarial transmission (Summerfelt, unpublished).

The multiplicative stages of the parasite have been previously

investigated, and the processes of schizogony and sporogony have been related to the shiner's ovarian cycle (Warner 1972). A close synchrony occurs between the parasite's development and ovary maturation. Maximum spore development approximately coincides with the shiner's spawning season. The abundance of certain parasitic stages is seasonal and depends upon a particular stage of ovary development.

In young-of-the-year shiners, P. ovariae is latent and cannot be detected by ordinary microscopic examination until the fish approaches sexual maturity. By the time spawning occurs, a full-blown parasitic infection is present. Thereafter, stages of the parasite can be found almost any time of the year; the stage of the parasite which is present depends upon which egg stages[1] are present. Schizogony occurs in stage III and IV oocytes; sporogony, in stage V, VI, and VII oocytes. The number of sporonts increases as shiner eggs mature. In Warner's study, the number of sporonts increased by a factor of 22 from stage IV to stage V oocytes.

Considering the seasonal nature of parasitic development, proper timing of chemotherapeutic treatment appears to be essential to obtain eradication or optimal control of P. ovariae in golden shiner ovaries. The most rational chemotherapy must be directed at parasites undergoing schizogony and sporogony, rather than at mature spores which are well protected from the external environment. The reproductive phases of the parasite, when it would be most susceptible to chemotherapy, would occur during February to April under natural conditions

---

[1]Warner (1972) adopted the terminology defined by Braekevelt and McMillan (1967) to describe oogenesis. Ova are classed from stage 0 to stage VII on the basis of size and nuclear, cytoplasmic, and membrane characteristics. Stage VII are mature and ready to spawn.

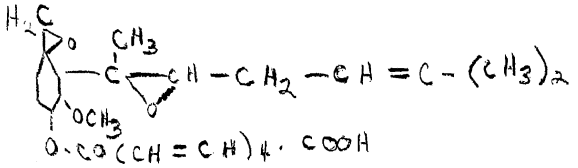
in most of the range where golden shiners are cultured. Meyer's experiments indicate that effective treatment would be obtained with a four week (February 15 to March 15) treatment. Four weeks is sufficient because microsporidian life cycles generally last for one to 24 days (Warner 1972). Warner observed a sharp increase in percent of the ovary affected by P. ovariae from a negligible amount in late April to over 60% in early June, the highest percentage coinciding with the second spawning as revealed by gonadal somatic index (gonad weight divided by body weight times 100%) determinations. This pattern of parasitism occurs even when the fish are removed from infected parents at an early age and raised apart from them.

Maturing ovaries increase rapidly in size, taking up materials abundantly as individual ova accumulate yolk and grow to spawning size. Ovary maturation of golden shiners in natural conditions begins in February at this location, but the gonadal somatic index (GSI) increases most rapidly during March and April, reflecting a great uptake of materials into the ovary. The water temperature during this period is 16 to 18°C (Warner 1972).

Drugs Used to Control Microsporidians

Microsporidians occur in only a few commercially important hosts; their occurrence in man was not known until recently, and research on their susceptibility to known parasitic chemotherapeutic drugs has not been extensively investigated. The first intensive research on chemotherapy of a disease of microsporidian etiology dealt with Nosema apis, an intestinal parasite of the honey bee, Apis mellifera. Nosema

disease was not controlled with available antibiotics, sulfa drugs, arsenicals, or anti-protozoan agents. A newly discovered antibiotic in 1952, fumagillin, an isolate from Aspergillus fumigatus H-3 did establish control of the bee parasite. Its structural formula is



First recognized as an antiphage agent (Hanson and Eble 1949), fumagillin was soon tested against a variety of parasites. Although it had shown little antiviral, antibacterial, or antifungal action, fumagillin had potent amebicide action and effectively subdued several other protozoans--Giarda lamblia, Chilomastix mesnili, Endolimax nana, Iodameba butschlii, Trichomonas hominus, and Plasmodium vivax (Killough et al. 1952 and McCowan et al. 1951). When fumagillin was fed to infected bees, a striking reduction in the Nosema apis infection was observed (Katznelson and Jamieson 1952). Further research substantiated that fumagillin arrested nosema disease without interferring with normal development of the bee colony (Jamieson 1955).

"Nosemack", a complex organic mercury compound whose active principle is merthiolate, was found to serve as a preventative for nosema disease in 1959 (Goetze and Zeutzschel 1959), but later it was shown to have toxic effects on the bees (Furgala and Boch 1970 and Gochnauer and Furgala 1969). Thus fumagillin remains the best control available for nosema disease in bees.

Fumagillin was later applied to other infections of microsporidian etiology. A Pleistophora species identified in a laboratory clone of Hydra littoralis was continuously exposed to a 200 mg/l solution of fumidil B for five weeks. At this time all hydra were

free of spores and remained uninfected for over a year when the report was written (Spangenberg and Claybrook 1961).

In 1970 fumidil B, the biocyclohexyl amine salt of fumagillin, was included in the diet of larval European corn borers, Ostrinia nubilalis, infected with Perezia pyraustae. At levels of 200 to 12,000 ppm, fumidil B significantly reduced the level of P. pyraustae infection without adversely affecting the hosts. P. pyraustae reduces percentage survival and fecundity and increases development completion time of laboratory cultures of the European corn borer, an economically important agricultural parasite (Lewis and Lynch 1970).

Fumidil B has also recently been applied to P. ovariae, the microsporidian parasite of the golden shiner and will be discussed in the next section.

Selected Drugs for Microbial Fish Diseases

It would be desireable to find an efficacious drug from among those presently being used to control other microbial fish diseases. Fumagillin has been applied in a few instances to protozoan parasites of fish. Fumagillin was effective in treatment of Hexamita (Protozoa: Mastigophora) from fingerling salmon without contraindicating side effects (Hawking 1963b). However, fumagillin administered orally to golden shiners was ineffective in subduing P. ovariae (Tonguthai 1972).

Oxytetracycline and sulfa drugs are widely used to treat bacterial diseases in fish (Snieszko 1954). Both, however, were ineffective in tests against Nosema apis, the microsporidian bee parasite (Gochnauer and Furgala 1969 and Furgala and Boch 1970).

Putz and McLaughlin (1970) reviewed drug treatments undertaken

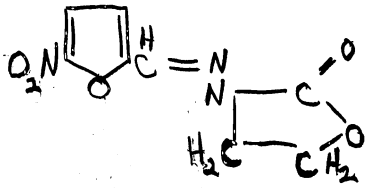
in Japan on salmonids infected with a Pleistophora species. The fish were treated with sulfaisomide, sulfamonomethoxine, sulfadimethoxine, nitrofurazone, and amprolium (1-(4-amino-2-propylpyrimidin-5-ymethyl)-2-picolinium chloride-HCl); the route of administration was not mentioned. Survival rate of the hosts increased with most drugs. Histological observations revealed that only amprolium eliminated schizogony, but an effective level of this drug yielded survival rates of only 52 to 58%.

Six drugs were tested in 1973 for control of Myxosoma cerebralis. This myxosporidian produces the economically crippling whirling disease in cold water hatchery fish. Oxytetracycline, sulfamerazine, furazolidone, nicarbazin, amprolium, and Merck experimental drug #930 were fed to trout in water known to be contaminated with M. cerebralis in an attempt to prevent infection; efficacy was measured with spore counts. Only furazolidone manifested reduced spore formation although trophozoites and granulomas were still present in some tissue sections. Growth of trout was less in this group, however, possibly due to the unpalatability of the furazolidone, the fish did not completely consume their feed rations (Taylor et al. 1973).

Nitrofurans are widely used in fish farming in Italy for treatment of bacterial infections because they are both economical and efficacious when given orally, a convenient application method for fish farmers (Ghittino 1972). Therapeutic concentrations can be established in body fluids at relatively low cost (Post 1959). Nitrofurans operate against a wide range of microorganisms, including certain fungi, protozoa, and bacteria (Burrows et al. 1968).

Several types of nitrofurans have been examined for treatment of

fish deseases. Furazolidone (Furoxone), a N-(f-nitro-2-furfurylidene-3-amine-2-oxazalidone, one of the most common nitrofurans is used mainly in connection with protozoan and microbial diseases. In fish it has been shown effective against vibrio (Seaman et al, 1969) and controls Aeromonas salmonicida, the pathogen causing furunculosis in trout (Post 1959, Heaton 1968, Warner 1969, and Ghittino 1972). The structural formula for furazolidone is



Furazolidone sometimes causes toxic symptoms in trout. At 2.3 mg/kg body weight, fish showed vertigo, paralysis, aimless movements, muscle tremors, and a higher mortality rate than control groups. Other test groups treated at the rates of 75 mg/kg and 100 mg/kg body weight showed similar toxic effects. Still other test groups, however, showed no toxic effects or excessive mortality for dosages ranging from 6.6 mg/kg to 450 mg/kg body weight (Post and Keiss 1962).

Another nitrofuran drug, nitrofurazone, has been used in the field of fish diseases. Unlike furazolidone which produces some deleterious effects, nitrofurazone apparently caused no harmful side effects when applied to golden shiners infected by P. ovariae (personal communication, 1973, Fred P. Meyer, Chief, Fish Control Laboratory, La Crosse, Wisconsin). Meyer's work is described here in detail because it is not generally known (unpublished) and because it served as an important antecedent experiment for the present study. Meyer mechanically mixed nitrofurazone with the feed. Drug adhered to feed particles was fed to golden shiners from February 15 to March 15, 1972,

at two dosage rates of 62.5 and 125 grams active ingredient per 100 pounds of feed. Pre-treatment and post-treatment examinations for the parasite by wet mount of the ovaries were made; similar examinations occurred at eight week intervals after the post-treatment check to observe any reoccurrence of the parasite. Initially there was 100% infection. After treatment there was a decrease in the percentage infection corresponding to drug concentration. No spores or sporoblasts were found and infection rates were significantly reduced.

Meyer repeated the experiment in 1973. Generally the same methods were employed; however, nitrofurazone was fed from March 15 to April 15 in seven treatment groups ranging from 10 to 125 grams active ingredient per 100 pounds of feed. Post-treatment examination of wet mounts showed a marked decline of sporonts in the ovary. Subsequent examinations indicated parasitic recovery, beginning with the appearance of sporonts, until pre-treatment levels of infection were again present as suggested by high intensity of spores.

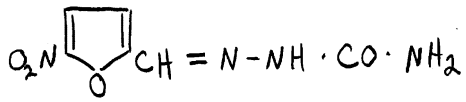
Meyer's observations provide substantive indication for efficacy of nitrofurazone in controlling P. ovariae. Also, salmonid fish infected with a Pleistophora species treated with nitrofurazone demonstrated an increased survival rate (Putz and McLaughlin 1970). Building on the observed cure by Meyer and first hand observations on the ovarian cycle of the golden shiner, the present study provides a definitive entry into efforts at chemotherapeutic control of P. ovariae infections of golden shiners.

Description of Nitrofurazone

Nitrofurazone, the oldest of the nitrofuran antibacterial agents,

is a widely used chemotherapeutic agent in veterinary medicine. Employed primarily as an antibacterial agent and local aneseptic, it is also effective in treatment of coccidiosis in poultry, lambs, and kids (Stephenson and Mettelstaedt 1965). At recommended dosages, toxicity is negligible, although if it is used continuously for over three months, supplements of vitamin B are recommended (Daykin 1960). Massive dosages can produce adverse side effects, but systemic poisoning at normal dosage levels is not likely (Sollman 1957).

