ASPECTS OF FATHEAD MINNOW REPRODUCTIVE BEHAVIOR

THESIS

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By

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Following a study of normal reproductive behavior of fathead minnows, Pimephales promelas, experiments were conducted to determine the stability/variability of behavior by sexually mature, territorial males under a variety of manipulated conditions. Collectively, these experiments indicate that although the individual behaviors of fathead minnows appear to be quite variable, the overall process, reproductive behavior, is stable.

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CHAPTER I

INTRODUCTION

Various aspects of the biology of the fathead minnow, Pimephales promelas Raf., have been studied including distribution (Lee et al. 1980), life-cycle (Markus 1934; Isaak 1961; McMillan and Smith 1974), and role as a bioassay subject (Rand and Petrocelli 1985). Although a large volume of literature exists, many unknown questions remain concerning fathead minnow biology. The reproductive behavior of fathead minnows has yet to be fully described (Cole and Smith 1987). This research examined several hypotheses concerning the reproductive biology of fathead minnows, including whether or not the reproductive behavior can be used as a bioassay (Rand 1985).

Reproduction is important to all organisms for survival of the species. A large variety of reproductive methods have evolved among vertebrates, from solitary to communal. Mating systems can be monogamous or polygamous (Willson 1984). Fishes have a large range of reproductive "strategies" and behaviors, often with variations present within the same family or genus (Breder and Rosen 1966). Reproductive behavior including courtship is an important aspect of reproduction. Courtship includes behaviors which bring both genders to the same spawning site. Courtship

behavior provides cues for species recognition, assessment of reproductive ability (Krebs and Davies 1981) and identification of gender.

Reproductive behavior in the fathead minnow has been described in the field and laboratory (McMillan and Smith 1972; Cole and Smith 1987). Males enter shallow waters along stream and lake margins to establish territories around potential spawning sites. Sites can be the undersides of floating or submerged objects including roots, logs, or rock cobble (Marcus 1934; Isaak 1961; Andrews and Flickinger 1973). Male courtship behavior has been described by Cole and Smith (1987) including "leading" behavior which a male leads a female to his prospective spawning site. Sexually mature female P. promelas which enter male territories will oviposit. The males guard the eggs until hatching.

The behavior of territorial male fathead minnows in the presence of mature females has been described (McMillan and Smith 1974; Cole and Smith 1987) but little research has addressed the behavior of a territorial male in the presence of males (mature or immature) and immature females. Part of this research was designed to determine if the behaviors of a territorial male towards immature females, immature and mature males were different than behaviors towards a mature female.

Male fathead minnows have been reported to suffer high post-spawning mortalities (Markus 1934; Flickinger 1973; Unger 1983). High post-spawning mortalities seem to indicate that spawning stresses individual fish. One method to assess spawning stress is to compare physiological performance of nonspawned and recently spawned fishes, such as their ability to withstand high temperatures. Critical thermal maximum (CTM) has been used to assess stress on animals by comparing the temperatures where loss of equilibrium occurs, while temperature is raised at a slow, constant rate (Silbergeld 1973; Paladino and Spotila 1978). A lower tolerance of high temperature might be expected of recently-spawned and hence stressed fish, as compared to fish which have never spawned.

Fish have been exposed to steroid hormones for both research and applied fishery management (Schreck 1974; Donaldson and Hunter 1981; Yamazaki 1983); however, the effect of estradiol-17β on gender change in fathead minnows is unknown. In addition, little is known of effects upon fish by administered steroid hormones other than gender change, particularly the effects upon behavior (Billy and Liley 1985). If behavioral changes occur, will these changes influence the success of these fish?

Behavior has been used as a bioassay technique to measure stress at sublethal and environmentally realistic

or observed concentrations of a toxicant (Rand 1985). Environmental concentrations of a chemical may have a negative effect on a behavior of an organism, such as preference/avoidance behavior (see reviews Giattina and Garton 1983; Beitinger and Freeman 1983), and mortalities may occur. Negative effects of a chemical on reproductive behavior may lead a population to extinction. The assay conducted in this research examined the reproductive behavior of fathead minnows after 24-hour exposures to three concentrations of selenate-selenium. These concentrations range from sublethal to lethal in previous studies (Adams and Johnson 1981; Watenpaugh and Beitinger 1985a). Selenium has been identified as a common pollutant in wastewaters of industry (Andren et al. 1975), and selenium has been found as high as 10 mg/l in reservoirs and lakes (Lemly 1985). The null hypothesis tested in these experiments was that sublethal concentrations of selenium will not cause a significant difference in reproductive behaviors of exposed fathead minnows. In total, this research addresses several previously unstudied basic and applied aspects of the reproductive biology of fathead minnows.

