VARIOUS ASPECTS OF REPRODUCTIVE CONTROL IN FRESHWATER FISHES

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX TABLES

INTRODUCTION

One of the problems in fisheries management is the control of fish populations. Control of reproduction and numbers of fish is the general theme of this thesis. I designed three projects. Two projects concern the prospects of sex reversal by employing an antiandrogenic compound, cyproterone acetate, on adult guppies (Poecilia reticulata) and larval Japanese medaka (Oryzias latipes). The third project is a study of the effects of different food amounts and different population densities on the growth and reproduction of fathead minnows (Pimephales promelas).

Control of numbers and reproduction in fish is an important management tool for many purposes: to prevent the overcrowding and, hence, stunting of fish growth; to control numbers of undesirable fish; to control exotic species while being tested in new habitats; to control the number of a species used for biological controls; and to control numbers of predators in the species balance of farm ponds. Manipulation of population number by control of sex or by limiting some biotic or abiotic factor in the ecosystem is a practice which is being studied further in fisheries science research today.

PART I

REPRODUCTIVE BEHAVIOR, COLORATION, AND GAMETOGENESIS IN GUPPIES (*POECILIA RETICULATA*) TREATED WITH METHYL TESTOSTERONE AND CYPROTERONE ACETATE

INTRODUCTION

Androgens are involved in the expression of primary and secondary sex characters in teleosts (Pickford and Atz 1957; Hoar 1962, 1969; Liley 1969). Pandey (1969a) found that administration of methyl testosterone to hypophysectomized adult guppies (Poecilia reticulata) effects spermatogenesis and the elaboration of male coloration. Other studies employing the antigonadotropic agent methallibure (I.C.I. 33, 828) on adult guppies have shown that spermatogenesis and secondary sex characters are, at least in part, dependent on gonadotropin secretion (Pandey 1970; Pandey and Leatherland 1970). The above reports of experiments employing androgen administration, hypophysectomy, and gonadotropin antagonism illustrate the relationship of the pituitary-gonadal axis with respect to expression of sexual characteristics in the guppy.

The development of cyproterone acetate (CA) (1,2-methylene-6-chloro-$\Delta^{4,6}$-pregnadiene-17α-ol-3,20-dione-17α-acetate), a potent antiandrogen in mammals (Neumann et al. 1967a,b) may provide a new approach in the research of the role of androgens in fishes. Typical female sexual behavior was induced by CA in male rats (Neumann and Elger 1965, 1966a). Reduced male sexual activity was observed in other male rodents treated with CA (Neumann et al. 1967c; Steinbeck et al. 1967). However, Whalen and Edwards (1969) and Whalen et al. (1969) reported no inhibitory effect of CA on the androgen-dependent mating behavior in male rats. Further, adult males treated with CA showed depressed spermiogenesis (Neumann and von Berswordt-Wallrabe

1966a; Neumann et al. 1968), whereas in females, cyproterone reduced the ovulatory inhibition of androgens, estrogens, and other sex steroids (Neumann and von Berswordt-Wallrabe 1966b). CA apparently affects the feedback mechanism of the pituitary-gonad axis by blocking the effects of testosterone and increasing gonadotropin secretion (Neumann 1966; Bloch and Davidson 1967). In fish, it is known that CA effectively blocks androgen uptake and/or retention in testes but not in ovaries of rainbow trout (Salmo gairdneri) (Schreck 1973). Rastogi and Chieffi (1975) showed that CA failed to block the masculinizing action of androgens on secondary sex characters in Xiphophorus helleri. CA did, however, inhibit the seminal vesicular hypersecretion caused by androgens in castrate Heteropneustes fossilis (Sundararaj and Nayyar 1969). The action of the antiandrogen in other classes of vertebrates is reviewed by Chieffi et al. (1974) and Rastogi and Chieffi (1975). CA possibly blocks androgens at both a central and peripheral level and, therefore, it is of interest to evaluate effects of CA administration on reproductive attributes in fishes.

The objective of this study was to establish the efficacy of CA in inhibiting the action of androgens in fishes. Effects of CA, methyl testosterone, and a mixture of the two steroids on behavioral, colorational, and gametogenic characteristics of intact, adult guppies were evaluated.

METHODS

Two male and three female adult guppies were placed into each of eight 20-liter aquaria. The fish were maintained at 28 C with a 16L:8D photoperiod. The aquaria were isolated from each other and observed through small observation windows. During a 4-day acclimation period, preliminary observations revealed typical reproductive behavior in the fish.

Replicated treatments were as follows: (1) control diet of Tetra Min^{R} standard fish food; (2) 1,000 ug methyl testosterone (MT)/g Tetra Min; (3) 1,000 ug CA/g diet; and (4) 1,000 ug MT and 1,000 ug CA/g diet. Steroids were added to the feed by the alcohol evaporation method; control diet was saturated in alcohol alone and dried by evaporation. Fish were fed all that they would consume in approximately 10 minutes, three times daily for 18 days.

Reproductive behavior was monitored beginning 48 h after commencement of treatment. The fish in each aquarium were observed every day for 10 min throughout the study. The sigmoid body configuration, the dorsal fin erection, and the vibrating-backing motion of the displaying male (Breder and Coates 1935; Liley 1966) were performed simultaneously and, therefore, were considered as one activity. Gonopodial swings and thrusts (Breder and Coates 1935; Clark and Aronson 1951; Liley 1966) were not always included as a part of this act. Therefore, 'swings' were not included in the evaluation of a sexual display, even if they were observed. Female behavior typically consisted of passive or evasive responses to male activity (Breder

and Coates 1935; Clark and Aronson 1951). Female positive response (Liley 1968; Liley and Wishlow 1974) was never observed.

At the conclusion of the experiment, pigmentation intensity and distribution were visually evaluated for all fish and ranked on a scale from 0 (pale color) to 3 (extremely brilliant) for males and 0 (normal, dull gray) to 3 (extra brilliance) for females. All fish were fixed in Bouin's solution, paraffin-imbedded, serially crossed-sectioned (7μ), and stained with Harris' hematoxylin and eosin stains. Stages of gametogenesis were quantified by determining the average number of cysts per tissue section for each gonadal sample.

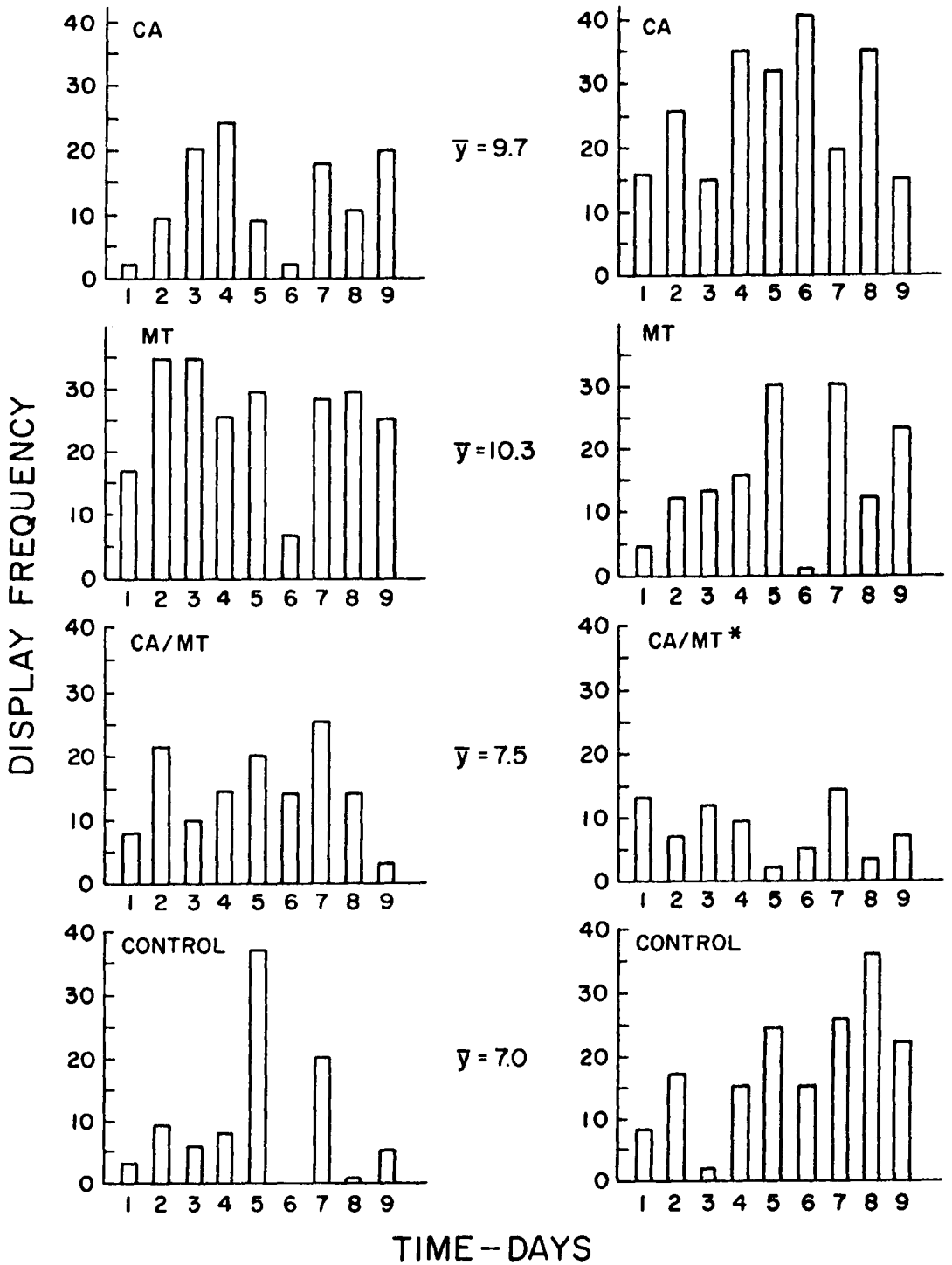
RESULTS

Male courtship behavior was observed prior to treatment and regularly in all tests for 8 days after onset of treatment. Ichthyophthirius was contracted by some fish on day 18 of treatment; hence, only the data up to day 16 are presented. Also, to overcome any differences due to adjustment periods to the treated diets, the behavioral results accumulated between days 8 and 16 are reported. The average frequency of male courtship display was not significantly different between treatments (Fig. 1). Hierarchical position of males with respect to dominance and territories was established by the fish in all treatments, except for MT in which co-dominance prevailed. Any apparent differences in frequencies of display within and among treatments may be due to natural variability in dominance relationships between fish.

In the MT and CA/MT treatments, females displayed male sexual behavior by adopting the sigmoid body configuration as well as the vibrating-backing motion. Dorsal fin erections did not always accompany this display. The masculinization in the female's behavior was noted on the 11th and 12th days in the MT treatment; whereas, in the CA/MT treatment, the masculinized behavior of females was observed on the 14th and succeeding days. CA did not block this androgen-induced activity.

At termination of the experiment, all surviving males and females were ranked according to color intensity (Table 1). Control and CA treated females exhibited no observable color change. However,

Fig. 1. Frequency of sexual display (dorsal fin erection, sigmoid body configuration, and vibrating-backing motion) in male guppies in replicate treatments with MT, CA, and CA/MT. Each bar consists of the total number of male displays per aquarium. The $\bar{Y}$'s = the average number of displays over the test period per day per treatment. The 9 days represent the 8th through the 16th day of treatment inclusively.



* one male died on day one.

Table 1. Effects of cyproterone acetate (CA), methyl testosterone (MT), and a combination of CA with MT on color elaboration in male and female guppies after 18 days treatment.