Nitrofurazone (Furacin) is a 5-nitro-2-furalsehyde semicarbazone, with a structural formula of



It is almost insoluble in water and only slightly more soluble in 95% ethanol or acetone; it will not dissolve in ether. Nitrofurazone is golden-yellow in color and has a melting point of 227 to 241°C. Solutions decompose upon contact with certain metals, such as iron and zinc (Paul and Paul 1964), or when exposed to light (Hawking 1963a).

Nitrofurazone interferes with the normal metabolic process by tying up electrons produced by the anaerobic pyruvate production from acetate, carbon dioxide and hydrogen. These electrons are needed for the reverse reaction; by absorbing them, nitrofurazone inhibits the metabolic economy of the organism. In vitro nitrofurazone acts to inhibit bacterial growth (Paul and Paul 1964).

Nitrofurazone prohibits the action of many enzymes, but it cannot be classed as a general enzyme poison because there are many which it does not affect. Nitrofurans may also interfere with acetylation steps in the formation of the acetyl amino compounds of

the bacterial cell wall, rendering the microorganism vulnerable to attack from the host's normal body defense.

In experiments with laboratory animals, such as mice, rats, dogs, and chickens, several deleterious effects have been observed: occurrence of transplantable mammary tumors, inhibition of spermatogenesis, failure of pregnancies, kidney and liver damage (in chickens at levels 20 times greater than the recommended dosage), vomiting, growth depressions (in rats at .25% of feed), and various neurological abnormalities like hyperirritability and convulsions in mice followed by death when they were given 250 mg/kg body weight for three days. All of these symptoms occurred at very high dosage levels or during unusually prolonged treatment (Paul and Paul 1964). When nitrofurazone was tested against vibrio and kidney disease in fish, it lacked efficacy and was relatively toxic to the fish (Seaman et al. 1969). However, shiners treated with nitrofurazone did not show any deleterious responses (personal communication, 1973, Fred P. Meyer, Chief, Fish Control Laboratory, La Crosse, Wisconsin).

Effects of Hormones on Ovary Maturation

Numerous workers have shown that ovarian development and ovulation of fish can often be stimulated by injection of pituitary hormones. Ovulation in ripe goldfish, white crappie, and channel catfish has been precipitated with hormone treatments (Sneed and Clemens 1959). Tafanelli (1972) enhanced goldfish GSI with injections of human chorionic gonadotropin (HCG). Gonads of goldfish stimulated with HCG were more seriously affected from cadium chloride injections than the inactive gonad. For this reason HCG injections were used in half of the

the laboratory fish under the assumption that gonadal uptake of nitrofurazone would be increased by HCG stimulation.

CHAPTER III

METHODS AND MATERIALS

Description of Experimental Groups

Golden shiners were obtained in December 1973 from an Oklahoma source (John Reeves) near Sulphur, Oklahoma. Fish from this source when sampled in 1970 were heavily infected with P. ovariae (Summerfelt and Warner 1970a). Fish from this source were stocked in five 0.1 hectare mud-bottom ponds near Lake Carl Blackwell; water is supplied from Lake Carl Blackwell. These ponds were constructed in 1972 and had never held golden shiners. The fish were approximately seven months old when placed in the ponds to overwinter. Early in February, 1974, fish from one of the ponds were collected and transported to the laboratory. The remaining four ponds of shiners were designated as a control group and three treatment groups of 50, 75, and 100 grams of active ingredient nitrofurazone per 45.4 kilograms of feed.

The laboratory group was divided among eight tanks, each containing 175 shiners with an average total weight of 676 grams. Four of the eight tanks of fish were used as controls; the other four were treated with nitrofurazone at a rate of 75 grams active ingredient per 45.4 kilograms feed. These shiners were maintained under a constant photoperiod of 12 hours per day. Temperature was regulated at 18°C

by mixing of cold, dechlorinated tap water with hot tap water. Due to deficiencies in the controls, the temperature in the tanks generally varied ± 2°C from 18°C; total range was from 14°C in February to a high of 26°C in July.

Drug Treatment

Beginning February 19, 1974, laboratory fish received treated feed at a rate of 2% of their body weight daily, 5 days a week. Nitrofurazone, obtained from Norwich Agricultural Products (a division of Morton-Norwich Products, Inc., Norwich, New York) in the form of Amifur (11% active ingredient, corn meal, soybean oil, and lecithin), was mixed with a commercial minnow feed of 40% protein concentrate. Observations indicated that Amifur and minnow meal were about the same particle size and sank at approximately the same rate; fish did not appear to discriminate between particles in feeding, both at the surface and at the bottom of the tank. Feeding treated feed in the laboratory to treatment groups continued until April 5, 1974, for a total of 35 days. Thereafter all groups were fed standard minnow meal throughout the experimental observation period.

Experimental fish in the ponds were fed mixtures of Amifur in the same minnow meal at levels of 50, 75, and 100 grams active ingredient per 45.4 kilograms feed respectively at a rate of 1% of their body weight per day, 5 days a week. Control fish were fed minnow meal without nitrofurazone under a similar schedule. The pond fish were given Amifur treated feed for 30 days from February 19 to March 30, 1974.

Table 1. Various expressions of dosage levels of nitrofurazone used in efficacy experiments

Drug/feed weight			Wt/Wt Proportion	Drug/Body weight	
50 g/45.4 kg	50 g/100 lb	1.75 oz/100 lb	1102 ppm	22.05 mg/kg	0.035 oz/100 lb
75 g/45.4 kg	75 g/100 lb	2.63 oz/100 lb	1653 ppm	33.07 mg/kg	0.052 oz/100 lb
100 g/45.4 kg	100 g/100 lb	3.50 oz/100 lb	2205 ppm	44.09 mg/kg	0.070 oz/100 lb

Hormone Injection

On February 28, 1974, and March 14, 1974, two tanks of control fish and two tanks of treated fish were injected with HCG in an attempt to stimulate gonadal development, thereby increasing drug uptake and perhaps enhancing the effects of nitrofurazone. The shiners were injected intraperitoneally at a dosage level of 2 international units (IU) per gram of average body weight per tank.

Sampling

Fish were sampled initially on February 14, 1974, to determine whether the P. ovariae infection was apparent at this time. Experimental sampling began after treatment with nitrofurazone ended and continued on a monthly basis through July to analyze the effects of HCG injections given to half of both control and treated groups. Total length (mm), wet(fresh) body weight (g), and wet gonadal weight (g) were obtained from each fish.

Histology

Ovaries were examined in histological sections to determine incidence and intensity of P. ovariae. This method has been demonstrated to give a significantly higher incidence of infection than wet mounts (Summerfelt and Warner 1970a). Moreover, experience with both wet mounts and histological sections indicates that schizonts cannot be recognized in wet mounts, making accurate quantification of incidence and intensity difficult without histological sections.

Ovaries from fish sampled from laboratory groups were fixed in

Heidenhain's Susa. This fixative was selected in a preliminary experiment set up to evaluate the effects of various fixatives on shiner ovaries. The fixatives which had been used previously include Schaudinn's (Summerfelt 1964), 10% formalin (Summerfelt and Warner 1970a), Bouin's (Summerfelt and Warner 1970b), and Kaformacet (Warner 1972). Schaudinn's is commonly used for protozoan smears, but produces excessive shrinkage in tissue sections (Humason 1964). Heidenhain's Susa, however, is specifically recommended for fish tissues (Galigher and Kozloff 1971 and Bucke 1972). In the preliminary experiment, five ovaries were fixed in each of the following fixatives: 10% formalin, Bouin's, Kaformacet, and Heidenhain's Susa. Thereafter all tissues were treated identically (except for special rinses which accompany individual fixatives at staining), moving through the traditional alcohol dehydration and toluene clearing to embedding in Paraplast. Four slides were prepared from each ovary; two were stained with hematoxylin and eosin, two with Mallory's triple stain. Examination of these slides revealed that those tissues fixed in Susa sectioned with less crumbling and maintained their integrity better than those fixed in other fixatives. Staining was also brighter with good differentiation for Susa fixed tissues.

Heidenhain's Susa contains 50 ml mercuric chloride (saturated solution in physiological saline), 2 g trichloracetic acid, 20 ml formalin, 4 ml glacial acetic acid, and 30 ml distilled water (Galigher and Kozloff 1971).

Fish collected from the ponds were immediately placed in 10% formalin. After measurements were recorded in the laboratory, ovaries were transferred to Susa overnight to improve staining.

From this point on, both batches of tissues--laboratory samples and pond samples--were treated identically. After 24 hours fixation, the tissue was washed and transferred to 50% isopropanol for preservation until further processing could be completed. According to standard procedures, the ovaries were dehydrated in isopropanol, cleared in toluene, infiltrated and embedded in Paraplast, sectioned at 6 to 7 microns, stained, then mounted with Permount. Four slides were prepared from each female. Two were stained with alum hematoxylin and eosin; the others, with Mallory's triple stain. Before staining, slides were treated with Lugol's solution in alcohol to remove all traces of mercuric chloride.

Evaluation

Ovaries were examined from prepared slides and evaluated by determining incidence of infection by *P. ovariae*, intensity of infection in each individual ovary, and frequency of oocyte stages in those samples taken to observe effects of HCG injections.

Incidence was measured through microscopic examination of two Mallory's stained slides. In cases in which presence of the parasite was questionable, one or both of the hematoxylin stained slides was also examined. Average incidence per group was calculated by summing the number of infected females and dividing by the total number of females in that group.

Intensity was expressed as the average percentage of the area of four longitudinal ovary sections affected by *Pleistophora ovariae*. The area affected included any eggs containing a developmental stage of the parasite (schizonts, sporonts, and/or spores), or the occurrence

of atretic spore masses. Using a grid overlay on an imaging screen mounted on the photo tube of a microscope, intensity was calculated by dividing the number of squares which were greater than 50% filled with infected oocytes or spore masses by the total number of squares counted. Total magnification of the screen using the 10X stage objective is 50 times the size of the section on the slide. One hundred squares were counted per field. Four fields of each ovary were counted from one of the Mallory stained slides; an average of these four percentages then was the measure of intensity for a particular fish.

To calculate an average intensity for a particular group, the average intensity of all the fish in the group were summed and divided by the total number of fish in the group. Uninfected fish were included in the group average using 0.00% intensity.

Frequency of oocyte stages was determined by classifying and counting all oocytes within one to three randomly selected fields until 100 oocytes were counted. Seven developmental stages were previously used to tabulate oocyte stages (Warner 1972). Stage I and II oocytes were counted together in this analysis. No stage VII eggs were observed in any of the slides examined. Hence, frequency is expressed for stages I-II through VI. The average cross-sectional area in a given slide occupied by all oocytes of a given stage was obtained as the product of frequency for each times their average cross-sectional area. Average percentages of area occupied by each stage was computed for each group.

CHAPTER IV

RESULTS

Laboratory Experiments

Influence of Hormone on Gonadal Development

Prior to the first injection of HCG (28 February), the mean GSI of 10 females was 3.72. One week after the first injection, the range of means of the GSI's of four groups receiving HCG was 4.40 to 5.50% (Table 2). The second hormone injection was given on 14 March, and the following week (21 March), the GSI's in the four groups receiving HCG ranged from 3.91 to 5.24%. The observed increase in GSI after hormone injection relative to the initial value on 14 February cannot be attributed to the gonadotropin. Analysis of variance of the average GSI's from the 7 and 21 March samples, which were the two sampling dates when HCG would most likely show an effect, revealed no significant difference between any of the groups (Table 3).

Mean GSI values for all sampling dates were nearly identical for the HCG injected fish and those which were not injected (Table 2). No overall difference in GSI was observed that would be related to HCG or treatment.