CHAPTER II

UPPER THERMAL TOLERANCE IN THE FATHEAD MINNOW, *PIMEPHALES PROMELAS*, AFTER SPAWNING

Previous researchers have commented on the high postspawning mortality in fathead minnows (Markus 1934; Unger 1983; Flickinger 1973) although not all reports have supporting data (Gale and Buynak 1982). Flickinger (1973) found large mortalities of these minnows between spring stocking and fall harvesting. Greatest mortalities occurred in ponds in which fathead minnows produced most offspring, and mortality rates as high as 91% were reported. Unger (1983) stated that a male fathead minnow "... might benefit by displaying epigamic features that exaggerate apparent size, conceal physical handicaps, or obscure deficient energy reserves." Perhaps "weak" males are not obvious to an observer until they die. The energetic demands of reproduction may be a primary factor of senescence and death in fishes (Woodhead 1979).

The objective of this research was to determine if a single spawning event influences the physiological well-being of post-spawning fathead minnows. Upper temperature tolerance measured as the critical thermal maximum (CTM) was used as a bioassay method to assess physiological well-being. Determination of CTM in a fish

involves increasing water temperature at a constant rate until a fish exhibits a definitive endpoint. Previous research by others (Silbergeld 1973; Paladino and Spotila 1978; Becker and Wolford 1980), and in our laboratory (e.g. Watenpaugh and Beitinger 1985a, Watenpaugh et al. 1985, Carrier and Beitinger in press; Rutledge and Beitinger in press) has shown the CTM of fishes to be sensitive to several abiotic factors. This research proposes to test a hypothesis that a biotic factor or process such as spawning, which decreases a species' ability to tolerate changes in temperature, would also adversely affect other physiological, ecological, and life-history processes and ultimately would contribute to the reported increase in post-spawning mortality. Previous research on costs of reproduction have measured reproductive output by the number of gametes produced per parent, the biomass of gametes produced per biomass of parent (see the review by Calow 1979), or have limited food input and measured size and egg production (Wooton 1977). No research in the area of effects of reproduction on thermal tolerance has been published to my knowledge.

Materials and Methods

Fathead minnows were obtained from a culture maintained

at North Texas State University. The fish were raised in dechlorinated tap water in aquaria (either 110 or 38 liter) at temperatures ranging from 22 to 26°C (controlled with heater units). All test fish were sexually mature (more than 6 months from date of hatch). Aquaria for spawning contained nesting sites consisting of PVC pipe (ID = 7 cm) about 8 cm long which were cut in half lengthwise. Control fish (not having spawned) were maintained under identical abiotic conditions but at higher densities and without nesting sites. All fish were fed twice daily with flake food supplemented with frozen and freshly hatched brine shrimp (Artemia).

Critical thermal maxima (CTM) were determined for 13 males (mean total length = 5.2 cm, SD = 0.7) and 14 females (mean total length = 4.0 cm, SD = 0.2) which had spawned within the previous 24 hours. CTM methods were those of Becker and Genoway (1979) with a 0.3°C·minute^{-1} rate of temperature increase and first loss of equilibrium as the endpoint (see Watenpaugh et al. 1985 for details). Also, data were collected for 12 males (mean total length = 4.9 cm, SD = 0.8) and 18 females (mean total length = 3.7, SD = 0.1) which had not spawned (controls). During CTM trials, water temperature was raised with two circulating heating units and monitored with a digital thermometer, sensitive to 0.01°C. Fish were placed individually in 2-liter

aquaria after spawning was witnessed or before testing of control fish. Trials occurred between 0900 and 1100 hours, and treatment (post-spawn) fish were then returned to aquaria with its original mate. No attempt was made to spawn the control fish. Gender was determined from body coloration, presence or absence of an enlarged urogenital structure (Flickinger 1969), or the ratio of anal fin length to total length as described by Lewis (1977). Following CTM trials, mass (to 0.1 g) and total length (to 1.0 mm) were measured, and Fulton's condition factor was calculated to determine if control and treatment fish had similar body conditions.

Data were subjected to parametric one- and two-way analyses of variance SAS (1985) after Shapiro-Wilk W tests showed both CTM and condition factor were normally distributed ($p = 0.09$ and 0.10, respectively).

Results and Discussion

Mean CTMs of the four fathead minnow groups ranged from 36.0 to 36.9 °C (Table 2) and were not significantly different (parametric one-way AOV, $F = 1.02$, $p = 0.39$). In addition, a parametric two-way AOV indicated no significant differences in mean CTMs between spawned or

Table 1.-Mean critical thermal maxima, CTM, and mean condition factor ± SD (n in parentheses) for fathead minnows recently spawned and not having spawned, by gender.