Color Rank	Treatments			
	Control	CA	MT	CA/MT
	Number of females			
Normal, dull gray	6	6	0	2
Slight iridescence	0	0	0	1
Brilliant-iridescence with black and orange blotches	0	0	1	3
Extra brilliant	0	0	5	0
	Number of males			
Pale color	1[a]	2[a]	0	1[a]
Normal, brilliant color	3	2	0	2
Extra brilliant	0	0	4[b]	0

[a] Subordinate males.

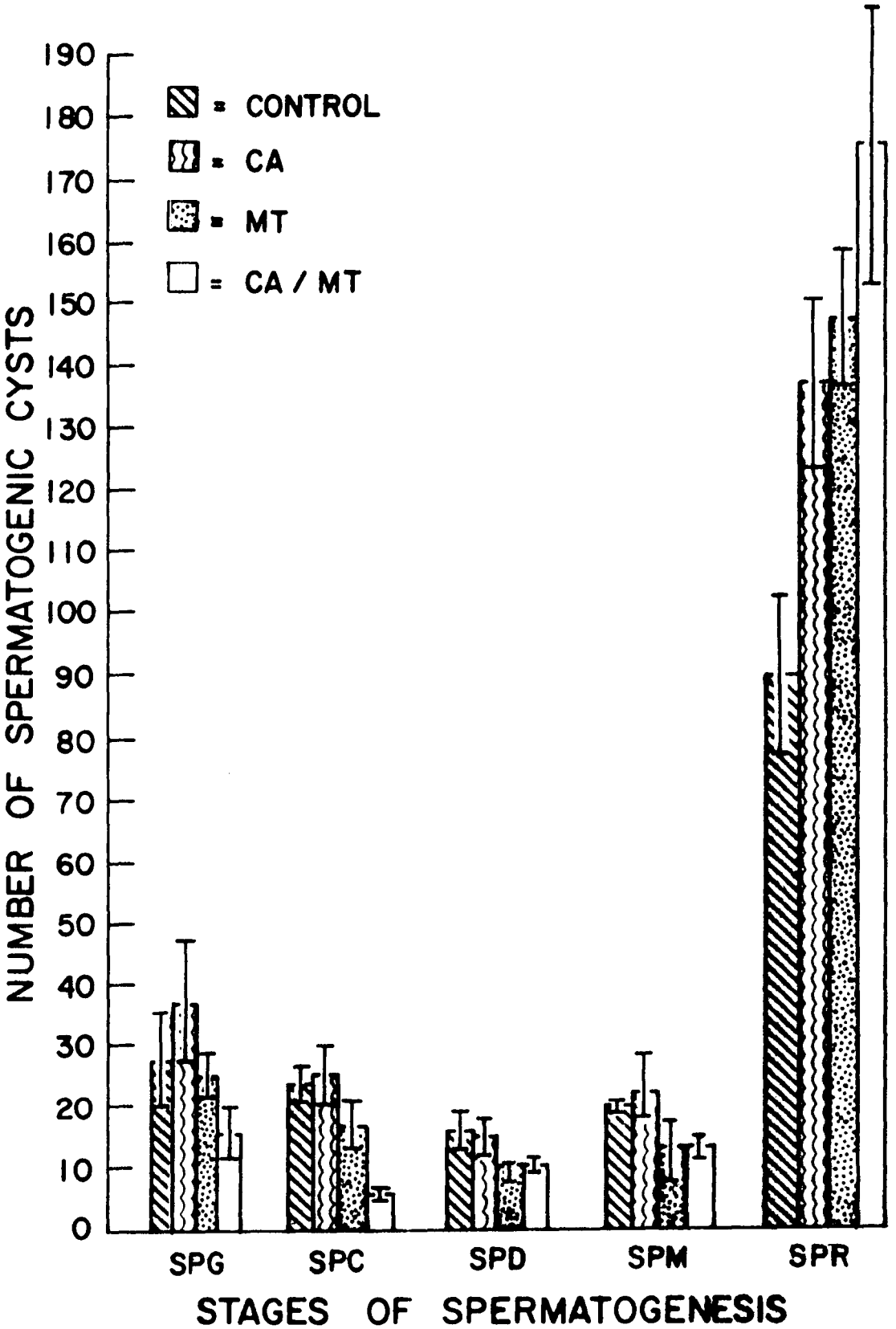
[b] Co-dominant males.

iridescense on the dorsal and caudal fins of MT treated females was observed by the 12th day of treatment. Iridescence was accompanied by black blotching which became more pronounced and widespread each succeeding day. Orange color became evident in the peduncle region of the body on approximately the 16th day and became more brilliant and extensive thereafter. It was difficult to distinguish males from females by their pigmentation by the end of the experiment. In the CA/MT treatment, females displayed a color range from dull gray to iridescent with orange and black blotches. Color change in these females began on the 8th day of treatment. CA may have partially inhibited the color-stimulating activity of MT.

Coloration of males in the control, CA, and CA/MT groups ranged from pale to brilliant. MT treated males were much more brilliant than normal, untreated male guppies. Color extended to the tip of the caudal fin, a quality not observed in males of the other treatments. Hierarchical position of males was related to color elaboration; i.e., dominant males were always more brilliant, whereas subordinate males lost much of their color. MT treated males shared co-dominance and were equally brilliant. CA alone did not influence the dominance role of the males but, in combination with MT, may have partially blocked the exogenous androgen's behavioral and colorational effects.

An inverse relationship existed between the number of cysts in the early spermatogenic stages and the number of spermatophores (Fig. 2). A highly significant difference ($P \leq 0.0001$) was found in the numbers of cysts in the spermatogenic stages (two-way analysis of variance). Treatment with MT, CA, or CA/MT caused no statistically

Fig. 2. Average number (± standard error) of cysts in each spermatogenic stage for each testicular sample in each treatment. SPG = spermatogonia; SPC = spermatocytes; SPD = spermatids; SPM = spermatozoa; SPR = spermatophores.


190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
= CONTROL
= CA
= MT
= CA / MT
NUMBER OF SPERMATOGENIC CYSTS
SPG
SPC
SPD
SPM
SPR
STAGES OF SPERMATOGENESIS

demonstrable difference from the control pattern.

Histological examination revealed the presence of eggs in the ovaries of all but two females (Table 2). The exceptions probably do not reflect differences due to treatment. Embryos were found in all females in the control and CA treatments. Only one female in the MT treatment and none in the CA/MT group had embryos present in its ovaries. A binomial test revealed a highly significant difference ($P \leq 0.003$) between proportions of females with embryos in the four treatment groups.

Table 2. Histological observations revealing treated females with eggs and/or embryos at termination of the experiment. CA = cyproterone acetate; MT = methyl testosterone.

Treatment	Number of Females	Number of Females with Eggs	Number of Females with Embryos
Control	6	6	6
CA	6	5	5
MT	6	5	1
CA/MT	4	4	0

DISCUSSION

Even though there was some difference in male sexual display between treatments, it appears that MT elicited male co-dominance. CA possibly inhibited the action of endogenous as well as exogenous androgens in both the CA and CA/MT treated males because co-dominance was not demonstrated. One-half of the females receiving the combined steroids displayed masculinized behavior, whereas all MT treated females displayed male courtship behavior. It is, therefore, probable that the antiandrogen partially inhibited the effects of MT in the CA/MT treated females. It is also possible that the co-dominance observed in the MT treated males was the result of the masculinization of the females in those tanks.

The antiandrogen did not inhibit the action of endogenous androgen in production of male coloration in the CA and CA/MT treated males. The antiandrogen, however, appeared to have blocked the action of the exogenous androgen because of the difference in coloration between MT and CA/MT treated fish of either sex. In all cases, the male exhibiting pale color was the subordinate. Where MT alone was administered, no territories were established and both males were co-dominant and exhibited equal brilliance. Changes in the males' color expression mostly involved the integumentary lipophores, whereas in the females, the iridocytes, melanophores, and lipophores were noticeably affected. This difference is probably due to the distinct dimorphic coloration that exists naturally.

Pandey (1969a,b) found that hypophysectomy completely blocks mitotic division in spermatogonia and inhibits development of spermatogonia into spermatocytes. Treatment with methyl testosterone restored and increased mitosis in spermatogonia and increased the number of spermatocytes (Pandey 1969a). Because CA acts as an antiandrogen to testosterone retention in piscine testicular tissue (Schreck 1973), one would expect gametogenesis to be inhibited. The relationships of spermatogonia and spermatocytes, as shown in Fig. 2, are approximately the same in all treatments, except for the control, suggesting that CA has no effect on the transformation of spermatogonia into spermatocytes in the fish treated with androgen. When administered alone, CA also had no effect. There were more spermatophores in all the treated males than in the controls, and only one female in the MT treatment and none in the CA/MT group had embryos present in its ovary. The higher number of spermatophores in the males and the absence of embryos in the females of the MT and CA/MT treatments are, perhaps, best explained by the masculinized or otherwise altered behavior and/or physiology on the part of the female. In general, CA appears, at least at the concentration employed, to be an extremely weak antiandrogen with respect to inhibiting sexual characteristics induced by endogenous and exogenous androgen in both male and female guppies.

PART II

THE EFFECT OF CYPROTERONE ACETATE ON SEX DETERMINATION IN THE JAPANESE MEDAKA (*ORYZIAS LATIPES*)

INTRODUCTION

Sex reversal is a phenotypic and functional sex change from that of an individual's genotype. Larval fish, treated with proper doses of androgens or estrogens prior to and during the stage of gonadal differentiation, successfully change to the sex appropriate for the sex steroid administered. Embryonic sex inductors, similar to sex steroids, may mediate sex determination in fish (Yamamoto 1953, 1955, 1958, 1959a,b, 1961, 1962, 1963, 1964a,b, 1965, 1968; Yamamoto and Kajishima 1968; Yamamoto and Matsuda 1963). Other studies concerning sex determination in fish have been reviewed and accompany a comprehensive reference chart of sex hormones and other steroids tested for their effects on sex of juvenile fish (Schreck 1974). By these experiments, it was shown that the gonads in fish respond to sex steroids and that man can direct the sex of certain teleostean fishes, irrespective of their genotype. However, in mammals treated with sex hormones, only the secondary and accessory sex characters are changed to the heterologous sex; the gonads are not changed. In contrast to studies on fish, mammalian research may support a non-steroid sex inductor theory.

Laboratory synthesis of cyproterone acetate (CA) (1,2α-methylene-6-chloro-$\Delta^{4,6}$-pregnadiene-17α-ol-3,20-dione-17α-acetate) a potent antiandrogen in mammals has afforded a new approach in studying the function of male sex hormones and the effects of inhibiting endogenous male sex steroids on sex determination. If embryonic sex inductors are similar to sex steroids, CA would be expected to block the

androgenic effect of a male sex inductor during sex differentiation.

Earliest studies demonstrating the efficacy of CA in inhibiting the male sex steroids in mammals are reviewed by Neumann and Elger (1966a) and Neumann et al. (1967a,b). Feminization of male secondary and accessory sex characters was induced when fetal male rabbits, mice, and rats were treated with CA via administration of the anti-androgen to their pregnant mothers (Elger and Neumann 1966; Elger et al. 1967; Neumann and Elger 1966b; Neumann et al. 1966a,b). Neumann et al. (1967b) suggested that CA attaches to androgen receptor sites, blocking endogenous androgens at target tissues. However, the gonads were not influenced by CA and were, therefore, assumed to be androgen-independent. These studies further supported a non-steroid-like sex inductor theory.