Mean GSI values of all laboratory groups declined abruptly between 16 May and 13 June (Fig. 1); the mean of the four group means on 16 May was 4.8 compared with 2.3 for the mean of the four group means on

Table 2. Mean gonadal somatic index of female golden shiners with and without hormone for fish given nitrofurazone (treated) and controls

	Control groups						Treatment groups					
	with HCG			without HCG			without HCG			with HCG		
Date	1	2	$\bar{x}$	3	4	$\bar{x}$	5	6	$\bar{x}$	7	8	$\bar{x}$
2/14				3.72								
3/ 7	4.77	4.04	4.40	4.71	4.08	4.39	4.41	3.92	4.16	5.50	4.49	5.00
3/21	5.00	3.91	4.45	5.66	4.21	4.93	4.71	5.46	5.08	4.40	5.24	4.82
4/20	4.90	4.69	4.80	5.13	4.64	4.88	3.86	5.03	4.44	5.29	5.14	5.22
5/16	6.17	4.00	5.08	3.86	3.86	3.86	5.10	6.35	5.72	4.87	4.21	4.54
6/13	2.36[a]	2.05	2.20	3.82	2.00	2.91	2.14	1.92	2.03	2.02	2.07	2.04
7/11	1.46[a]	1.91	1.70	1.86	1.85	1.86	2.09[a]	1.77	1.92	1.48	1.51	1.50
Mean	4.19	3.43	3.80	4.17	3.44	3.80	3.75	4.08	3.82	3.93	3.78	3.85

[a]Each sample contained ten females except for those collected 6/13 and 7/11 which contained nine. Each of the group means ($\bar{x}$) contained twenty females except those in which the odd number samples played a part.

Table 3. Computation of analysis of variance of group means of gonadal somatic index of laboratory fish on 7 and 21 March

Source	df	SS	MS	F
7 March sample				
Total	79	111.77		
Among groups	3	7.64	2.55	0.93
Between tanks	4	10.96	2.74	
Within tanks	72	93.17	1.29	
21 March sample				
Total	79	158.00		
Among groups	3	4.34	1.45	0.25
Between tanks	4	22.68	5.67	
Within tanks	72	130.98	1.82	

13 June. In pond populations, a decline of 3 to 4 percentage points is usually interpreted to indicate spawning. The GSI of laboratory groups in this study declined less than 3 percentage points, and spawning was not detected during daily observations. No spawned ova or fry were observed, nor did histological preparations from ovaries collected 7 and 21 March indicate the presence of mature ova (stage VII).

To further evaluate effects of HCG injections upon ovary development, frequency of occurrence of different egg stages was determined from the 7 and 21 March samples (Table 4). This was the percentage of the total number of eggs that a particular egg stage occupied in a microscope field of 100 eggs. For example, if a differential count from a particular ovary slide contained 14 stage II, 48 stage III, 17 stage IV, 2 stage V, and 19 stage VI oocytes, the percentage frequency of occurrence would be equal to the counts.

Compared to the initial 14 February sample (14.47% stage II, 68.53% stage III and 17.00% stage IV), the frequency of occurrence of stage II oocytes decreased in every group of both 7 and 21 March samples. After the first HCG injection, the range of stage II oocytes was 5.90 to 18.40%. After the second injection stage II oocytes ranged from 5.20 to 11.60%. Similarly fewer stage III oocytes were observed in all groups of the 7 March sample (45.70 to 63.40%) and the 21 March sample (47.10 to 57.70%) than in the initial February sample (68.53%). Conversely, an increase in frequency of stage IV oocytes occurred after the initial sample. The 7 March sample included from 23.30 to 45.30% stage IV oocytes; 21 March, from 22.80 to 34.10%. Both March samples had some groups which contained

Figure 1. Change in mean gonadal somatic index of shiners held in the laboratory related to time and mean water temperatures

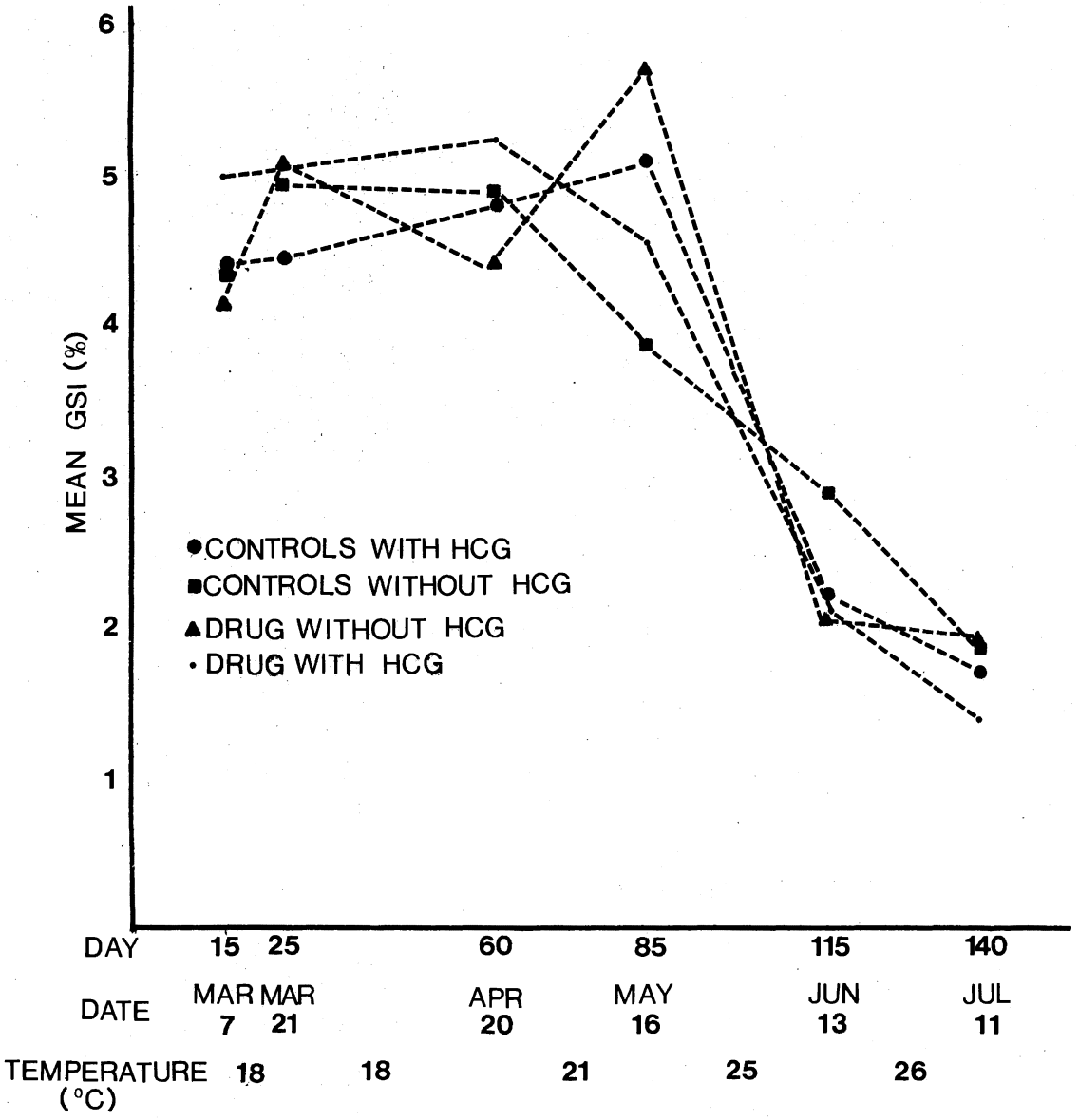

6
5
4
3
2
1
MEAN GSI (%)
●CONTROLS WITH HCG
■CONTROLS WITHOUT HCG
▲DRUG WITHOUT HCG
• DRUG WITH HCG
DAY 15 25 60 85 115 140
DATE MAR 7 MAR 21 APR 20 MAY 16 JUN 13 JUL 11
TEMPERATURE (°C) 18 18 21 25 26

Table 4. Mean frequency of occurrence of individual egg stages

Group name	Tank	7 March sample					21 March sample				
		II	III	IV	V	VI	II	III	IV	V	VI
Control with HCG	1	11.90	46.20	27.10	11.50	2.76	8.60	52.90	27.70	4.90	5.90
	2	12.80	48.70	30.00	8.40	0.10	11.60	57.70	22.80	3.80	4.10
Control without HCG	3	13.40	53.50	27.80	5.20	0.10	7.50	47.10	31.40	7.30	6.70
	4	11.70	58.30	25.40	4.60	0.00	8.20	54.40	31.50	4.30	1.60
Treatment without HCG	5	13.30	58.80	27.70	0.00	0.20	7.40	53.70	28.00	6.70	4.20
	6	13.20	63.40	23.30	0.10	0.00	7.60	48.60	34.10	5.30	4.40
Treatment with HCG	7	5.90	45.70	45.30	2.20	0.90	9.90	54.70	27.40	5.50	2.50
	8	6.50	54.30	38.10	1.10	0.00	5.20	50.60	29.00	6.20	9.00

some stage V and VI oocytes in contrast to the 14 February sample which had none. This decrease of immature egg stages (II and III) and the corresponding increase of more mature egg stages indicates only a small degree of maturation occurred within laboratory-held fish.

Looking more specifically at stages V and VI, the stages which are most demonstrative of maturation, a trend denoting a positive effect of the hormone injections was observed in the 7 March sample. In that sample, both the control and treatment fish which were injected with hormone had higher frequencies of stage V and VI ova than their respective control groups, not injected with HCG. In the 21 March sample, however, the frequencies of stage V and VI oocytes are similar in injected and uninjected groups.

In order to determine whether the injected hormone or environmental conditions produced an increased developmental effect, further analysis was undertaken. The average percent of the total ovary area occupied by each egg stage was computed by multiplying the frequency of occurrence by the average area of that particular egg stage (computed from diameters defined by Warner 1972). For example, if occurrence was 14% stage II, 48% stage III, 17% stage IV, 2% stage V, and 19% stage VI, the average percent total area was computed by summing the products of 14% by 4299 microns, (the average area of one stage II oocyte), 48% by 24,041 microns, 17% by 58,505 microns, 2% by 98,930 microns, and 19% by 214,720 microns and then finding the percent composition of each egg stage from the total area of 64,863 microns. For this example the computed average percentages of the total area are as follows: 0.93% stage II, 17.79% stage III, 15.33% stage IV, 3.05% stage V, and 62.90% stage VI oocytes.

In comparison with the 14 February sample (2.45% stage II, 63.20% stage III, and 34.35% stage IV), a lower percent of immature egg stages (II and III) and higher percent of more mature egg stages (V and VI) occurred in the 7 and 21 March samples (Table 5). In the 7 and 21 March samples, the maximum percentage of stage II oocytes of the total ovary area was 1.92% and 1.42% respectively. In a like manner the maximum percentage of stage III oocytes was 51.85% in the 7 March sample and 39.75% in the 21 March sample. In both March samples, all groups contained less area occupied by stage II and III oocytes than the 14 February sample, and a greater percent of the ovary was occupied with stage IV eggs. Stage IV oocytes for 7 and 21 March ranged from 37.91% to 64.36% and 31.88% to 47.81% respectively in contrast to 34.35% in the February sample. Only in tank 8, the treatment group with HCG, did percentage area of stage IV oocytes not exceed that of the initial sample (34.35%), but high percentages of stage V and VI oocytes were observed, indicating that this group had undergone more maturation than the others.

Area of stage V and VI oocytes repeated the trend observed in the frequency measurements. In the 7 March sample the injected fish in both control and treated groups demonstrated larger areas occupied by these more mature egg stages than their respective controls, indicating that HCG did promote ovary maturation. In the 21 March sample, however, area percentages were similar in injected and uninjected groups. As in the case of the frequency of occurrence parameter, these changes could be attributed to HCG, or to sample date and environmental stimuli, or even to drug treatment.