Little is known about the effects of CA in teleosts. In a preliminary study, Schreck (1973) demonstrated the blocking effectiveness of CA on the uptake and retention of labelled testosterone in rainbow trout (Salmo gairdneri) testes. Nothing has been done to demonstrate the effects of CA in sex determination of larval fish. In nonmammalian experiments, Chieffi et al. (1974) demonstrated that undifferentiated tadpoles treated with testosterone or with CA developed into male frogs. The present experiment was designed to demonstrate the effects of CA in larval fish and to test whether embryonic sex inductors in fish are similar to sex steroids.

MATERIALS AND METHODS

Experiment 2. Japanese medaka (*Oryzias latipes*) eggs of the cultivated golden strain were obtained from Carolina Biological Supply Company, Burlington, North Carolina. Eggs were incubated in petri dishes containing an embryo-rearing solution to minimize mortality (Kirchen and West 1969). Upon hatching, fry were equally distributed among 12 4-liter jars for the first 3 weeks after hatching. Fish from each of the 12 jars were then transferred to 12 100-liter aquaria and reared to maturity in 25 C aerated water under 14L:10D photoperiod.

Three dosage levels, 50, 250, and 500 ug CA/g Tetra Min^{R}, standard fish diet were prepared. The steroid was added to the food by the alcohol evaporation method. Control diet, without steroid, was saturated in alcohol and dried by evaporation. Three groups of fish were fed control diet; three groups were fed 50 ug CA/g diet; three groups were fed 250 ug CA/g diet; and three groups were fed 500 ug CA/g diet. Feedings, three times daily, began within 24 h after hatching and continued for 12 weeks. Thereafter, all fish were fed standard diet of dried food supplemented with mashed egg or liver.

The experiment was terminated after 27 weeks. Total length and wet weight were measured for each fish. Histological examinations were made on the gonads of each fish. Fish were fixed in Bouin's solution and embedded in paraffin. Serial cross-sections (10u) were stained with Harris' hematoxylin and eosin. Ratio of

males to females was used as an index of the effect of CA.

Interpretation of the results of this experiment was not definitive. Based on observations of sex ratios, I could not assume that sex reversal had, in fact, been established. Therefore, the experiment was repeated using a strain of medaka with a sex-indicative genetic marker.

Experiment 3. Eggs of the d-rR strain Japanese medaka (Oryzias latipes) were obtained from the Department of Anatomy, State University of New York, Brooklyn. This strain of medaka produces sex-linked dimorphically colored fish: red males and white females. Eggs were incubated in petri dishes containing embryo-rearing solution. Hatched fish were equally distributed among eight 20-liter aquaria.

Two treatment diets were prepared. CA was added to Tetra Min^{R} standard fish food by the alcohol evaporation method in concentrations of 500 and 1,000 ug CA/g diet. Control diet, without steroid, was saturated in alcohol and dried by evaporation. Two groups of fish were fed control diet; two groups were fed 500 ug CA/g diet; and four groups were fed 1,000 ug CA/g diet three times daily. Exogenous feedings began immediately upon hatching of fish and continued for 12 weeks. Thereafter, fish were fed fish diet without steroid until the end of the experiment.

The project was terminated in 6 months. Wet weight and total length were measured for each fish. Sex ratios in each treatment were determined by three methods: dimorphic coloration; dimorphic secondary sex characters, especially the dorsal fin configuration described by Yamamoto (1953) and Kirchen and West (1969); and

histological examination.

RESULTS

Experiment 2. Histological examination revealed 9 male and 12 female fish in the control group (Table 3). Average length for these fish was 2.6 cm and average weight was 0.22 g. Sex frequencies for fish treated with 50 ug CA/g diet were 18 male and 11 female fish. These fish averaged 2.7 cm length and 0.24 g weight. Fourteen male and 13 female fish were counted in the group fed 250 ug CA/g diet. Average length was 2.8 cm and average weight was 0.28 g for this group. For fish fed 500 ug CA/g diet, 5 male and 13 female fish were counted. These fish averaged 2.7 cm length and 0.22 g weight. No significant differences (chi-square, $P \leq 0.25$; Snedecor and Cochran 1967) were found in the sex ratios of treated fish from those of control fish.

Mortality was approximately the same for all treatment groups (Fig. 3). Groups treated with 50, 250, and 500 ug CA/g diet had 64, 66, and 78 percent mortality, respectively, in comparison to the control group which had 74 percent mortality.

Experiment 3. Sex reversal was not established in the fish treated with CA. Sex, determined histologically, agreed in all cases with the sex identified by dimorphic coloration and by dorsal fin configuration (Table 4). One control fish and one fish treated with 1,000 ug CA/g diet could not be sexed histologically. Gonads were not found in either fish. Mortality was high in all treatment groups. Eighty-five and 82.5 percent mortality were found in the 500 and 1,000 ug CA/g diet groups compared to 75 percent found in the control group.

Table 3. Total length (cm), wet weight (g), and sex, histologically determined, at the end of the experiment, for each fish fed different concentrations of cyproterone acetate (CA)/ diet.

Treatment-Control				Treatment-50ug CA/g Diet			
Fish No.	L	W	Sex	Fish No.	L	W	Sex
1	3.2	0.31	M	1	2.3	0.17	M
2	2.1	0.11	F	2	2.7	0.23	M
3	2.8	0.22	M	3	2.4	0.12	F
4	2.7	0.21	F	4	2.7	0.25	F
5	2.0	0.15	F	5	2.0	0.09	F
6	2.1	0.13	F	6	3.2	0.40	F
7	2.5	0.22	F	7	2.5	0.17	M
8	2.6	0.18	M	8	2.1	0.09	M
9	3.2	0.26	M	9	3.2	0.41	M
10	3.3	0.33	M	10	2.9	0.23	M
11	3.1	0.31	F	11	1.8	0.05	F
12	2.4	0.13	F	12	2.9	0.25	M
13	2.0	0.06	F	13	2.9	0.30	M
14	2.5	0.14	M	14	3.2	0.35	F
15	2.8	0.47	F	15	2.9	0.24	F
16	2.6	0.14	M	16	3.1	0.33	M
17	3.3	0.45	F	17	2.9	0.25	M
18	2.5	0.19	F	18	2.8	0.19	M
19	2.8	0.24	M	19	3.5	0.40	M
20	2.6	0.18	F	20	3.1	0.30	F
21	2.2	0.11	M	21	2.8	0.20	M
				22	2.8	0.23	M
$\bar{Y}$ =	2.6	0.22		23	2.9	0.25	M
				24	3.2	0.37	F
No. of Males = 9				25	2.8	0.20	M
No. of Females = 12				26	1.9	0.06	M
				27	2.9	0.25	M
				28	3.4	0.51	F
				29	1.9	0.13	F
				$\bar{Y}$ =	2.7	0.24	
				No. of Males = 18			
				No. of Females = 11			

Table 3. Total length (cm), wet weight (g), and sex, histologically determined, at the end of the experiment, for each fish fed different concentrations of cyproterone acetate (CA)/ diet (continued).

Treatment-250ug CA/g Diet				Treatment-500ug CA/g Diet			
Fish No.	L	W	Sex	Fish No.	L	W	Sex
1	2.9	0.33	F	1	2.8	0.23	F
2	3.2	0.40	F	2	2.5	0.28	F
3	3.0	0.34	F	3	2.2	0.16	F
4	3.2	0.42	F	4	3.2	0.33	F
5	2.8	0.30	M	5	2.7	0.27	F
6	1.9	0.08	F	6	2.6	0.18	M
7	2.7	0.31	M	7	3.2	0.45	F
8	2.5	0.20	F	8	2.5	0.14	F
9	2.2	0.13	F	9	2.4	0.11	F
10	3.2	0.39	F	10	2.1	0.10	F
11	2.9	0.27	M	11	2.0	0.08	?
12	3.1	0.34	M	12	1.7	0.04	M
13	3.1	0.31	M	13	2.5	0.15	F
14	3.2	0.33	F	14	3.5	0.37	M
15	3.2	0.49	F	15	2.8	0.22	F
16	3.5	0.37	M	16	2.9	0.27	F
17	3.0	0.30	F	17	2.9	0.27	M
18	2.9	0.28	M	18	2.9	0.32	F
19	2.0	0.09	M	19	3.1	0.29	M
20	2.1	0.13	F				
21	3.1	0.29	M	$\bar{Y}$ =	2.7	0.22	
22	3.2	0.34	M	No. of Males = 5			
23	3.0	0.27	M	No. of Females = 13			
24	3.1	0.31	M				
25	2.9	0.23	M				
26	2.7	0.21	F				
27	2.1	0.09	M				
$\bar{Y}$ =	2.8	0.28					
No. of Males = 14							
No. of Females = 13							

Fig. 3. Number of live fish in each treatment at the start (S) and at the finish (F) of Experiment 2.

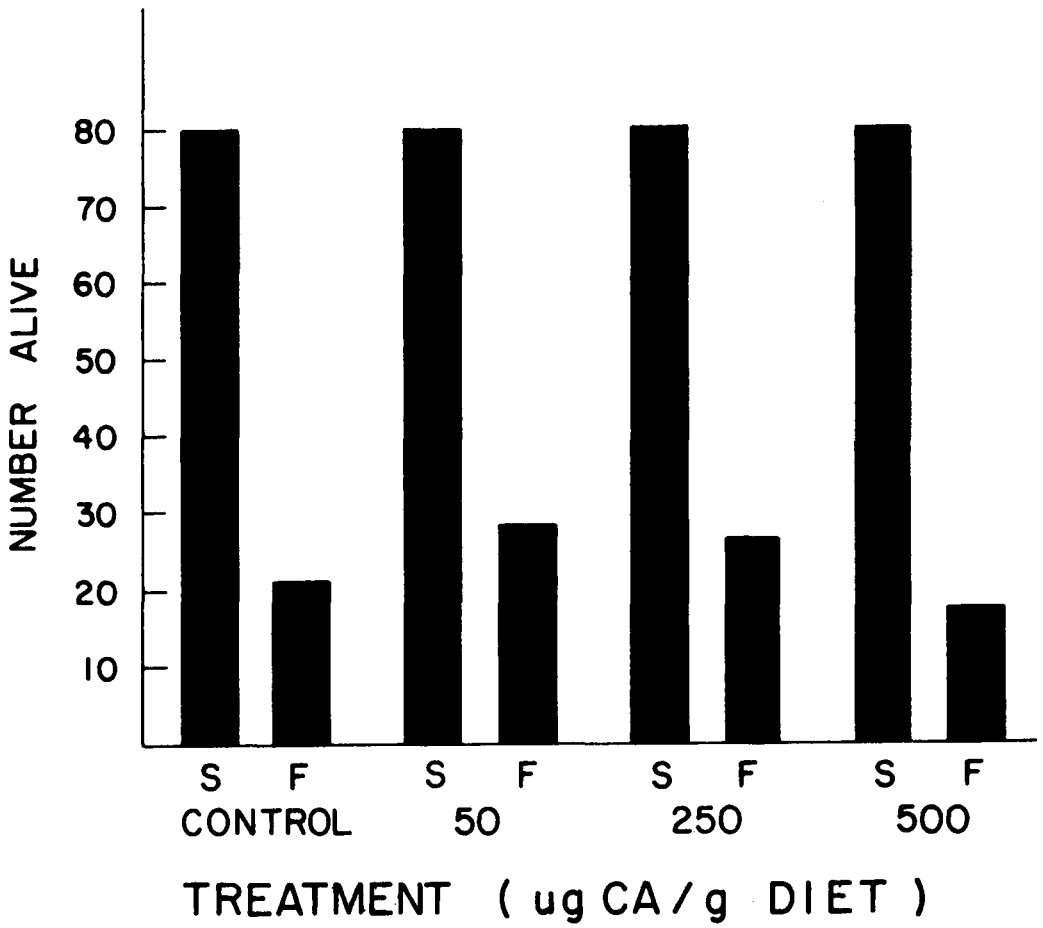

NUMBER ALIVE
80
70
60
50
40
30
20
10
S F S F S F S F
CONTROL 50 250 500
TREATMENT (ug CA / g DIET)

Table 4. Sex identified by dimorphic coloration, dorsal fin configuration, and histological examination for Japanese medaka fed different concentrations of cyproterone acetate (CA)/diet.