To determine whether differences in ovary maturation were caused

Table 5. Mean percents of total ovary area for individual egg stages

Group names	Tank	7 March sample					21 March sample				
		II	III	IV	V	VI	II	III	IV	V	VI
Control with HCG	1	1.15	25.63	37.91	22.26	13.05	0.85	31.19	38.97	9.86	19.13
	2	1.52	31.57	46.30	20.08	0.54	1.42	39.75	34.81	7.97	16.05
Control without HCG	3	1.64	37.28	46.20	14.26	0.62	0.72	23.91	38.16	13.95	23.26
	4	1.54	44.37	42.49	11.60	0.00	0.90	34.48	47.81	9.84	6.97
Treatment without HCG	5	1.87	46.09	50.92	0.00	1.12	0.80	32.10	39.14	13.15	14.81
	6	1.92	51.85	45.91	0.33	0.00	0.80	27.93	43.88	10.66	16.73
Treatment with HCG	7	0.66	27.46	64.36	4.48	3.05	1.11	34.13	41.21	12.68	10.87
	8	0.78	35.91	60.19	3.12	0.00	0.42	22.84	31.88	11.19	33.67

by drug, hormone, time period, or any combination of these, an analysis of variance of the developmental egg stages was calculated in terms of the following measurements: frequency of occurrence of stage II, III, IV, V, VI oocytes; a development factor; and an area factor. The development factor was computed by multiplying the number of the egg stage by the frequency of occurrence of that egg stage, then summing the products for all egg stages and dividing by 100. An example of this computation would be calculated as follows: ((2 x 11.90) + (3 x 46.20) + (4 x 27.10) + (5 x 11.50) + (6 x 2.76)) ÷ 100. This process was applied to each of the tanks and tabulated in Table 4, for both samples, yielding 80 development factors. The area factor was similarly computed by first multiplying the number of the egg stage by the mean average percent of total ovary area (Table 5), then summing those products and dividing by 100, for example: ((2 x 1.15) + (3 x 25.63) + (4 x 37.91) + (5 x 22.26) + (6 x 13.05)) ÷ 100.

One to several significant differences were obtained for each of the measurements used to examine ovary development (Table 6). Average frequencies of stage II oocytes differed at the $p < 0.05$ level due to the drug, drug x hormone, period, and hormone x period. As was anticipated, the factor of period or time produced a pattern of significant differences in all of the parameters except frequency of stage III and IV oocytes. The dynamics of the process suggests equal number of oocytes were entering these stages as were leaving via the maturation process, resulting in a stable level of stage III and IV oocytes. Differences between development for the groups given HCG and their controls were non-significant and the observed difference could not be attributed to the hormone alone. The differences observed in

Table 6. Mean squares computed for analysis of variance of frequency of occurrence of five oocyte stages observed in laboratory females following hormone injections

Source	df	Frequency of oocyte stages					Development factor[a]	Area factor[a]
		II	III	IV	V	VI		
Drug	1	174.3[b]	75.6	532.9[b]	327.8[b]	0.3	0.0	1.7
Hormone	1	61.3	455.6	207.0	63.8	47.3	0.4	66.6
Drug x hormone	1	204.7[b]	81.2	774.4[b]	11.6	0.8	0.1	6.9
Tank (drug hormone)	4	20.6	137.6	27.2	20.3	52.8	0.1	29.6
Period	1	322.1[b]	52.9	102.4	74.3[b]	726.8[b]	1.1[b]	340.8[b]
Drug x period	1	16.3	250.0	225.6	551.3[b]	11.6	0.2	33.3
Hormone x period	1	228.0[b]	1638.4[b]	1849.6[b]	170.2[b]	0.2	0.7	73.0
Drug x hormone x period	1	54.1	0.4	297.0	56.4	7.7	0.0	1.2
Period x tank (drug hormone)	4	24.0	166.8	159.2	9.5	51.3	0.1	32.4

[a]Development and area factors are defined in the results section of the text.

[b]The computed F's associated with these mean squares were significant at the 0.05 level.

the individual egg stage frequencies are in any case difficult to evaluate because the direction of development is not indicated. Thus, it is difficult to determine if the oocytes are maturing or if resorbtion of developing ova is occurring, but development and area factors seem to be more indicative of the developmental status of the entire ovary. The only significant difference identified within these parameters was due to period, which apparently is due to an increase in abundance of stage V and VI oocytes in the 21 March sample compared to the 7 March sample (Table 5).

In combination, the results of the analysis of variance conducted upon the measurements of GSI, frequency of oocyte stages and development and area factors indicate that the HCG injections had no long-term or significant effect upon ovary development.

Effects of Nitrofurazone on P. ovariae

Pleistophora ovariae was found in only two of 496 females sampled from laboratory groups (Table 7). The two infected fish in the laboratory populations were observed in the 21 March sample. One of the infected females was in the control group receiving no hormone; the other was in a treatment group receiving HCG and nitrofurazone at the rate of 75 grams active ingredient per 45.4 kilograms feed. Intensity of infection in each of these individuals was 46.50% and 66.25% respectively. Because of the low incidence observed in these laboratory groups, no conclusions can be drawn as to the efficacy of nitrofurazone as a treatment for P. ovariae from this data.

During the course of the experiment, laboratory fish were observed closely to detect possible deleterious effects of the nitrofurazone

Table 7. Incidence (%)[a] of *Pleistophora ovariae* infection in female golden shiners held in the laboratory

	Control groups				Treatment groups[b]			
	with HCG		without HCG		without HCG		with HCG	
Date	1	2	3	4	5	6	7	8
2/14			0.00[c]					
3/ 7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3/21	0.00	0.00	10.00	0.00	0.00	0.00	0.00	10.00
4/20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5/16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6/13	0.00[c]	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7/11	0.00[c]	0.00	0.00	0.00	0.00[c]	0.00	0.00	0.00

[a]Incidence is expressed as an average number of infected females per total number of females in the group.

[b]The dosage in the treatment groups was 75 g/45.4 kg feed.

[c]All samples contained ten females except these footnoted entries. The 14 February sample contained 19 females; the other three each contained nine females.

treatment. Mortality from causes other than sampling (Table 8) was recorded. During the time when nitrofurazone was being fed to the fish, ten fish in the control groups died, and nine fish died in experimental treatment groups. Some of these mortalities probably were due to stress causes by the hormone injections on 28 February and 14 March; 19 fish given HCG died compared to only 8 in the groups not given HCG. Groups not injected with hormone were not given saline injections and therefore were not stressed by handling as the fish that were given hormone. Ten mortalities were observed in the treatment groups (tanks 5 to 8) compared to 17 in the control groups (tanks 1 to 4).

There is some indication that the drug may have retarded oocyte maturation in the 7 March sample. Frequency of occurrence and area percentage of stage V and VI oocytes were substantially less in treated groups (injected with HCG and uninjected) than in control groups, whereas in the 21 March sample the frequencies and area percentages of stage V and VI eggs were similar in control and treatment groups. Thus the effect observed in the 7 March sample was not verified, however, if nitrofurazone retarded or inhibited development, this may contrabalance its possible positive effects for parasite control.

Pond Experiments

Effects of Nitrofurazone on P. ovariae

No *P. ovariae* were observed in any of the 85 fish from the controls and treatment groups in the 29 March pond sample (Table 9).

Table 8. Mortality of laboratory fish from causes other than sampling during the nitrofurazone experiment

		Control groups				Treatment groups			
		with HCG		without HCG		without HCG		with HCG	
Date	Day	1	2	3	4	5	6	7	8
2/19	1								
2/21	3					1	1		
2/25	7						1		
2/28	10	2	3					2	2
3/ 6	16		2						2
3/14	24	1	2						
*									
5/ 9	80	1							
5/16	87							1	
6/13	115			1					
6/24	126			1	1				
6/26	128				1				
7/ 1	133		1						
7/ 3	135			1					
Totals		4	8	2	3	1	2	3	4

*April 1 was the end of feeding with medicated feed in the laboratory aquaria.

Table 9. Incidence of *Pleistophora ovariae* in pond-reared shiners

	Controls	Treatment Levels		
	0 g/45.4 kg	50 g/45.4 kg	75 g/45.4 kg	100 g/45.4 kg
		Pre-spawning		
3/29	0.00 (0/19)	0.00 (0/27)	0.00 (0/18)	0.00 (0/21)
4/26	0.00 (0/ 9)	5.88 (1/17)	13.04 (3/23)	0.00 (0/32)
Subtotals	0.00 (0/28)	2.27 (1/44)	7.32 (3/41)	0.00 (0/53)
		Post-spawning		
5/28	83.33 (25/30)	96.00 (24/25)	82.14 (23/28)	70.83 (17/24)
6/21	93.10 (27/29)	88.00 (22/25)	95.65 (22/23)	72.41 (21/29)
7/19	92.00 (23/25)	93.33 (28/30)	69.23 (9/13)	88.89 (24/27)
Subtotals	89.29 (75/84)	92.50 (74/80)	84.38 (54/64)	77.50 (62/80)
Grand totals	66.96 (75/112)	60.48 (75/124)	54.28 (57/105)	46.62 (62/133)

Four of 81 females sampled had patent infections of P. ovariae on 26 April. All four infected fish were in groups which received nitrofurazone in feed. In subsequent samples all treatment and control groups contained some infected fish. In the controls and in drug treatment groups, the incidence of infection increased substantially between the 26 April and 28 May samples, with levels of infection fluctuating about high levels thereafter. The abrupt, nearly explosive increase in infection as yearling shiners approach their first spawning is a normal part of the parasite's cycle. Patent infections do not appear in young-of-the-year fish; only as ovaries mature for the first spawning does the parasite appear, then reproducing prolifically. The first two samples, therefore, may be misleading when the efficacy of nitrofurazone is evaluated. Fish of this age and state of maturity cannot be expected to display parasitic infection although a hidden latent infection must be presnet in order for the full-blown patent infection to occur later.

Measures of intensity are also affected by spawning. Intensity will appear higher after spawning because uninfected (and some infected) eggs have been expelled from the ovary, leaving a higher ratio of infected eggs in the fish. Considering the marked differences between incidence and intensity of pre-spawning and post-spawning fish, the statistical analysis was done separately for each. The post-spawning or spent fish can ordinarily be designated on the basis of GSI values. With fish infected by P. ovariae, however, a high GSI may also indicate a fish so heavily infected that it will not spawn; the ovary will resorb eggs as they undergo atresia. Histological examination of the ovaries still requires subjective decisions as to which

fish are approaching spawning (for the first or second time in the same season), which are spent, and which will never spawn. Therefore, for evaluation of the effects of nitrofurazone in this experiment, the samples were classed as pre-spawning or post-spawning (spent or resorbing). The sharp decrease in GSI (Fig. 2) indicates that most of the fish had spawned by the 28 May sample. Hence, 29 March and 26 April comprise the pre-spawning class, and 28 May, 21 June, and 19 July make up the post-spawning class. Statistical analysis was applied to both the composites of these two classes for the overall effect of nitrofurazone and to the post-spawning class for a more precise estimate of the drug's effect because unequal numbers of fish in the first two sampling dates when included with the spent fish could mask the true treatment effects.

Pooled totals for the entire experimental period show that incidence of P. ovariae in the 50 gram level treatment group was not significantly different from the controls ($X^2 = 1.31$, $p = 0.25$). Incidence at both the 75 and 100 gram levels, however, was significantly different from that of the controls ($X^2 = 7.24$, $p = 0.007$ and $X^2 = 26.63$, $p < 0.001$ respectively). In both cases the percent of females infected by P. ovariae was less than the control group. A linear relationship of incidence and drug level was obtained ($r = -0.98$, $R^2 = 95.45\%$) (Fig. 3). Although there is no way to determine the parasite's response beyond the observed treatment levels, a linear extrapolation indicates an incidence of zero would occur at a drug level of 343.53 grams active ingredient per 45.4 kilograms of feed. Further experiments are planned for dosage levels of 150, 250, and 350 grams per 45.4 kilograms feed to test this extrapolation.