Treatment	Fish Number	Sex Color	Sex Fins	Sex Histology
Control	1	M	M	M
	2	M	?	?
	3	F	F	F
	4	M	M	M
	5	M	M	M
500 ugCA/gDiet	1	F	F	F
	2	M	M	M
	3	M	M	M
1,000ugCA/gDiet	1	F	F	F
	2	M	M	M
	3	M	M	M
	4	M	M	?
	5	F	F	F
	6	M	M	M
	7	M	M	M

Evidence of the effects of CA on sex determination in fish was definitive. Three male and one female fish were identified in the control group; two male and one female fish were found in the group treated with 500 ug CA/g diet; and four male and two female fish were counted in the group treated with 1,000 ug CA/g diet.

DISCUSSION

It appears, by the results of Experiment 2 and Experiment 3, that the embryonic sex inductor in fish is not similar to a male sex hormone. However, very recently, while my experiment was in progress, Rastogi and Chieffi (1975) showed that CA did not inhibit the masculinization effects of testosterone proprionate or of 11-keto-testosterone on secondary sex characters when administered concurrently to female swordtail fish (Xiphophorus helleri). In support, the experiment in Part I of this thesis showed that CA was an extremely weak antiandrogen with respect to inhibiting sex characteristics induced by endogenous and exogenous androgen in both male and female guppies (Poecilia reticulata). It is apparent, with this added information, that CA does not block the effects of androgens on sex characters that are known to be androgen dependent in fish. It is, therefore, assumed that CA would not be effective in blocking a steroid sex inductor, if present, in the larval fish.

PART III

GROWTH, FECUNDITY, AND TOLERANCE DENSITY IN THE FATHEAD MINNOW (*PIMEPHALES PROMELAS*): EFFECTS OF FOOD AND POPULATION DENSITY

INTRODUCTION

A study designed to determine the impact of different harvesting intensities on wild bait species has been conducted on Rich Creek, West Virginia (Brandt and Schreck 1975). Data for 2 years (1973 and 1974) showed these harvesting pressures to have no measurable effect on the densities of the bait fish populations.

Two variables that influence recruitment to bait fish stocks are food and population density. These factors could influence both growth and fecundity of the fish (Bagenal 1971). Generally, a direct relationship exists between food abundance and growth rate, and an inverse relationship is present between population density and growth rate (Le Cren 1965). However, there may be no relationship between food abundance and growth when a space-limiting effect is operating on the population (Johnson 1965). As a partial explanation for the reduction in reproduction and growth under crowded conditions, several people have suggested the presence of a water-borne, fish produced represser factor that inhibits reproduction (Swingle 1953; Rose and Rose 1965) and reduces growth rate (Rose and Rose 1965; Yu and Perlmutter 1970; Francis et al. 1974). Pfuderer et al. (1974) partially purified this substance. However, Glaser and Kantor (1974) showed that spawning rate in medaka (Oryzias latipes) can be inhibited by social factors of crowding, irrespective of chemical conditions of the water. The present experiment was designed to separate the variables of population density and nutrition and to test their effects on growth and fecundity of fish.

MATERIALS AND METHODS

Experiment 4. Fathead minnow (Pimephales promelas) eggs were obtained by placing several terra cotta pots in the shallows of a pond in Blacksburg, Virginia. Minnows spawned in the pots, and the eggs were incubated in the laboratory. Resultant fry were fed finely pestled commercial flake diet (Tetra Min^{R}) ad libitum after hatching. Three- to four-week-old fish were randomly divided into nine groups. Each group of fish was placed in a 90-liter aquarium containing aged, dechlorinated tapwater. Three groups contained 25 fish each, three groups contained 50 fish each, and three groups contained 100 fish each. One group of 25 fish was fed ad libitum (all they could eat in 20 minutes), the second group of 25 fish was fed 66 percent of the ad libitum amount and the third group of 25 fish was fed 33 percent of the ad libitum amount. The same procedure was followed with each of the other two population densities. Feedings were twice daily. Food rations were recalculated weekly to compensate for growth and for any mortality of fish in the aquaria. Calculated food amounts are tabulated in Appendix I. Fish were maintained on a 16L:8D photoperiod at 21±1C. Each aquarium was equipped with incurrent and excurrent waterflow. A complete water exchange was effected in approximately 24 h to ensure adequate water quality in the aquaria but prevent a total depletion of potential pheromones. Population densities and water chemistries were monitored monthly. Dissolved oxygen was measured by the azide modification of the Winkler method and ranged between 7 and 8.5 ppm in all aquaria during the experiment.

The pH fluctuated between 7.1 and 7.7 as determined on a Corning™ Model 10 pH meter. Total alkalinity ranged between 36 and 48 mg/liter. Total ammonia, measured by the Nesslerization method, remained at less than 0.5 mg/liter.

The research plan called for female fish to be sacrificed when they appeared gravid and ready for spawning. However, 9 months after beginning the study, even though the temperature and photoperiod were optimum to induce maturation and spawning, the fish showed no indication of having reached this condition. Water temperature was then increased to 25 C. Females still did not appear gravid after an additional 2 months. This experiment was then terminated. Total length and wet weight were measured for each fish. Total number of eggs were to be counted. However, the ovaries, in many cases, were not well developed. Dissection of the entire ovary, necessary in fecundity measurements, was impossible. Fifty eggs were then randomly sampled from each female fish and measured with an ocular micrometer.

Because these fish from wild parents did not reach maturity by the end of the experiment, some requirement for successful reproduction apparently was not being satisfied under laboratory conditions. Consequently, a second experiment was designed to use a laboratory stock of fathead minnows with known success in spawning under laboratory conditions.

Experiment 5. A laboratory stock of fathead minnow fry, 3 to 7 days old, was obtained from the National Water Quality Laboratory, Duluth, Minnesota. Fish were acclimated for one month in five 40-liter aerated aquaria at 18 C. At the end of this time period mortality

was no longer significant. Finely pestled, freeze-dried brine shrimp were fed six times daily to all fry during the first 2 weeks of acclimation. During the last 2 weeks of the acclimation period the fish were fed frozen brine shrimp nauplii four times daily supplemented with freeze-dried brine shrimp.

At the end of the acclimation period the fish were randomly divided among eight 160-liter aquaria containing aged dechlorinated water. Fifteen fish were placed in each of four aquaria. Forty-five fish were placed into each of the remaining four aquaria. Two of the groups of 15 fish and two of the groups of 45 fish were fed twice daily. The remaining two groups of each population density were fed six times daily. Feedings were ad libitum (all the fish could eat in 20 minutes) and consisted of frozen adult brine shrimp. Fish were maintained on a 16L:8D photoperiod. Water temperature was held at 25±1C. An incurrent and excurrent waterflow provided an approximate 24 h water exchange rate to help maintain good water quality but prevent a total loss of possible pheromones.

By 19 weeks the fish appeared in spawning condition. The males had the characteristic enlarged black heads with nuptial tubercles while females appeared robust in girth. Procedures for measurements and data collection were the same as in Experiment 4.

Statistical tests were conducted for each experiment separately. A two-way factorial analysis of variance with unequal cell sizes designed for Statistical Analysis System (SAS, Barr and Goodnight 1972) was used to evaluate differences in lengths, weights, and egg sizes due to population density, food abundance, and their interaction.

In addition, SAS procedures for regression analyses were performed on the data to determine what relationship egg size had to the variables length and weight.

RESULTS

Experiment 4. Pooled data on growth of fish in all three population densities fed ad libitum were compared to the pooled data for those fish fed 66 percent of the ad libitum amount. These data in turn were each compared to the pooled data for fish fed 33 percent of the ad libitum amount. In all cases, fish fed greater amounts of food were larger and weighed more than those fish fed lesser amounts of food. Mean lengths for fish fed ad libitum, 66 percent, and 33 percent of the ad libitum amount were 59, 53, and 46 mm, respectively (Table 5), which were significantly different from each other ($P \leq 0.01$). Mean weights were significantly different from each other ($P \leq 0.01$) and were 2.3, 1.6, and 1.0 g for fish fed ad libitum, 66 percent, and 33 percent of the ad libitum amount, respectively (Table 5).

Similar comparisons on pooled growth data for female fish only and for male fish only were made. Male fish were consistently larger than female fish in all treatments. Male fathead minnows, in nature, are generally larger than the female fish of the same age. Average lengths for female fish fed ad libitum, 66 percent, and 33 percent ad libitum were 54, 51, and 44 mm, respectively (Table 6), which were significantly different from each other ($P \leq 0.05$); average weights, 1.6, 1.3, and 0.9 g (Table 6) were found significantly different ($P \leq 0.05$). Average lengths for male fish fed ad libitum, 66 percent, and 33 percent ad libitum were 62, 55, and 47 mm, respectively (Table 7), which were significantly different from each other

Table 5. Means for total length (mm) and wet weight (g) of fathead minnows (Pimephales promelas) subjected to different feeding rates and population densities. N = pooled number of all male and female fish in three aquaria corresponding to a particular food abundancy or population density treatment at the end of the experiment. SD = standard deviation.

Experiment 4

Fish Growth Data

Treatment	N	Length	SD	Weight	SD
Food Abundancy					
ad libitum	48	59^{x}	7	2.3^{x}	0.9
66% ad libitum	56	53^{y}	7	1.6^{y}	0.7
33% ad libitum	36	46^{z}	8	1.0^{z}	0.5
Initial Population Density[a]					
25	45	53^{b}	9	1.6^{b}	0.9
50	42	53^{b}	9	1.7^{b}	0.9
100	53	54^{b}	9	1.6^{b}	0.9

[a]Initial population density = number of fish in each of three aquaria at the start of the experiment.

[x,y,z]Means with different superscripts in the same column (food abundancy treatment) are significantly different from each other at the $P \leq 0.01$ level.

[b]Means with the same superscript in the same column (population density treatment) are not significantly different from each other.

Table 6. Means for total length (mm), wet weight (g), and egg size (mm) of female fathead minnows (Pimephales promelas) subjected to different feeding rates and population densities. N = pooled number of all female fish in three aquaria corresponding to a particular food abundancy or population density treatment at the end of the experiment. SD = standard deviation.

Experiment 4

Female Fish Growth and Egg Size Data

Treatment	N	Length	SD	Weight	SD	Egg Size	SD
Food Abundancy							
ad libitum	17	54^{x}	4	1.6^{x}	0.4	0.33^{b}	0.12
66% ad libitum	30	51^{y}	5	1.3^{y}	0.4	0.31^{b}	0.18
33% ad libitum	13	44^{z}	8	0.9^{z}	0.6	0.24^{b}	0.19
Initial Population Density[a]							
25	23	51^{c}	7	1.3^{c}	0.5	0.33^{c}	0.16
50	18	50^{c}	8	1.3^{c}	0.6	0.28^{c}	0.18
100	19	51^{c}	6	1.3^{c}	0.5	0.27^{c}	0.17

[a]Initial population density = number of fish in each of three aquaria at the start of the experiment.

[x,y,z]Means with different superscripts in the same column (food abundancy treatment) are significantly different from each other at $P \leq 0.05$ level.

[b]Means with the same superscript in the same column (food abundancy treatment) are not significantly different from each other.

[c]Means with the same superscript in the same column (population density treatment) are not significantly different from each other.

Table 7. Means for total length (mm) and wet weight (g) of male fathead minnows (Pimephales promelas) subjected to different feeding rates and population densities. N = pooled number of all male fish in three aquaria corresponding to a particular food abundancy or population density treatment at the end of the experiment. SD = standard deviation.

Experiment 4

Male Fish Growth Data

Treatment	N	Length	SD	Weight	SD
Food Abundancy					
ad libitum	31	62^{x}	7	2.6^{x}	0.9
66% ad libitum	26	55^{y}	9	1.8^{y}	0.8
33% ad libitum	23	47^{z}	8	1.0^{z}	0.5
Initial Population Density[a]					
25	22	55^{b}	11	1.9^{b}	1.2
50	24	56^{b}	9	2.0^{b}	0.9
100	34	56^{b}	9	1.9^{b}	1.0

[a]Initial population density = number of fish in each of three aquaria at the start of the experiment.

[x,y,z]Means with different superscripts in the same column (food abundancy treatment) are significantly different from each other at $P \leq 0.01$ level.

[b]Means with the same superscript in the same column (population density treatment) are not significantly different from each other.

($P \leq 0.01$); average weights were 2.6, 1.8, and 1.0 g, respectively (Table 7), and were significantly different from each other ($P \leq 0.01$).

Pooled data on egg size for female fish fed different food rations were also compared in like manner as the growth data. Mean egg sizes, 0.33, 0.31, and 0.24 (Table 6), for female fish fed ad libitum, 66 percent, and 33 percent ad libitum, respectively, were not found to differ statistically ($P \leq 0.2018$).

Three different population density treatments were compared to test if fish growth and reproduction are related to population size. Pooled data on growth of fish fed the three different food rations and raised in the initial population density of 25 fish, were compared to pooled data for those fish raised in the initial population density of 50 fish. Each of these data, in turn, were compared to the pooled data for fish raised in the initial population density of 100 fish. Population density had little or no effect on the parameters measured. Average lengths and average weights (Table 5) were not significantly different ($P \leq 0.9678$, 0.9083, respectively) for fish raised in the three population densities.

Similar comparisons on pooled growth data for female fish only and for male fish only were made. Female fish showed no significant differences ($P \leq 0.6212$, 0.6134) in their lengths and their weights, respectively, with different population densities (Table 6). Correspondingly, no significant differences were found for lengths and weights ($P \leq 0.9495$, 0.7706, respectively) of male fish raised in different population densities (Table 7).

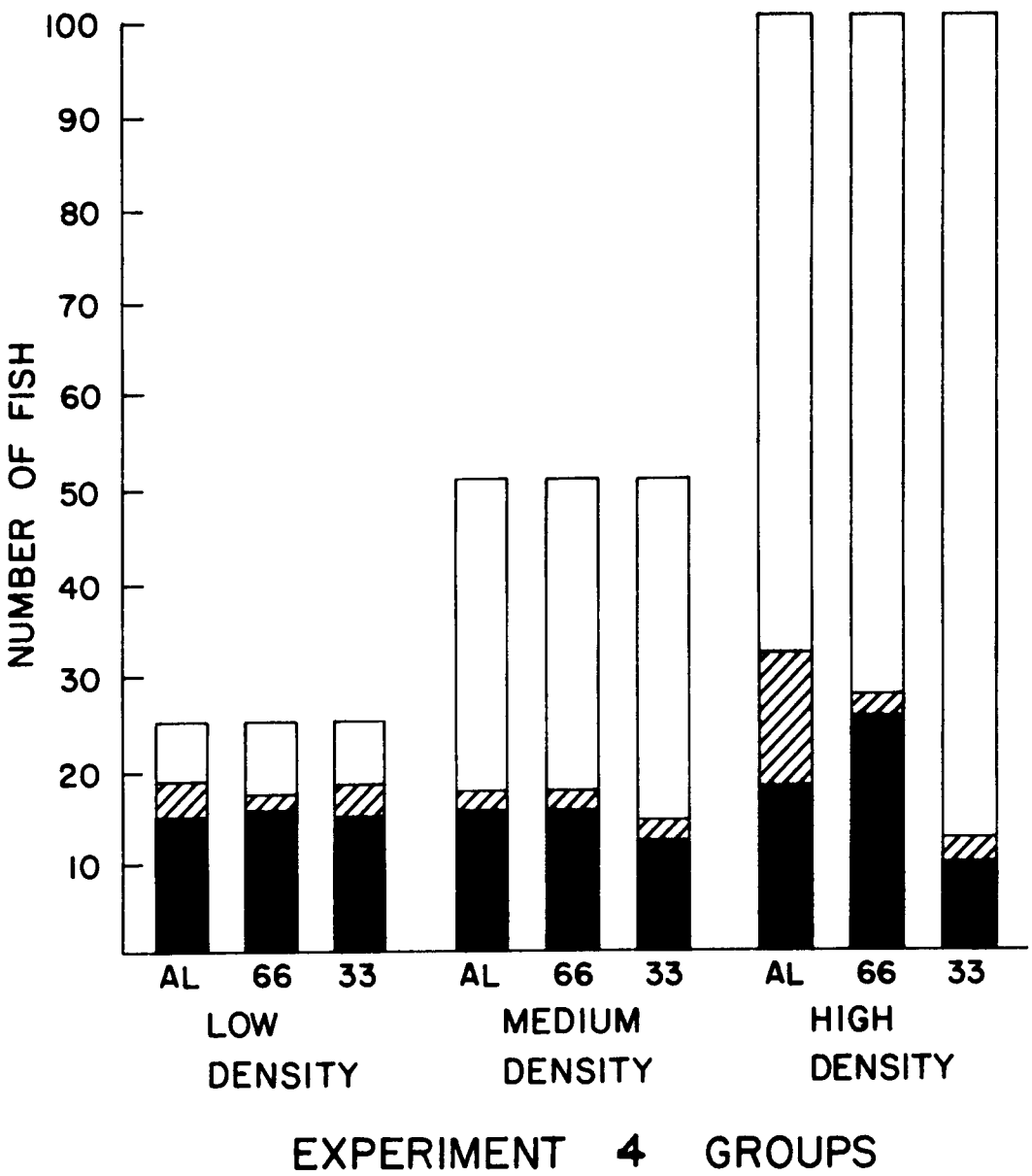
Pooled data on egg size from female fish stocked at different initial population densities were also compared. A different trend in egg size with different population densities was evident. Female fish produced eggs with average diameters of 0.33, 0.28, 0.27 when raised in the initial population densities of 25, 50, and 100, respectively (Table 6). However, these differences were not found to be statistically different ($P \leq 0.1933$). No interaction effect, due to nutrition and population density, was found for any of the factors tested.

High mortality in all experimental groups early in the study may be an explanation for finding no differences in growth of the fish raised under different initial population densities. Population levels under all experimental conditions became nearly equal during the first month of the study. Numbers of fish decreased from 100, 50, and 25 to numbers between 10 and 25 fish (Fig. 4) by the end of the study. Few fish were found dead, and it was the smaller fish that were discovered missing from each aquarium. I suspect cannibalism for the reduction in fish numbers.

Regression analyses of egg size on lengths and egg size on weight of female fish were performed and a low R^2 (0.36091, 0.37441, respectively) was reported for both variables. Regression analyses were then performed on transformed data. Length and weight were found to be exponentially related to egg size. Weight was related to egg size ($R^2 = 0.99551$) by the model:

$$\text{egg size} = b_0 10^{b_1 W^2}$$

Fig. 4. Numbers of fish (Experiment 4) in the initial population densities (plain bar); after the first month (hatched bar); and at the end of the experiment (black bar) for each experimental group. Numbers below bars indicate food ration given each experimental group. AL = ad libitum; 66 = 66 percent of ad libitum amount; 33 = 33 percent of ad libitum amount.


NUMBER OF FISH
100
90
80
70
60
50
40
30
20
10
AL
66
33
AL
66
33
AL
66
33
LOW DENSITY
MEDIUM DENSITY
HIGH DENSITY
EXPERIMENT 4 GROUPS

where $b_0 = 0.10686$ and $b_1 = 0.19357$. Length was related to egg size ($R^2 = 0.82224$) by the model:

$$\text{egg size} = 10^{L(b_0+b_1L)}$$

where $b_0 = -0.05946$ and $b_1 = 0.00092$. Figs. 5 and 6 illustrate the linear relationship of the transformed data of egg size on length and on weight, respectively.

Experiment 5. Pooled data on growth of fish in the two population densities fed twice daily were compared to fish fed six times a day. Growth was consistently greater for fish fed six times daily than for fish fed twice daily. Average lengths for fish fed twice and six times daily were 41 and 46 mm, respectively (Table 8), and were significantly different from each other ($P \leq 0.0003$). Mean weights were 0.8 and 1.1 g for fish fed twice and six times daily, respectively (Table 8), and were statistically different from each other ($P \leq 0.0003$).

Similarly, pooled data on growth for male fish only and for female fish only were compared. Male fish were consistently larger than female fish. In nature, male fathead minnows are generally larger than female fish of the same age. Average lengths for female fish fed twice and six times a day were 40 and 44 mm, respectively (Table 9), and were found significantly different from each other ($P < 0.01$). Mean weights, 0.6 and 0.9 g (Table 9) for female fish given different food rations were significantly different from each other ($P < 0.01$). Male fish fed twice and six times daily exhibited mean lengths of 43 and 50 mm, respectively (Table 10), and were found

Fig. 5. Plot of transformed data of Experiment 4; $\log_{10}$ egg size/length^2 on 1/length. A = one observation, B = two observations.