Figure 2. Change in mean gonadal somatic index of pond-reared shiners, divided by treatment groups, related to time and mean water temperatures

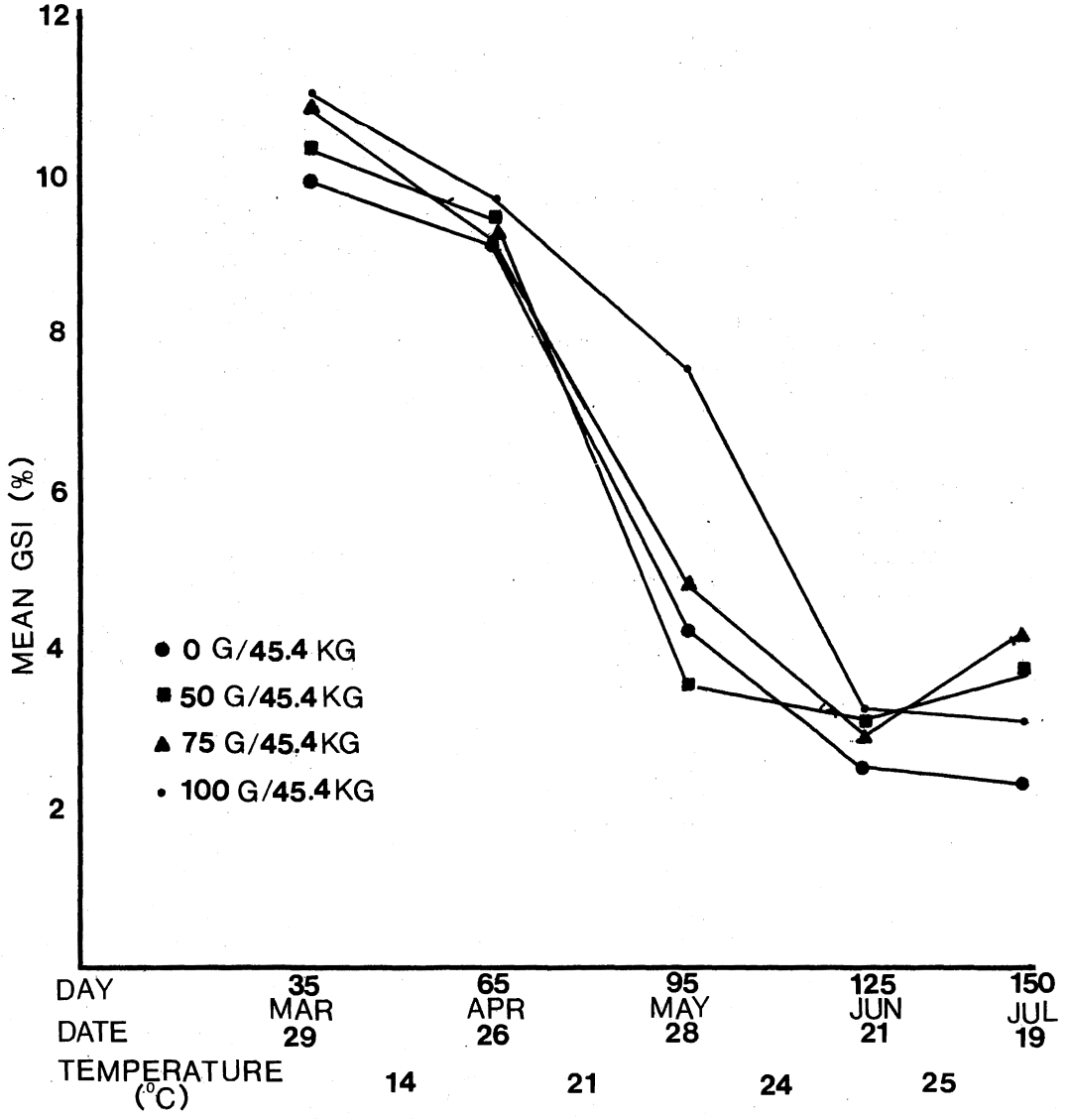
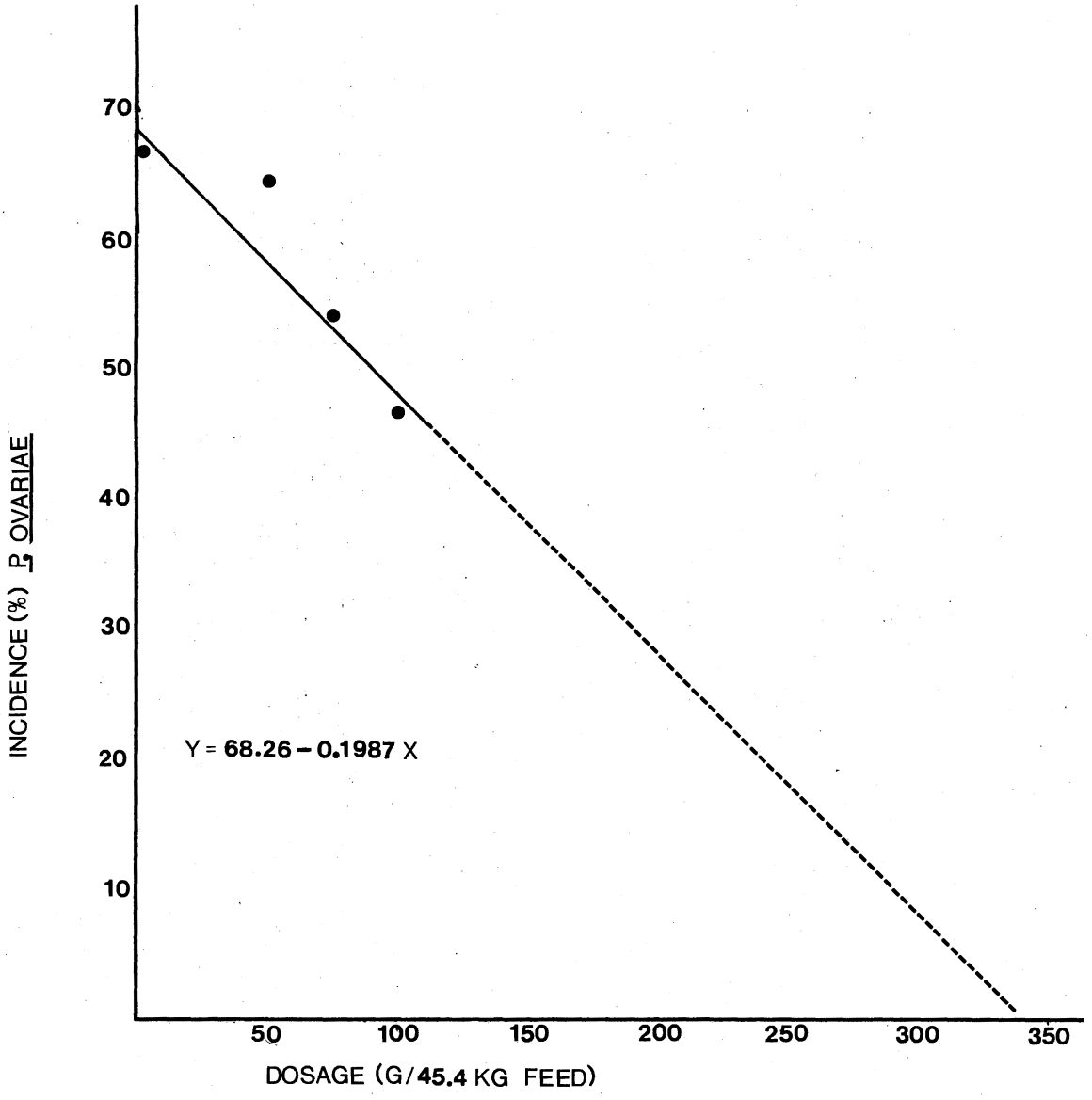

12
10
8
6
4
2
MEAN GSI (%)
● 0 G/45.4 KG
■ 50 G/45.4 KG
▲ 75 G/45.4 KG
• 100 G/45.4 KG
DAY
DATE
TEMPERATURE (°C)
35
MAR
29
65
APR
26
95
MAY
28
125
JUN
21
150
JUL
19
14
21
24
25

Figure 3. Relationship of incidence of P. ovariae to nitrofurazone dosage


70
60
50
40
30
20
10
INCIDENCE (%) P. OVARIAE
Y = 68.26 – 0.1987 X
50
100
150
200
250
300
350
DOSAGE (G/45.4 KG FEED)

Analysis of the post-spawning class of fish revealed similar results. Incidence at the 50 and 75 gram treatment levels was not significantly different from that of the controls (X^2 = 1.13, p = 0.29 and X^2 = 1.45, p = 0.23 respectively). The 100 gram treatment level still shows a significant reduction in incidence from the controls (X^2 = 10.14, p = 0.001). The relationship of incidence and drug level was insignificant (r = 0.75, R^2 = 56.39%).

Average levels of intensity pooled over all five samples showed a general decreasing trend as nitrofurazone dosage was increased. Mean intensity of the controls was 50.01%; for the 50 gram group, 42.79%; for the 75 gram group, 44.38%; for the 100 gram group, 33.69% (Table 10). Intensity showed the same pattern as incidence over time with a large increase in infection between 26 April and 28 May samples and with fluctuating patterns after the 28 May sample. In the composite data, no significant difference from the controls was observed except at the 100 gram treatment level (t = 3.16, p = 0.002). The average intensity of the 100 gram group was much less than that of the control due primarily to the large number of uninfected fish in the 29 March and 26 April samples. Re-examining data for the post-spawning fish, the intensity level in the treatment groups did not differ significantly from that of the controls though the 100 gram group came very close to the p = 0.05 significance level (t = 1.94, p = 0.054). Intensity thus followed the scheme of incidence--both showed a tendency of decreased infection at the 100 gram treatment level.

Intensity measurements were also evaluated according to their frequency of occurrence in the following intensity classes; 0-10%, 10-50%, 50-90%, and 90-100%. The classes were defined as they appear

Table 10. Average intensity of Pleistophora ovariae in pond-reared shiners

Date	Controls 0 g/45.4 kg	Treatment groups 50 g/45.4 kg	75 g/45.4 kg	100 g/45.4kg
		Pre-spawning		
3/29	0.00	0.00	0.00	0.00
4/26	0.00	0.82	10.67	0.00
Subtotal	0.00	0.32	5.99	0.00
		Post-spawning		
5/28	72.28	83.66	76.65	65.75
6/21	74.32	70.22	73.95	55.27
7/19	51.10	48.19	43.05	46.15
Subtotal	66.68	66.15	68.97	56.01
Grand total	50.01	42.79	44.38	33.69

to avoid classes with zero frequencies. Intensity patterns were analyzed using the composite and post-spawning classes. Significant differences between every treatment group and the controls were present in both strata. For the composite data, the 50, 75, and 100 gram groups were all significantly different from the controls ($X^2 = 8.23$, $p = 0.04$; $X^2 = 11.47$, $p = 0.01$; $X^2 = 24.77$, $p < 0.001$ respectively). Also in the post-spawning data class, the 50, 75, and 100 gram treatment groups were all significantly different from the controls ($X^2 = 8.57$, $p = 0.036$; $X^2 = 8.05$, $p = 0.045$; and $X^2 = 9.61$, $p = 0.022$).[2] These differences reflect the occurrence of lower frequencies of high intensities and higher frequencies of low intensities, observed in treatment groups in comparison to the control group.