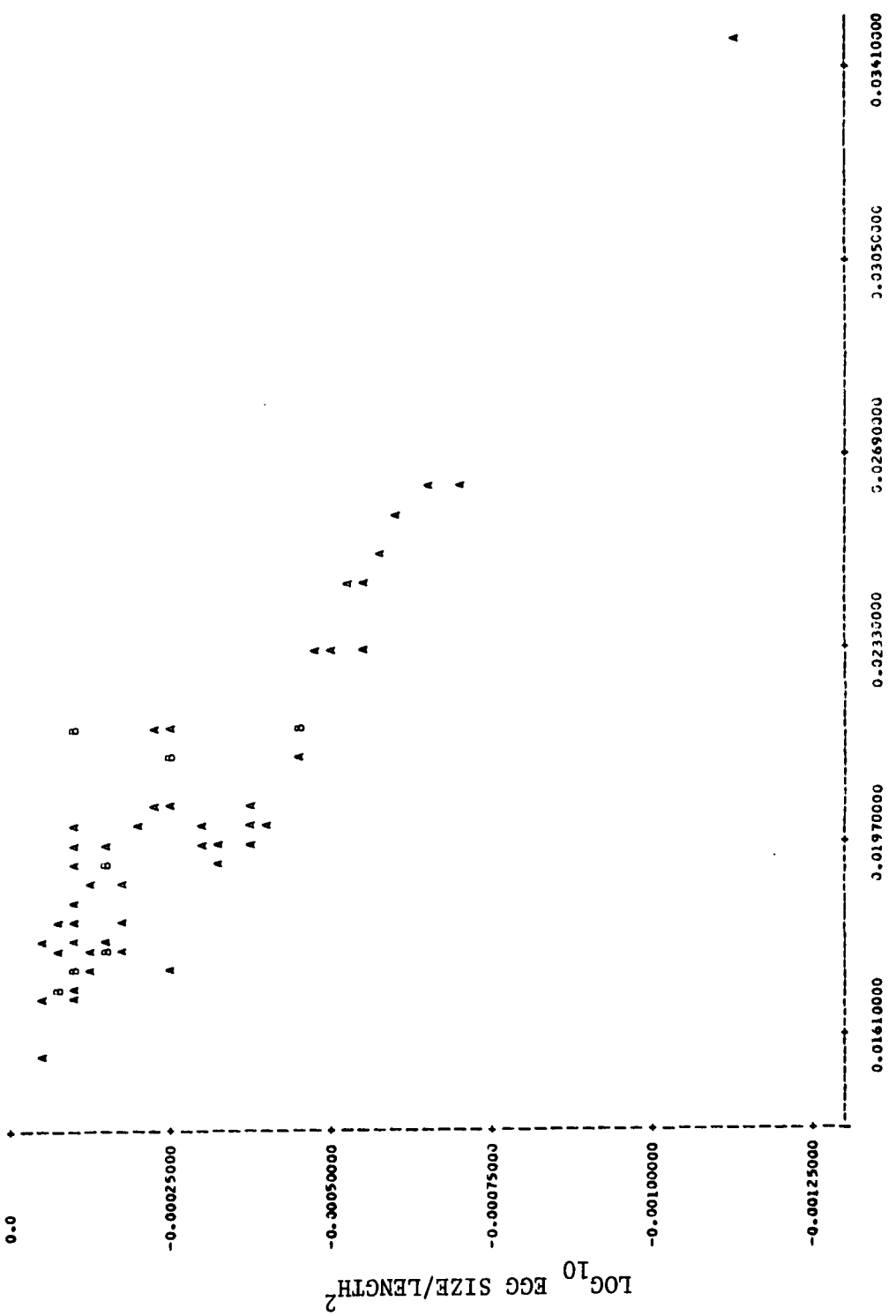

PLOT OF LEGL2 VS RECLEN
0.0
-0.00025000
-0.00050000
-0.00075000
-0.00100000
-0.00125000
0.01610000
0.01970000
0.02330000
0.02690000
0.03050300
0.03410300
LOG10 EGG SIZE/LENGTH2
1/LENGTH

Fig. 6. Plot of transformed data of Experiment 4; $\log_{10}$ egg size/weight^2 on 1/weight^2. A = one observation, B = two observations, C = three observations, D = four observations, H = eight observations, T = twenty observations.

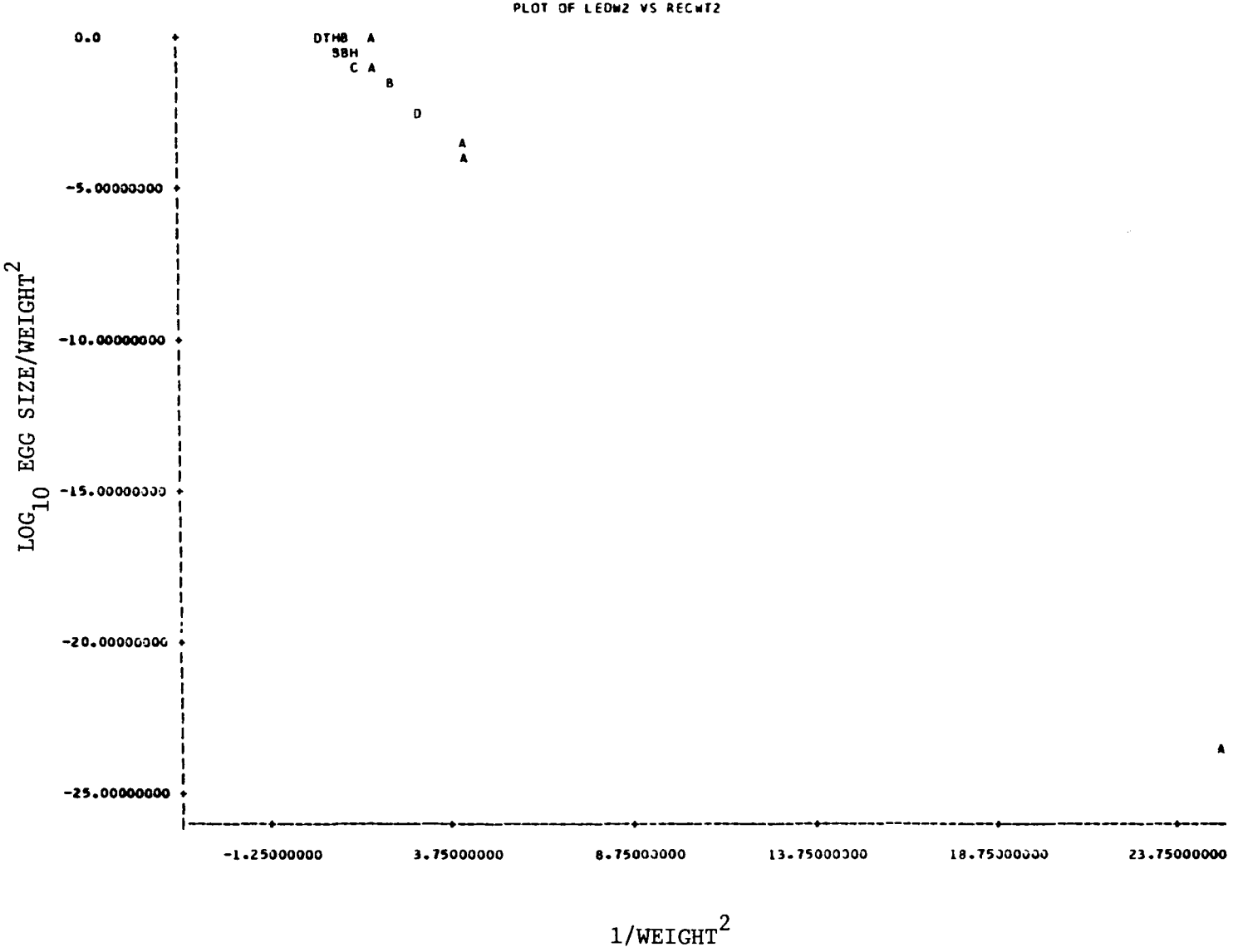

PLOT OF LEDW2 VS RECWT2
0.0
-5.00000000
-10.00000000
-15.00000000
-20.00000000
-25.00000000
DTHB A
SBH
C A
B
D
A
A
A
-1.25000000
3.75000000
8.75000000
13.75000000
18.75000000
23.75000000
LOG10 EGG SIZE/WEIGHT2
1/WEIGHT2

Table 8. Means for total length (mm) and wet weight (g) of fathead minnows (Pimephales promelas) subjected to different feeding rates and population densities. N = pooled number of all male and female fish in four aquaria corresponding to a particular feeding rate or population density treatment at the end of the experiment. SD = standard deviation.

	Experiment 5				
		Fish Growth Data			
Treatment	N	Length	SD	Weight	SD
Number of Feedings Daily					
2	109	41^{m}	9	0.8^{m}	0.6
6	111	46^{n}	8	1.1^{n}	0.7
Initial Population Density[a]					
15	55	47^{p}	8	1.2^{p}	0.7
45	165	42^{q}	9	0.8^{q}	0.6

[a]Initial population density = number of fish in each of four aquaria at the start of the experiment.

^{m,n}Means with different superscripts in the same column (number of daily feedings treatment) are significantly different from each other at $P \leq 0.0003$.

^{p,q}Means with different superscripts in the same column (population density treatment) are significantly different from each other at $P \leq 0.0003$.

Table 9. Means for total length (mm), wet weight (g), and egg size (mm) of female fathead minnows (Pimephales promelas) subjected to different feeding rates and population densities. N = pooled number of all female fish in four aquaria corresponding to a particular feeding rate or population density treatment at the end of the experiment. SD = standard deviation.

Experiment 5

Female Fish Growth and Egg Size Data

Treatment	N	Length	SD	Weight	SD	Egg Size	SD
Number of Feedings Daily							
2	52	40^{m}	6	0.6^{m}	0.3	0.19^{b}	0.16
6	69	44^{n}	5	0.9^{n}	0.3	0.25^{b}	0.20
Initial Population Density[a]							
15	31	43^{r}	5	0.9^{w}	0.4	0.28^{r}	0.21
45	90	41^{s}	6	0.7^{x}	0.3	0.21^{s}	0.17

[a]Initial population density = number of fish in each of four aquaria at the start of the experiment.

[m,n]Means with different superscripts in the same column (number of daily feedings treatment) are significantly different from each other at $P < 0.01$.

[r,s]Means with different superscripts in the same column (population density treatment) are significantly different from each other at $P < 0.05$.

[w,x]Means with different superscripts in the same column (population density treatment) are significantly different from each other at $P < 0.01$.

[b]Means with the same superscript in the same column (number of daily feedings treatment) are not significantly different from each other.

Table 10. Means for total length (mm) and wet weight (g) of male fathead minnows (Pimephales promelas) subjected to different feeding rates and population densities. N = pooled number of all male fish in four aquaria corresponding to a particular feeding rate or population density treatment at the end of the experiment. SD = standard deviation.

Experiment 5

Male Fish Growth Data

Treatment	N	Length	SD	Weight	SD
Number of Feedings Daily					
2	57	43^{m}	11	0.9^{m}	0.7
6	42	50^{n}	10	1.4^{n}	1.0
Initial Population Density[a]					
15	24	51^{w}	9	1.6^{w}	0.9
45	75	44^{x}	11	1.0^{x}	0.8

[a]Initial population density = number of fish in each of four aquaria at the start of the experiment.

[m,n]Means with different superscripts in the same column (number of daily feedings treatment) are significantly different from each other at $P < 0.003$.

[w,x]Means with different superscripts in the same column (population density treatment) are significantly different from each other at $P < 0.003$.

significantly different from each other ($P \leq 0.003$). Average weights were 0.9 and 1.4 g (Table 10) and were significantly different from each other ($P < 0.003$) for male fish fed twice and six times a day, respectively.

Pooled data on egg size for female fish given different food allowances were also analyzed in a similar manner as the growth data. Mean egg diameters were 0.19 and 0.25 mm for female fish fed twice and six times daily (Table 9) and were not statistically different from each other ($P \leq 0.2376$).

Similar comparisons on growth for fish raised with different population numbers were made. Pooled data on growth of fish fed twice and six times daily and raised in the initial population density of 15 fish were compared to pooled data for fish raised in the initial population density of 45 fish. Statistical results concerning effects of population density on growth were different from those found in Experiment 4. An inverse relationship is indicated between growth and population density. Length and weight were consistently greater for fish raised with 15 fish than for those raised with 45 fish. Average lengths for fish raised in initial population density of 15 fish and 45 fish were 47 and 42 mm, respectively (Table 8), and were significantly different from each other ($P \leq 0.0003$). Mean weights of 1.2 and 0.8 g were significantly different from each other ($P \leq 0.0003$) for fish raised with 15 fish and 45 fish, respectively (Table 8).

Similarly, pooled data on growth, each for female fish and for male fish raised in different initial population densities, were

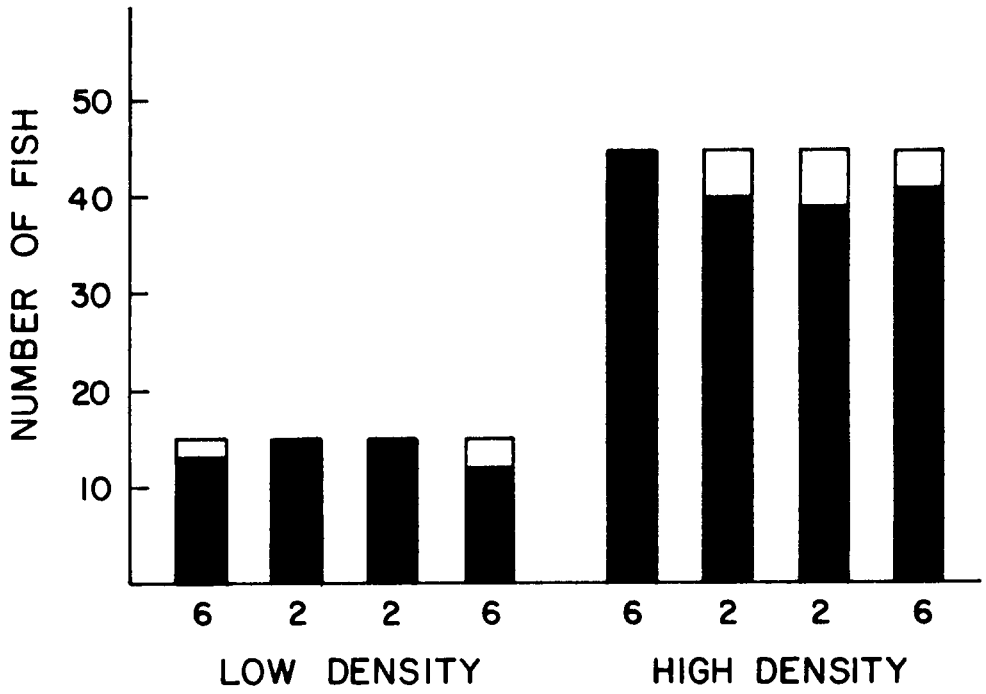
compared. Mean lengths for female fish reared in densities of 15 and 45 fish were 43 and 41 mm, respectively (Table 9), and were statistically different from each other ($P < 0.05$); average weights were 0.9 and 0.7 g (Table 9) and were significantly different from each other ($P < 0.01$). Male fish raised with 15 and 45 fish exhibited mean lengths of 51 and 44 mm, respectively (Table 10), and were statistically different from each other ($P < 0.003$). Average weights for males reared with 15 and 45 fish were 1.6 and 1.0 g, respectively (Table 10), and were statistically different from each other ($P < 0.003$).

Pooled data on egg size were also compared for female fish raised in the two initial population densities. An inverse relationship of egg size to population density is revealed by the data. Egg diameters averaged 0.28 and 0.21 for female fish raised with 15 and 45 fish, respectively (Table 9), and were found statistically different from each other ($P < 0.05$). No interaction effect, due to nutrition and population density, was found for any of the factors tested.

I believe differences between Experiment 4 and Experiment 5 with respect to effects of population density on growth and egg size were due to different mortality rates found in the two experiments. Unlike the high mortality found in Experiment 4, few fish died in Experiment 5 (Fig. 7); differential densities were maintained throughout the experiment.

Regressions of egg size on length and egg size on weight of female fish were performed and a low R^2 (0.36939 and 0.49033, respectively) was found for both analyses. The data were then transformed

Fig. 7. Numbers of fish (Experiment 5) in the initial population density of each experimental group are indicated by the height of the bar; black bars show number of fish at end of the study. Numbers under bars indicate number of feedings per day.



EXPERIMENT 5 GROUPS

and regression analyses were repeated. Length and weight were found to be exponentially related to egg size. Weight was related to egg size (R^2 = 0.98555) by model:

$$\text{egg size} = b_0 10^{b_1 W^2}$$

where b_0 = 0.09809 and b_1 = 0.33347. Length was related to egg size (R^2 = 0.82835) by model:

$$\text{egg size} = 10^{L(b_0+b_1 L)}$$

where b_0 = -0.07161 and b_1 = 0.00125. Figs. 8 and 9 show the linear relationship of the transformed data of egg size on length and egg size on weight, respectively.

Fig. 8. Plot of transformed data of Experiment 5; $\log_{10}$ egg size/length^2 on 1/length. A = one observation, B = two observations, C = three observations, D = four observations.

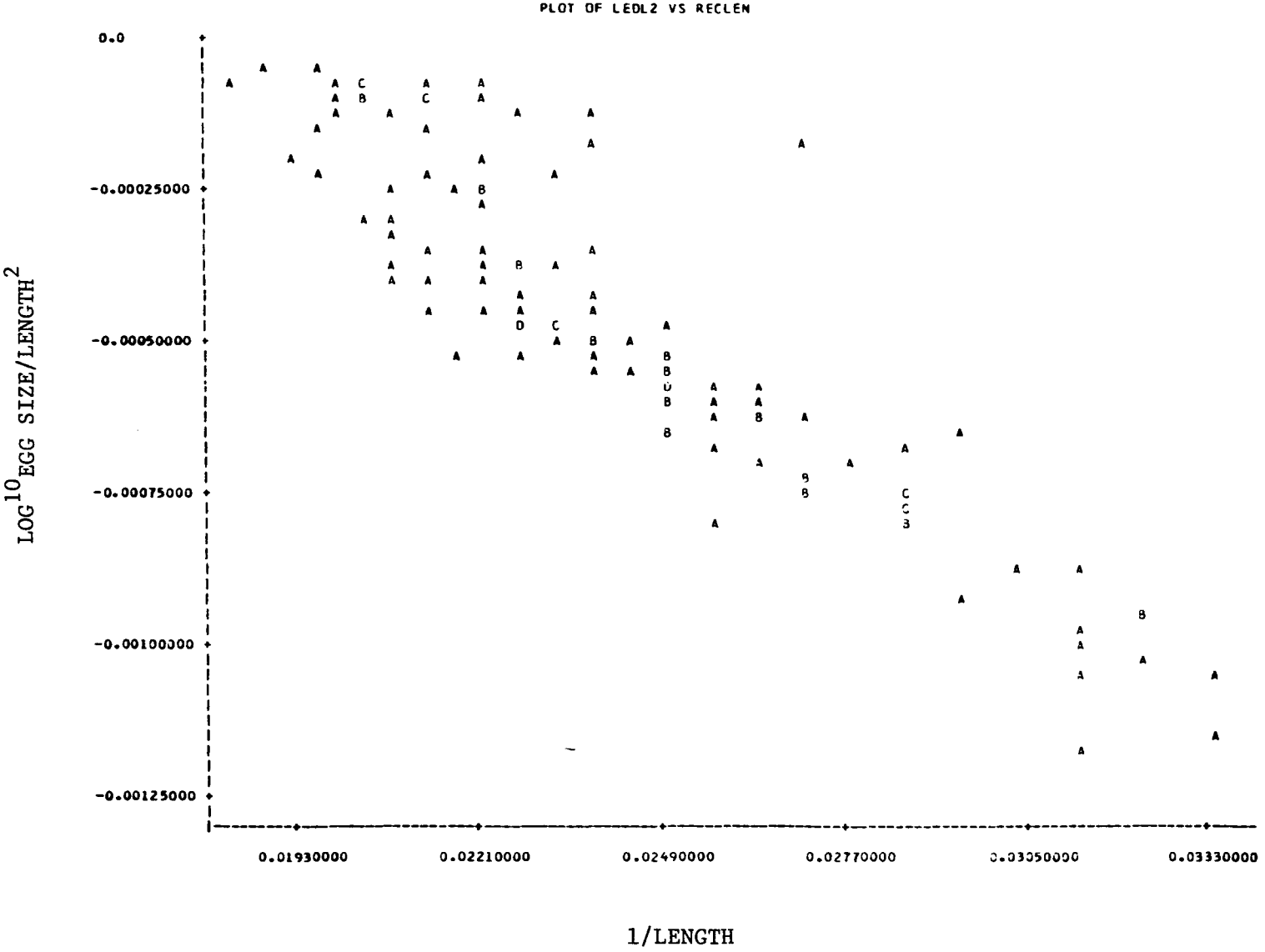
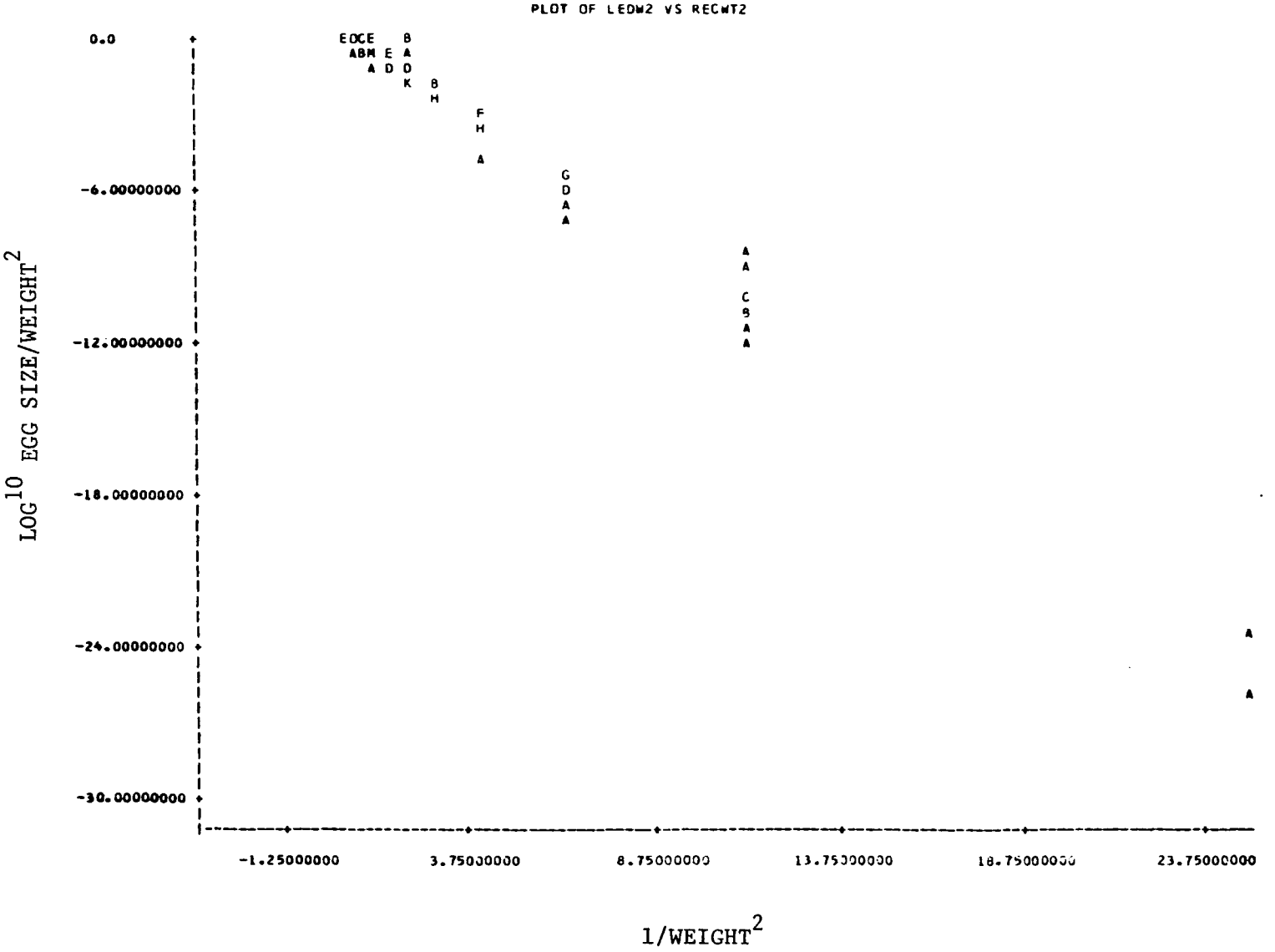

PLOT OF LEDL2 VS RECLEN
0.0
-0.00025000
-0.00050000
-0.00075000
-0.00100000
-0.00125000
LOG10EGG SIZE/LENGTH2
0.01930000
0.02210000
0.02490000
0.02770000
0.03050000
0.03330000
1/LENGTH

Fig. 9. Plot of transformed data of Experiment 5. $\log_{10}$ egg size/weight^2 on 1/weight^2. A = one observation, B = two observations, C = three observations, D = four observations, E = five observations, F = six observations, G = seven observations, H = eight observations, K = eleven observations, M = thirteen observations, O = fifteen observations.