The analyses of incidence and intensity of P. ovariae together indicate nitrofurazone was somewhat efficacious. A negative effect of the drug upon the minnow was also noted in examination of the relationship between dosage and GSI, a measure of fecundity or the reproductive potential (Fig. 2). Ovary development, as reflected by mean GSI's for each group follows the normal pattern for fish maintained in ponds for the controls, and the 50 and 75 gram treatment groups, with a sharp decrease in GSI between 26 April and 28 May when spawning occurred. The GSI of the 100 gram group, however, decreased in two stages between the 26 April and 28 May samples and between the 28 May and 21 June samples. However, the GSI values of the 100 gram treatment group (Table 11) were noticeably different only in the 28 May sample. An AOV confirms that there is a significant

---

[2] Computation of respective X^2 values appears in the Appendix.

Table 11. Average gonadal somatic index of female golden shiners held in ponds

	Controls	Treatment groups[a]		
Date	0 g	50 g	75 g	100 g
3/29	9.99	10.34	10.84	11.10
4/26	9.15	9.43	9.16	9.70
5/28	4.24	3.61	4.94	7.26
6/21	2.48	3.08	2.87	3.01
7/19	2.30	3.76	4.08	3.07

difference ($f = 5.90$, $p < 0.05$) between groups for that sample. Histological examination shows that on 28 May, the high mean GSI of the 100 gram treatment group was a result of two factors. First, a large number of stage VI oocytes in the ovaries of some fish indicates that they had not yet spawned. If nitrofurazone delayed oogenesis, then the possibility exists that the prevalence of immature ova in an ovary prevented maturation of the parasite and would have effected the incidence and intensity of infection. Second, some of the females in this 100 gram treatment group with high GSI's (one as large as 15.54%) were massively infected with P. ovariae, to an extent that spawning would never occur; if these ova were ovulated, they would not be viable.

Production data indicate that high drug levels reduced fecundity. Net production when ponds were drained in the fall of 1974 was 5.57 pounds for the control group, 12.44 pounds for the 50 gram group, 11.76 pounds for the 75 gram group, and 2.00 pounds for the 100 gram troup. Because these ponds were not fed or observed on a regular basis, production is considerably lower than would be expected in commercial culture, and other factors may account for the lower production in the 100 gram treatment group. However, the negative effect of nitrofurazone indicated by both the GSI's and production data necessitate careful balancing the potentially negative effects with the positive effect of reduced P. ovariae infection. Erradicating the parasite will produce no benefit for minnow farmers if the fecundity of their brood stock is impaired by the drug.

Additional Observations

Occurrence of Myxobolus argenteus

On 14 February, the initial experimental sample, a myxosporidian infection was noted in four of 36 fish. The parasite was subsequently identified as Myxobolus argenteus, first described by Lewis (1968) from minnow hatcheries at Gorham, Illinois, and Paragould, Arkansas. The M. argenteus infection persisted at a low level among laboratory fish through the 21 April sample. Throughout this time period, 14 of 1082 fish (males and females combined) were infected by M. argenteus. The average number of cysts per infected fish (intensity) was 2.07. Disregarding treatment and hormone variations, males seemed to be more heavily affected than females. Incidence among males was 1.37% and the average intensity was 2.62 cysts per infected fish. Because of the low numbers of infected fish (Table 12), no statistical analysis was attempted to discern effects of gonadotropin or nitrofurazone on incidence or intensity of this parasite.

Myxobolus argenteus was also observed in the 29 March and 26 April pond samples. A total of 17 out of 921 pond fish were infected with an average of 3.41 cysts per infected fish. Disregarding treatment groups, males were again seen to be more infected than females. Incidence among males was 3.18% compared to 0.62% for females. Average number of cysts per fish was 3.93 for males and 1.00 for females. Incidence and intensity were also tabulated according to treatment groups (Table 13), but no statistical analysis was attempted because of the low incidence of infection.

Table 12. Incidence and intensity of Myxobolus argenteus in shiners held in the laboratory

	Control groups		Treatment groups	
	Incidence	Intensity	Incidence	Intensity
Without HCG				
Males	4/192	16/4 = 4.00	1/117	1/1 = 1.00
Females	4/139	6/4 = 1.50	1/119	4/1 = 4.00
With HCG				
Males	3/130	4/3 = 1.33	0/143	0/0 = 0.00
Females	0/118	0/0 = 0.00	1/120	1/1 = 1.00
Males				
Without HCG	4/192	16/4 = 4.00	1/117	1/1 = 1.00
With HCG	3/130	4/3 = 1.33	0/143	0/0 = 0.00
Females				
Without HCG	4/139	6/4 = 1.50	1/119	4/1 = 4.00
With HCG	0/118	0/0 = 0.00	1/120	1/1 = 1.00

Table 13. Incidence and intensity of *Myxobolus argenteus* in shiners held in ponds

	Male	Female
Controls		
Incidence	5/108	1/119
Intensity	11/5 = 2.20	1/1 = 1.00
Treatments		
50 g/45.4 kg		
Incidence	3/106	0/124
Intensity	6/3 = 2.00	0/0 = 0.00
75 g/45.4 kg		
Incidence	3/132	1/105
Intensity	19/3 = 6.33	1/1 = 1.00
100 g/45.4 kg		
Incidence	3/94	1/133
Intensity	9/3 = 3.00	1/1 = 1.00

Occurrence of Lernaea cyprinaceae

The 28 May, 21 June, and 19 July pond samples contained a fairly heavy infection of Lernaea cyprinaceae. Incidence and intensity (number of Lernaea per infected fish) were pooled over these three sample dates (Table 14). In this composite, 24.59% of the total 846 fish harbored at least one L. cyprinaceae adult. The number of this copepod parasite per infected fish ranged from one to fourteen, median being one or two parasites. L. cyprinaceae infection did not occur until after administration of nitrofurazone had ceased; obviously it was not affected by the nitrofurazone treatment. A statistical analysis was undertaken, pooled across treatment groups, to see if difference in infection levels existed between males and females, and to see if there was a relationship between Lernaea infection and infection with P. ovariae. Incidence of L. cyprinaceae in males and females was not found to be significantly different (X^2 = 0.96, p = 0.32). Intensity, however, was significantly different (t = 2.45, p = 0.015) with males carrying a higher average number of parasites per infected fish than females.

Correlation coefficients were computed for relationships between 1) incidence of Pleistophora to incidence of Lernaea, 2) average intensity of Pleistophora to average intensity of Lernaea, 3) incidence of Pleistophora to intensity of Lernaea, 4) incidence of Pleistophora to number of Lernaea per fish and 5) average intensity of Pleistophora to number of Lernaea per fish. None of the computed correlation coefficients were significant ($p < 0.05$). Thus, the occurrence and intensity of infections of golden shiners with Lernaea cyprinaceae

Table 14. Incidence and intensity of *Lernaea cyprinaceae* in shiners held in ponds

	Male	Female
Incidence	96/372 = 25.81	112/474 = 23.63
Intensity	210/96 = 2.01	185/112 = 1.69

would not have any apparent effect in the nitrofurazone treatment to control Pleistophora ovariae in golden shiners.

CHAPTER V

DISCUSSION

Efficacy of Nitrofurazone

Because of the low incidence of P. ovariae in shiners maintained in the laboratory, the efficacy of nitrofurazone for control of P. ovariae could not be measured. Only 0.40% of all females sampled from the laboratory were infected with P. ovariae, whereas 56.75% of all females collected from the ponds had patent infections. All of these fish originally came from the same infected source (code 05, Summerfelt and Warner 1970a), and were overwintered in ponds constructed in 1972 which had never before held golden shiners. Thus both the laboratory and pond groups had the same potential for becoming infected. If no laboratory shiners had displayed infections of P. ovariae, one would be led to believe that the supply of shiners was originally uninfected, and that those fish maintained in the ponds contracted the parasite from the environment. Two laboratory fish, however, did have P. ovariae infections, indicating that all shiners in the laboratory had the potential for developing P. ovariae infections without further exposure.

Examination of environmental conditions maintained in the laboratory offers some explanation to why P. ovariae failed to develop more extensively among the laboratory population. Warner (1972) did not observe P. ovariae infection until the fish became sexually mature,

even when they were separated from infected parents at an early age to prevent transmission of the parasite. The parasitic life cycle is correlated to ovary maturation so that spores of P. ovariae are ready to be released to the environment at the time the host spawns. Anything, therefore, that would disrupt ovary maturation could be expected as well to affect the parasite's life cycle.

Deviation in average GSI's for pond and laboratory females indicates that the ovaries of laboratory fish did not mature. In pond groups, the weighted average GSI by samples was highest (9.80) at the 26 April sample (Fig. 2), but for the laboratory fish, the highest mean GSI was 5.72. The laboratory group treated with HCG followed the pattern of pond fish, peaking at the 20 April sample, although its high value was considerably lower than that in the pond groups (Fig. 1). An abrupt decrease occurred in GSI of laboratory populations before May and June samples. Sharp decline in GSI would ordinarily signal spawning, but no spawned ova were observed and the decline in GSI was less than the 3 to 5% usually observed in pond fish (Warner 1972, and Summerfelt 1964). The water temperature (25°C) at the time when GSI declined sharply was within the range of golden shiner spawning, 21 to 27°C, but laboratory fish had not been subjected to the same kind of temperature rhythm as were pond fish. Pond water temperatures from February to June ranged from 6 to 25°C in contrast to a range of 16 to 26°C in the laboratory during the same time interval. At all times the laboratory temperatures exceeded those at the ponds, although near the end of the experiment the gap was being narrowed. The laboratory fish commenced but did not complete the maturation process and it seems, conditions in the laboratory were too

monotonous, lacking in appropriate environmental stimuli.

Photoperiod also affects gonadal development. No measure of photoperiod was made in the field, but in the laboratory a constant photoperiod of 12 hours per day was maintained to simulate the natural day length at the time of spawning. Under typical conditions shiners would spawn during a lengthening photoperiod. The ovarian cycle of laboratory fish was affected by their environment, as shown by differences in average GSI values, and therefore, the parasite did not complete development.

Whatever the reason, the fish held in the laboratory failed to develop adequate infection by P. ovariae to allow any measure of the efficacy of nitrofurazone. The pond groups, however, all developed ample infections and, with the consideration of changes in incidence and intensity due to spawning, provide a quantitative appraisal of nitrofurazone's efficacy for treatment of P. ovariae in golden shiners.

In the ponds, treatment at the 50 gram level was not sufficient to provide a significant difference in infection levels from those of the controls at either the cummulative or post-spawning data. The 75 gram level did significantly reduce incidence in the cummulative data; but looking also at the post-spawning data, this significant difference was seen to be a function of the unequal numbers of zeros in the pre-spawning group. A significant reduction in incidence was observed in the 100 gram group by analysis of both strata. Intensity of infection did not significantly differ from the control group for any of the treatment groups except the 100 gram treatment group in the cummulative data analysis. These results indicate nitrofurazone was somewhat efficacious in controlling and corroborate observations made by Meyer.

Meyer (personal communication, 1973, Chief, Fish Control Laboratory, La Crosse, Wisconsin) obtained decreased infection rates of P. ovariae when infected golden shiners were fed a nitrofurazone feed mix at rates of 62.5 and 125 grams active ingredient per 45.4 kilograms of feed. Japanese observations (Putz and McLaughlin 1970) also indicate that nitrofurazone increased host survival rates by acting negatively against another Pleistophora species.