PLOT OF LEDW2 VS RECWT2
0.0
-6.00000000
-12.00000000
-18.00000000
-24.00000000
-30.00000000
LOG10 EGG SIZE/WEIGHT2
-1.25000000
3.75000000
8.75000000
13.75000000
18.75000000
23.75000000
1/WEIGHT2

DISCUSSION

Genetic composition of the experimental fish, water temperature, population density, and nutrition are variables which may have affected growth and maturation rates between wild and laboratory fish stocks employed in the two experiments. Genetic differences between native pond fish used in Experiment 4 and laboratory reared minnows used in Experiment 5 may have partially been responsible for growth rate differences found in the two experiments because laboratory fish are selected for rapid growth and early maturity under laboratory conditions. Different diets used in Experiment 4 and Experiment 5 may have also been responsible for different growth rates between the experiments. Exogenous factors such as photoperiod and temperature influence reproductive cycles (De Vlaming 1972). Photoperiods were identical in both experiments, however, a difference in water temperatures of 5 C existed between Experiment 4 and Experiment 5 (20 and 25 C, respectively). Fathead minnows spawn within the range 18-30 C (Dobie et al. 1956; Flickinger 1971). The experimental conditions were well within this range. However, fish raised at 20 C water grew slower and failed to reach spawning condition by the end of one year, whereas, fish raised at 25 C water grew faster and appeared ready to spawn after 4 months. Alm (1953) and Le Cren (1965) found fish with a higher growth rate mature earlier than slower growing fish. Moreover, Le Cren (1965) stated that maturity is a function of size rather than age of fish. My data showed egg size was closely related to growth (i.e., length and weight) of the

fish. Most larger fish had actively developed eggs (i.e., eggs of various sizes), whereas smaller fish had small undeveloped eggs. Female fish with the larger eggs in both experiments were approximately the same length and weight. An optimum size is apparently necessary to stimulate the onset of maturation in fathead minnows. Although larger females had a greater propensity to have developing eggs, average egg size was comparatively smaller for the faster growing female fish (Experiment 5) than for the slower growing females (Experiment 4). Faster growing fish seemed to utilize available energy for growth, whereas, slower growing fish were also able to channel energy into egg production.

In addition to the different growth rates observed between fish in both experiments, I found growth to be directly related to food ration. Even though a statistical significance was not found, I believe a direct relationship of egg size with food ration also exists. Obviously, nutrition is required for energy supply for growth and gamete development. Also, my data appeared to indicate that increased population density in Experiment 5 may have limited growth and perhaps gamete development regardless of food abundance.

Factors establishing the final densities in the aquaria appeared to be operating. Water volume (i.e., space) may be an ultimate limiting factor for the optimum number of fish which can be supported within a contained area. Increased food increased the average weight per fish but not the number of fish which was supported by a particular volume of water in Experiment 4 and perhaps Experiment 5 (Figs. 4, 7

and Tables 5, 6, 7, 8, 9, 10). All treatments in Experiment 4 stabilized at nearly the same population density, which was an average of 0.17 (± 0.016 S.E.) fish/liter. This tolerance density (Dasmann 1964) of the aquaria was established almost completely during the first month of the study (Fig. 4). There was only very slight mortality evident in the fish stocked at low density in Experiment 5 (Fig. 7) where the mean number of fish/liter was 0.09 (± 0.005 S.E.). There was slight mortality of the fish stocked at the higher density, the average tolerance density appearing to be 0.26 (± 0.008 S.E.) fish/liter. This value may be somewhat of an underestimation of the actual tolerance density for this laboratory stock, because mortality was limited; i.e., the upper limits of tolerance density may not have been reached. Nevertheless, one would not expect any mortality of the fish stocked at the lower density. Such mortality was not seen, therefore, supporting my findings. Although the tolerance densities established within the two experiments were slightly different (t' -test, P = 0.01; Snedecor and Cochran 1967), they still are in extremely close agreement, considering the vastly different genetic histories of the stocks, the physical conditions, and feeds.

I was interested in comparing the tolerance densities of the fish populations in this study with the suggested rearing densities for fathead minnows under intensive fish culture conditions in ponds. Data presented by Dobie et al. (1956) and Flickinger (1971) were used to develop the following argument. The optimum stocking density of fathead minnow fry into ponds is 100,000-300,000 fish/0.4 ha (1 acre), with the maximum suggested for commercial farming being 600,000 fish/

0.4 ha. The average depth of a pond for rearing these fish was 91.4 cm (3 ft). If 600,000 fry were stocked into a 0.4 ha pond, 91.4 cm deep, then the density of fish would be 0.16 fish/liter. This density derived from fish farming ponds is in remarkably close agreement with the tolerance density of 0.17 fish/liter established in the small, 100-liter laboratory tanks.

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APPENDIX

Appendix Table I. Weekly food calculations.

Treatment	Food Amounts (GM)						
Food Ration/ Initial Pop. Density	8/11-8/17	8/18-8/25	8/26-8/31	9/1-9/7	9/8-9/14	9/15-9/21	9/22-9/29
AL[a]/25	0.0029	0.0029	0.0039	0.0049	0.0114	0.0114	0.0230
33%[b]/25	0.0007	0.0007	0.0013	0.0016	0.0035	0.0035	0.0073
66%[c]/100	0.0057	0.0057	0.0105	0.0131	0.0099	0.0099	0.0200
33%/50	0.0014	0.0014	0.0026	0.0033	0.0052	0.0052	0.0105
AL/100	0.0115	0.0115	0.0157	0.0198	0.0255	0.0255	0.0513
66%/50	0.0029	0.0029	0.0052	0.0066	0.0067	0.0067	0.0135
66%/25	0.0014	0.0014	0.0026	0.0033	0.0063	0.0063	0.0129
33%/100	0.0029	0.0029	0.0052	0.0066	0.0033	0.0033	0.0067
AL/50	0.0057	0.0057	0.0079	0.0099	0.0145	0.0145	0.0292
	9/30-10/5	10/6-10/12	10/13-10/19	10/20-10/26	10/27-11/2	11/3-11/9	11/10-11/16
AL/25	0.0375	0.0685	0.0922	0.1110	0.1220	0.1806	0.2250
33%/25	0.0118	0.0215	0.0226	0.0272	0.0300	0.0444	0.0552
66%/100	0.0325	0.0592	0.0803	0.0968	0.1064	0.1576	0.1968
33%/50	0.0171	0.0311	0.0339	0.0409	0.0450	0.0667	0.0552
AL/100	0.0834	0.1522	0.1562	0.1881	0.2067	0.3061	0.2616
66%/50	0.0220	0.0401	0.0607	0.0731	0.0805	0.1192	0.1174
66%/25	0.0209	0.0381	0.0515	0.0620	0.0682	0.1010	0.1329
33%/100	0.0110	0.0200	0.0190	0.0229	0.0252	0.0374	0.0509
AL/50	0.0475	0.0868	0.0827	0.0996	0.1096	0.1622	0.1910
	11/17-11/23	11/24-11/30	12/1-12/7	12/8-12/14	12/15-12/21	12/21-1/4	1/5-1/11
AL/25	0.2373	0.2373	0.2504	0.2686	0.2863	0.2863	0.2913
33%/25	0.0583	0.0583	0.0615	0.0660	0.0700	0.0700	0.0711
66%/100	0.2076	0.2076	0.2191	0.2350	0.2286	0.2286	0.2326
33%/50	0.0583	0.0583	0.0615	0.0660	0.0642	0.0642	0.0654
AL/100	0.2759	0.2759	0.2912	0.3123	0.2863	0.2863	0.2913
66%/50	0.1238	0.1238	0.1307	0.1402	0.1493	0.1493	0.1519
66%/25	0.1402	0.1402	0.1480	0.1587	0.1682	0.1682	0.1711
33%/100	0.0537	0.0537	0.0567	0.0608	0.0548	0.0548	0.0558
AL/50	0.2014	0.2014	0.2126	0.2280	0.2262	0.2262	0.2301

Appendix Table I. Weekly food calculations (continued).

Treatment	Food Amounts (GM)				
Food Ration/ Initial Pop. Density	1/11-1/18	1/19-1/25	1/26-2/1	2/1-2/7	2/8-2/13
AL/25	0.2913	0.3090	0.2880	0.2880	0.2880
33%/25	0.0711	0.0755	0.0703	0.0703	0.0703
66%/100	0.2326	0.2794	0.2604	0.2604	0.2604
33%/50	0.0654	0.0642	0.0599	0.0599	0.0599
AL/100	0.2913	0.3090	0.2880	0.2880	0.2736
66%/50	0.1519	0.1611	0.1502	0.1502	0.1502
66%/25	0.1711	0.1815	0.1692	0.1692	0.1692
33%/100	0.0558	0.0540	0.0540	0.0540	0.0540
AL/50	0.2301	0.2441	0.2275	0.2275	0.2275
	2/14-3/14	3/15-3/22	3/23-3/29	3/30-4/13	4/14-5/14
AL/25	0.2880	0.2933	0.2933	0.3405	0.3405
33%/25	0.0703	0.0716	0.0716	0.0832	0.0832
66%/100	0.2604	0.2652	0.2652	0.3079	0.3079
33%/50	0.0599	0.0610	0.0610	0.0708	0.0708
AL/100	0.2736	0.2700	0.2700	0.3235	0.3235
66%/50	0.1502	0.1529	0.1529	0.1775	0.1775
66%/25	0.1692	0.1723	0.1723	0.2000	0.2000
33%/100	0.0540	0.0513	0.0513	0.0596	0.0596
AL/50	0.2275	0.2205	0.2205	0.2690	0.2690

[a] AL = ad libitum.

[b] 33% = 33 percent of the ad libitum amount.

[c] 66% = 66 percent of the ad libitum amount.

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VARIOUS ASPECTS OF REPRODUCTIVE CONTROL IN FRESHWATER FISHES

by

Helen T. Smith

(ABSTRACT)

Male and female guppies (Poecilia reticulata) were treated with either methyl testosterone, cyproterone acetate (CA) (an antiandrogen) or a combination of the two steroids to determine effects on behavior, coloration, and gametogenesis. The antiandrogen did not fully block the effects of endogenous or exogenous textosterone in enhancing male sexual behavior and coloration in males or females. An inverse relationship existed between the numbers of cysts in the early stages of spermatogenesis and the number of spermatophores for males receiving any of the steroids. Only one female in the testosterone and combined steroid treatments had embryos present in its ovaries.

CA was administered orally to juvenile Japanese medaka (Oryzias latipes) in two experiments to determine the possible role of the antiandrogen on sex determination. Sex ratios were determined in the mature fish by dimorphic coloration, secondary sex characters, and histological examination. Sex reversal was not established in the fish.

The effects of population density and nutrition on growth and fecundity of fathead minnows (Pimephales promelas) were studied. Length and weight of the fish increased with increased food availability.

Perhaps a direct relationship of egg size with food ration also existed. Egg size was found exponentially related to growth of fish. Increased population density appeared to limit growth and perhaps gamete development regardless of food abundance. Additionally, water volume appears to limit the number (tolerance density) of fish which can be supported within a specific area. Increased food increased weight per fish but not number of fish in a particular volume of water.