Thus nitrofurazone shows some potential for reduction of P. ovariae infection levels in golden shiners. However, some contraindications of the drug have also been observed. Spawning was delayed and production reduced in the 100 gram treatment group in the field; the maturation of ova to stage V and VI was delayed in the 75 gram treatment group in the laboratory as illustrated by egg counts of the 7 and 21 March samples. Before nitrofurazone is incorporated routinely into minnow feed, additional research is needed to determine if a larger dosage of nitrofurazone can better control the parasite at a reasonable cost and to better define the drug's effect upon fecundity. Care should be taken also to avoid indiscriminate treatment with nitrofurazone. Another protozoan, Eimeria tenella, which can be controlled with nitrofurazone, has shown resistance to the drug when host chicks were fed suboptimal levels of nitrofurazone for five parasite passages. As tolerance developed, the growth rate of the chicks decreased, and pathological changes became more evident (Gardiner and McLoughlin 1963).

Effects of Hormone Injection

In contrast to the response usually observed with ripe fish taken from ponds or natural environments, injection of human chorionic gonadotropin (HCG) produced no change in ovary maturation in golden shiners under laboratory conditions. Although non-injected groups contained less stage V and VI eggs in the 7 March sample for both control and nitrofurazone treated groups, there was no significant difference between the frequency of each oocyte stage or the areas occupied by them due to hormone injection. The GSI was similar among all groups. Ovaries began to mature but never completed development. No maturation could be attributed to HCG alone. Since the shiners were not ripe when injected, they could not be expected to spawn from this application of HCG. The hormone injections may have increased drug absorption; however, the lack of patent infections of *P. ovariae* among laboratory fish does not allow evaluation of this possibility.

CHAPTER VI

SUMMARY

1. Injection of golden shiners with human chorionic gonadotropin at a rate of 2 IU per gram body weight twice at an interval of two weeks produced no significant changes in ovary maturation (as measured by mean GSI, frequency of occurrence, and percent area occupied by individual egg stages) under laboratory conditions of constant photoperiod (12 hrs/day) and an average water temperature of 18°C.
2. Analysis of post-spawning fish indicated a significant reduction of incidence of P. ovariae due to nitrofurazone, but only at the 100 level. The intensity of infection was not significantly reduced from that of the control at any treatment level. Using pooled data for the entire sampling period, 75 and 100 gram treatments significantly reduced incidence in comparison to the controls, and intensity was significantly reduced at only the 100 gram level. Although these data are believed to be biased due to latency of infection in the first two samples, they do show a general overall effect of nitrofurazone to reduce incidence as dosage is increased. In order to test this hypothesis and to further examine possible detrimental effects of the drug to the fish implied by delayed spawning and reduced net production in the 100 gram treatment group, more experiments are planned for 150, 250, and 350 gram treatment groups to evaluate feeding nitrofurazone for control of P. ovariae.

SELECTED BIBLIOGRAPHY

Bailey, W. M., F. P. Meyer, J. M. Martin, and D. L. Gray. 1974. Farm fish production in Arkansas during 1972. Proc. Southeast. Assoc. Game Fish Comm. 27 (1973): 750-755.

Braekevelt, C. R. and D. B. McMillan. 1967. Cyclic changes in the ovary of the brook stickleback Eucalia inconstans (Kirtland). J. Morphol. 123(4): 373-395.

Bucke, D. 1972. Some histological techniques applicable to fish tissues. Pages 153-189 in L. E. Mawdesley-Thomas, ed. Diseases of fish. Symp. Zool. Soc. London 30.

Burrows, W., J. W. Moulder, R. M. Lewert, and J. W. Rippen. 1968. Textbook of microbiology. W. B. Saunders Co., Philadelphia. 974 pp.

Daykin, P. W. 1960. Veterinary applied pharmacology and therapeutics. Bailliere, Tindall, and Cox, London. 767 pp.

Eble, T. E., and F. R. Hanson. 1951. Fumagillin, an antibiotic from Aspergillus fumigatus H-3. Antibiotics and chemotherapy 1: 54-58.

Furgala, B., and R. Boch. 1970. The effect of fumidil B, Nosemack, and Humatin on Nosema apis. J. Apic. Res. 9(2): 79-85.

Galigher, A. E., and E. N. Kozloff. 1971. Essentials of microtechnique. Lea and Febiger, Philadelphia. 531 pp.

Gardiner, J. L., and D. K. McLoughlin. 1963. Drug resistance in Eimeria tenella. IV. The experimental development of a nitrofurazone resistant strain. J. Parasitol. 49(6): 947-950.

Ghittino, P. 1972. The principal aspects of bacterial fish diseases in Italy. Pages 25-83 in L. E. Mawdesley-Thomas, ed. Diseases of fish. Symp. Zool. Soc. London 30.

Gochnauer, T. A., and B. Furgala. 1969. Chemotherapy of nosema disease: compatibility of fumagillin with other chemicals. Am. Bee J. 109(8): 309-311.

Goetze, G., and B. Zeutzschel. 1959. Nosema disease of honeybees and its control with drugs: review of research work since 1954. Bee World 40(9): 217-225.

Hanson, F. R., and E. J. Eble. 1949. An antiphage agent isolated from Aspergillus sp. J. Bact. 58: 527-529.

Hawking, F. 1963a. Chemotherapy of trypanosomias. Pages 131-142 in R. J. Schnitzer and F. Hawking, eds. Experimental chemotherapy I. Academic Press, New York.

_____. 1963b. Chemotherapy of miscellaneous infections. Pages 691-699 in R. J. Schnitzer and F. Hawking, eds. Experimental chemotherapy I. Academic Press, New York.

Heaton, L. H. 1968. Tissue residue and oral safety of furazolidone in four species of trout. Prog. Fish-Cult. 30(4): 208-215.

Herman, R. L. 1968. Fish furunculosis 1952-1966. Trans. Am. Fish. Soc. 97(3): 221-230.

Hoffman, G. L. 1967. Parasites of North American fishes. U. of California Press, Berkeley. 486 pp.

Humason, G. 1964. Animal tissue techniques. W. H. Freeman and Co., San Francisco. 468 pp.

Jamieson, C.A. 1955. Nosema disease and its control with fumagillin. Am. Bee J. 95:94.

Katznelson, H., and C. A. Jamieson. 1952. Control of nosema disease of honeybees with fumagillin. Science 115: 70-71.

Killough, J. H., G. B. McGill, and R. C. Smith. 1952. The treatment of amebiasis with fumagillin. Science 115: 71-72.

Leteux, F. J. 1974. Personal communication. Ozark Fisheries, Stoutland, Missouri.

Lewis, L. C., and R. E. Lynch. 1970. Treatment of Ortrinia nusitalis larvae with fumidil-B to control infection caused by Perezia pyraustae. J. Invert. Pathol. 15: 43-48.

Lewis, S. D. 1968. Myxobolus argenteus sp. n. (Protozoa: Myxosporida), a parasite of the golden shiner. J. Parasitol. 54(5): 1034-1037.

Lewis, W. M., and R. C. Summerfelt. 1964. A myxosporidian, Myxobolus notemigoni sp. n., parasite of the golden shiner. J. Parasitol. 50(3): 386-389.

Malone, J. M. 1970. Golden shiners and disease. Am. Fish. Farmer 1(2): 17.

Marler, E. E. J. 1967. Pharmacological and chemical synonyms. Exerpta Medica Foundation, New York. 349 pp.

McCowen, M. C., M. E. Callender, and J. F. Lawlis, Jr. 1951. Fumagillin (H-3), a new antibiotic with amebicidal properties. Science 113: 202.

Meyer, F. P. 1967. The impact of disease on fish farming. Am. Fish U. S. Trout News 11(6): 6, 18, 19.

_____. 1973. Personal communication. Chief, Fish Control Laboratory, La Crosse, Wisconsin.

Paul, H. D., and M. F. Paul. 1964. Chemotherapeutic properties.

Pages 307-370 in R. J. Schnitzer and F. Hawking, eds. Experimental chemotherapy II. Academic Press, New York.

Pittinger, C., and R. Adamson. 1972. Antibiotic blockade of neuromuscular function. Pages 169-184 in H. W. Elliot, ed. Annual Review of Pharmacology 12.

Post, G. 1959. A preliminary report on the use of nitrofuran compounds for furunculosis of trout with special emphasis on Furoxone. Prog. Fish-Cult. 21(1): 30-33.

_____. 1962. Furazolidone (nF-180) for control of furunculosis in trout. Prog. Fish-Cult. 24(4): 182-184.

Post, G., and R. E. Keiss. 1962. Further laboratory studies on the use of furazolidone for the control of furunculosis of trout. Prog. Fish-Cult. 24(1): 16-21.

Putz, R. E., and J. J. A. McLaughlin. 1970. Biology of Nosematidae (Microsporida) from freshwater and euryhaline fishes. Pages 124-132 in S. F. Snieszko, ed. A symposium on diseases of fishes and shellfishes. Am. Fish. Soc. Spec. Publ. No. 5.

Seaman, E. A., V. S. Norton, and T. E. DeVaney. 1969. Registration and clearance of chmicals for fish culture and fishery management. A symposium arranged by the Bureau of Sport Fisheries and Wildlife for the 99th Annual Meeting of the American Fisheries Society. New Orleans, Louisiana. September 12, 1969.

Sollman, T. 1957. A manual of pharmacology and its applications to therapeutics and toxicology. W. B. Saunders Co., Philadelphia. 1535 pp.

Sneed, R. E., and H. P. Clemens. 1959. The uses of human chorionic

gonadotropin to spawn warmwater fishes. Prog. Fish-Cult. 21(3): 117-120.

Snieszko, S. F. 1954. Therapy of bacterial fish diseases. Trans. Am. Fish. Soc. 83: 313-330.

Spangenberg, D. B., and D. L. Claybrook. 1961. Infection of hydra by microsporidia. J. Protozool. 8(2): 151-152.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics with special reference to the biological sciences. McGraw-Hill Book Co., Inc., New York. 481 pp.

Stephenson, H. C., and J. G. Mittelstaedt. 1965. Veterinary drug encyclopedia and therapeutic index. The Reuben H. Donnelley Corp., New York. 236 pp.

Stevens, R. E. 1966. Hormone-induced spawning of striped bass for reservoir stocking. Prog. Fish-Cult. 28(1): 19-28.

Summerfelt, R. C. 1964a. A new microsporidian parasite from the golden shiner, Notemigonus crysoleucas. Trans. Am. Fish. Soc. 93(1): 6-10.

_____. 1964b. Blood proteins, their pattern, variations, and functions in the golden shiner, Notemigonus crysoleucas, Cyprinidae. PhD Thesis, Southern Illinois University, Carbondale, Illinois. 155 pp. (Unpublished)

Summerfelt, R. C., and M. C. Warner. 1970a. Incidence and intensity of Pleistophora ovariae, a microsporidian parasite of the golden shiner, Notemigonus crysoleucas. Pages 142-160 in S. F. Snieszko, ed. A symposium on diseases of fishes and shellfishes. Am. Fish. Soc. Publ. No. 5.

_____. 1970b. Geographical distribution

and host-parasite relationships of Pleistophora ovariae in Notemigonus crysoleucas. J. Wildl. Diseases 6: 457-465.

Tafanelli, R. 1972. Effects of intraperitoneal injections of cadmium chloride on gonads, liver, and kidney of goldfish, Carassius auratus (Linnaeus). PhD Thesis, Oklahoma State University, Stillwater, Oklahoma. (Unpublished)

Taylor, R. E. L., J. C. Sebastion, and D. R. Junell. 1973. Attempts to control whirling disease by continuous drug feeding. J. Wildl. Diseases 9: 302-305.

Tonguthai, K. 1972. Experiments to control Microsporidea and Myxosporidea in fish using fumidil-B. M.S. Thesis, Auburn University, Auburn, Alabama. (Unpublished)

Warner, M. C. 1969. The efficacy of furazolidone on furunculosis in rainbow trout (Salmo gairnderi) at 17°C. M.S. Thesis, Utah State University, Logan, Utah. (Unpublished)

_____. 1972. The seasonal cycle of oogenesis and parasitism in golden shiners, Notemigonus crysoleucas, infected with the microsporidian Pleistophora ovariae. PhD Thesis, Oklahoma State University, Stillwater, Oklahoma. (Unpublished)

Woolfe, G. 1963. Chemotherapy of amebiasis. Pages 355-444 in R. J. Schnitzer and F. Hawking, eds. Experimental chemotherapy I. Academic Press, New York.

APPENDIX

Table 15. Average total length (mm) of female golden shiners held in the laboratory

	Control groups				Treatment groups			
	with HCG		without HCG		without HCG		with HCG	
Date	1	2	3	4	5	6	7	8
2/14			81.05					
3/ 7	80.20	70.50	75.40	81.30	80.70	69.30	79.80	80.40
3/21	71.10	68.10	76.70	81.00	74.60	81.10	79.20	81.70
4/20	70.40	75.70	66.40	83.90	76.60	75.50	72.00	84.40
5/16	77.30	64.50	67.00	84.40	77.00	87.80	86.30	84.70
6/13	75.78	75.50	74.30	85.10	82.80	84.20	80.90	90.10
7/11	72.33	77.30	72.70	84.70	76.00	80.80	82.30	85.70

Table 16. Average body weight (g) of female golden shiners held in the laboratory

	Control groups				Treatment groups			
	with HCG		without HCG		without HCG		with HCG	
Date	1	2	3	4	5	6	7	8
2/14			4.29					
3/ 7	4.17	2.90	3.61	4.22	4.23	2.60	4.19	4.15
3/21	2.87	2.64	3.57	4.31	3.62	4.22	3.75	4.29
4/20	2.90	3.43	2.48	4.20	3.76	3.70	2.99	5.06
5/16	3.95	2.34	2.71	4.99	3.77	5.43	5.27	5.17
6/13	3.48	3.68	3.51	4.93	4.52	4.71	4.25	5.92
7/11	2.94	3.82	3.03	4.86	3.61	4.04	4.29	5.00

Table 17. Average gonadal weight (g) of female golden shiners held in the laboratory

	Control groups				Treatment groups			
	with HCG		without HCG		without HCG		with HCG	
Date	1	2	3	4	5	6	7	8
2/14			0.17					
3/ 7	0.21	0.13	0.17	0.17	0.20	0.10	0.23	0.20
3/21	0.15	0.11	0.20	0.19	0.20	0.24	0.16	0.23
4/20	0.15	0.18	0.13	0.20	0.15	0.19	0.16	0.26
5/16	0.27	0.09	0.12	0.20	0.24	0.35	0.28	0.24
6/13	0.08	0.08	0.12	0.10	0.09	0.09	0.09	0.13
7/11	0.04	0.08	0.05	0.09	0.07	0.07	0.06	0.08

Table 18. Average total length (mm) of female golden shiners held in ponds

	Controls	Treatment groups[a]		
Date	0 g	50 g	75 g	100 g
3/29	72.35	71.04	71.28	74.86
4/26	82.33	71.12	80.43	70.22
5/28	83.13	77.60	83.54	79.67
6/21	91.24	80.00	87.26	88.66
7/19	86.92	90.50	80.85	84.41

[a]The treatment groups are defined by dosage of nitrofurazone in grams per 45.4 kg feed.

Table 19. Average body weight (g) of female golden shiners held in ponds

	Controls	Treatment groups[a]		
Date	0 g	50 g	75 g	100 g
3/29	4.49	7.60	4.71	5.58
4/26	6.65	4.27	2.53	4.02
5/28	5.47	4.45	5.64	5.11
6/21	7.16	4.65	5.61	6.41
7/19	5.75	7.82	4.61	5.77

[a]The treatment groups are defined by dosage of nitrofurazone in grams/ 45.4 kg feed.

Table 20. Average gonadal weight (g) of female golden shiners held in ponds

Date	Controls	Treatment groups[a]		
	0 g	50 g	75 g	100 g
3/29	0.53	0.59	9.55	0.63
4/26	0.61	0.41	0.57	0.39
5/28	0.26	0.16	0.31	0.41
6/21	0.18	0.16	0.16	0.20
7/19	0.13	0.30	0.19	0.18

[a]The treatment groups are defined by dosage of nitrofurazone in grams/45.4 kg feed.

Table 21. Computation of X^2 of incidence of *P. ovariae* in pond-reared shiners (composite data)

Dosage	Infected	Non-infected	Total
50 g/45.4 kg	75	49	124
75 g/45.4 kg	57	48	105
100 g/45.4 kg	62	71	133
Control	75	37	112
Totals	270	204	474

$X^2 = 11.31$ ($p = 0.01$)

Table 22. Computation of X^2 of incidence of *P. ovariae* in pond-reared shiners (post-spawning data)

Dosage	Infected	Non-infected	Total
50 g/45.4 kg	74	6	80
75 g 45.4 kg	54	10	64
100 g/45.4 kg	62	18	80
Controls	75	9	84
Totals	265	43	308

Table 23. Computation of X^2 of incidence of P. ovariae in controls compared to individual treatment groups[a] (composite data)

	O	E	O-E	$(O-E)^2$	$(O-E)^2/E$
		Treatment = 50 g/45.4 kg			
Infected	77	83	-6	36	0.43
Non-infected	47	41	6	36	0.88
Totals	124	124	0		$X^2 = 1.31$
					(p = 0.25)
		Treatment = 75 g/45.4 kg			
Infected	57	70	-13	169	2.41
Non-infected	48	35	13	169	4.83
Totals	105	105	0		$X^2 = 7.24$
					(p = 0.007)
		Treatment = 100 g/45.4 kg			
Infected	62	89	-27	729	8.19
Non-infected	71	44	27	729	11.57
Totals	133	133	0		$X^2 = 24.76$
					(p = 0.000046)

[a]Observed corresponds to treatment groups; the expected, to the proportion observed in the control groups.

Table 24. Computation of X^2 of incidence of *P. ovariae* in controls compared to individual treatment groups[a] (post-spawning data)

	O	E	O-E	$(O-E)^2$	$(O-E)^2/E$
		Treatment = 50 g/45.4 kg			
Infected	74	71	3	9	0.13
Non-infected	6	9	-3	9	1.00
Totals	80	80	0		$X^2 = 1.13$
					($p = 0.29$)
		Treatment = 75 g/45.4 kg			
Infected	54	57	-3	9	0.16
Non-infected	10	7	3	9	1.29
Totals	64	64	0		$X^2 = 1.45$
					($p = 0.23$)
		Treatment = 100 g/45.4 kg			
Infected	62	71	-9	81	1.14
Non-infected	18	9	9	81	9.00
Totals	80	80	0		$X^2 = 10.14$
					($p = 0.001$)

[a]Observed corresponds to treatment groups; the expected, to the proportion observed in the control group.

Table 25. Computation of X^2 of intensity classes of *P. ovariae* in pond-reared shiners (composite data)

% class	Controls	50 g	75 g	100 g	Totals
0-10	38	49	48	72	207
10-50	12	18	6	9	45
50-90	37	41	23	33	134
90-100	25	16	28	19	88
Totals	112	124	105	133	474

$X^2 = 24.45$ ($p = 0.0036$)

Table 26. Computation of X^2 of intensity classes of *P. ovariae* in pond-reared shiners (post-spawned data)

% class	Controls	50 g	75 g	100 g	
0-10	10	6	10	19	45
10-50	12	17	6	9	44
50-90	37	41	20	33	131
90-100	25	16	28	19	88
Totals	84	80	64	80	308

$X^2 = 23.47$ ($p = 0.0052$)

Table 27. Computation of X^2 of intensity classes of *P. ovariae* in controls compared to individual treatment groups[a] (composite data)

% classes	O	E	O-E	$(O-E)^2$	$(O-E)^2/E$
	Treatment = 50 g/45.4 kg				
0-10	49	42	7	49	1.17
10-50	18	13	5	25	1.92
50-90	41	41	0	0	0.00
90-100	16	28	-12	144	5.14
Totals	124	124	0		$X^2 = 8.23$
					(p = 0.041)
	Treatment = 75 g/45.4 kg				
0-10	48	36	12	144	4.00
10-50	6	11	-5	25	2.27
50-90	23	35	-12	144	4.11
90-100	28	23	5	25	1.09
Totals	105	105	0		$X^2 = 11.47$
					(p = 0.0094)
	Treatment = 100 g/45.4 kg				
0-10	72	45	27	729	16.20
10-50	9	14	-5	25	1.79
50-90	33	44	-11	121	2.75
90-100	19	30	-11	121	4.03
Totals	133	133	0		$X^2 = 24.77$
					(p = 0.00002)

[a]Observed corresponds to treatment groups; the expected, to the proportions observed in control groups.

Table 28. Computation of X^2 of intensity classes of *P. ovariae* in controls compared to individual treatment groups[a] (post-spawning data)

% classes	O	E	O-E	$(O-E)^2$	$(O-E)^2/E$
	Treatment = 50 g/45.4 kg				
0-10	6	10	-4	16	1.60
10-50	17	11	6	36	3.27
50-90	41	35	6	36	1.03
90-100	16	24	-8	64	2.67
Totals	80	80	0		$X^2 = 8.57$
					(p = 0.0356)
	Treatment = 75 g/45.4 kg				
0-10	10	8	2	4	0.50
10-50	6	9	-3	9	1.00
50-90	20	28	-8	64	2.29
90-100	28	19	9	81	4.26
Totals	64	64	0		$X^2 = 8.05$
					(p = 0.043)
	Treatment = 100 g/45.4 kg				
0-10	19	10	9	81	8.10
10-50	9	11	-2	4	0.36
50-90	33	35	-2	4	0.11
90-100	19	24	-5	25	1.04
Totals	80	80	0		$X^2 = 9.61$
					(p = 0.0222)

[a]Observed corresponds to treatment groups; the expected, to the proportions observed in control groups.

Table 29. Computation of X^2 of incidence of Lernaea cyprinaceae in pond-reared shiners

	Infected	Non-infected	Total
Controls	35	85	120
50 g/45.4 kg	62	168	230
75 g/45.4 kg	60	177	237
100 g/45.4 kg	51	173	224
Totals	208	603	811

$X^2 = 2.66$ ($p = 0.45$)

Table 30. Analysis of variance of mean gonadal somatic index from post-spawning pond fish from 28 May sample

Source	df	SS	MS	F
Total	106	1282.85		
Among treatments	3	188.06	62.69	5.90
Within treatments	103	1094.79	10.63	

VITA

Mary Louise Nagel

Candidate for the Degree of

Master of Science

Thesis: EFFICACY OF FEEDING NITROFURAZONE FOR CONTROL OF THE MICROSPORIDIAN PARASITE PLEISTOPHORA OVARIAE

Major Field: Zoology

Biographical:

Personal Data: Born in Cuero, Texas, September 12, 1951, the daughter of A. Harlan and Edieth A. Nagel.

Education: Graduated from Cuero High School, Cuero, Texas in May, 1969; received Bachelor of Arts degree in biology from Texas Lutheran College, Seguin, Texas, in May, 1973; completed requirements for a Master of Science at Oklahoma State University in May, 1975.

Professional Experience: Biological aid, Bureau of Sport Fisheries and Wildlife, Yellowstone National Park, June to August, 1972; graduate research assistant, Oklahoma Cooperative Fishery Research Unit, July, 1973, to present.

Professional Affiliations: American Fisheries Society; Wildlife Disease Association.