	CTM (°C)	Condition Factor
Male		
Non-spawn	36.17 ± 1.22 (12)	1.13 ± 0.24 (9)
Post-spawn	36.67 ± 1.38 (13)	1.30 ± 0.18 (12)
Female		
Non-spawn	36.91 ± 0.86 (18)	1.16 ± 0.30 (18)
Post-spawn	36.77 ± 1.40 (13)	1.36 ± 0.32 (13)

or female fishes (F = 1.89, p = 0.17). Also interaction between gender and reproductive status was not significant (F = 0.98, p = 0.33). Statistical significance was not gained despite the observation that coefficients of variation for control males and females were only 3.4% and 2.4%, respectively. Neither guarding a territory nor spawning a single clutch of eggs appear to weaken male or female fathead minnows such that an observable difference in thermal tolerance occurs.

Surprisingly, mean condition factors were not significantly different between non-spawning and post-spawning females (t = 1.79, p = 0.08) or males (t = 1.82, p = 0.08). Reproductive males might be expected to have different condition factors from nonreproductive males

from the presumed energetic costs of defending a territory, physiological changes, and spawning. Unger (1983) showed that some males gain water weight possibly to increase their apparent size to rival males. A gain in weight but not length would increase the condition factor. Competition for size would not be expected among females, and no difference was seen in condition factor for spawning and non-spawning females. Also, CTMs and condition factors were not correlated ($r = 0.17$, $p = 0.22$), suggesting that minnows with higher condition factors do not have greater thermal tolerances. This finding was expected, since neither CTM nor condition factor were highly variable in this research.

Finally and importantly, 9 of 13 males (69.2%) and 10 of 13 females (76.9%) spawned within 5 days following CTM testing. Exposure to CTM-level temperature does not appear to impair the future reproductive ability of minnows.

My results show that one spawning event does not decrease thermal tolerance of male or female fathead minnows. Temperature tolerance of fathead minnows may not be decreased by one spawning, but possibly by many spawning events.

CHAPTER III

THE BEHAVIOR OF MALE FATHEAD MINNOWS IN THE PRESENCE OF AN ADDITIONAL MALE OR FEMALE FATHEAD MINNOW

The reproductive behavior of the fathead minnow, Pimephales promelas, has been described both in the laboratory (McMillan and Smith 1974; Cole and Smith 1987) and field (Isaak 1961). McMillan and Smith (1974) observed spawning behavior, egg-laying and fertilization of fathead minnows in the laboratory. Male courtship behavior in the laboratory was described by Cole and Smith (1987) as leading behavior, a "rapid, straight-line or a distinctive zig-zag swimming motion from the female to the mouth of, or interior of, the pot" i.e., male territory. To my knowledge, no attempt has been made to compare courtship behavior by the male in the presence of a female relative to the behavior of a male in the presence of another male fathead minnow.

Agonistic behavior by territorial males toward males has been witnessed (Markus 1934; McMillan and Smith 1974; Unger 1983). Aggressiveness is related to body coloration and presence or absence of eggs (McMillan 1972). I attempted to determine if courtship-like behaviors of a territorial male occur in the presence of another male.

Methods

Fathead minnows, 6 to 10 months old, were obtained from a stock culture at North Texas State University. The fish were raised in dechlorinated tap water in aquaria (either 110 or 38 liter) at 22 to 26°C (controlled with electric heater units) under a 18L:6D photoperiod. Fish were fed twice daily with TetraMin staplefood flake food supplemented with frozen and freshly hatched brine shrimp (Artemia).

Behavioral data from observation trials (1000 seconds duration) were collected manually on a portable computer. The program, written by Dr. J. Baylis (Department of Zoology, University of Wisconsin, Madison) recorded behaviors and the time of occurrence, such that frequencies and durations of behaviors were available from the output. A dither effect was provided by five white cloud minnows, Tanichthys albonubes. This technique involves supplying a continuous stimulus so that the behavior of the observation animals is not affected by the presence of an observer (see Barlow 1977; Cole and Smith 1987). The 38-liter aquaria used for behavioral observation contained a nesting site consisting of PVC pipe (7 cm ID) about 8 cm long, cut in half lengthwise. One territorial male exhibiting breeding coloration, tubercles, and dorsal pad was added to each of

two observation aquaria; each male subsequently defended the PVC pipe nesting site. A single additional (= test) fish, either male or female, was added at least 30 minutes prior to the collection of behavioral data. After each recording session, the gender of the test fish was determined by internal gonad inspection. Gonadal somatic indices (GSI) were determined by dividing the wet weight of the gonad by the wet weight of the fish. GSI's were not determined for territorial males. The numbers of trials in which the added (test) fish were female and male were 33 and 34, respectively. A video camera recorded trials, and playback sessions helped data acquisition by allowing remote data collection in one aquarium and direct visual observation in another. All sessions occurred from either 0700 to 1000 or 1600 to 1800 and at least 30 minutes after feeding. Territorial males appeared to be most aggressive following feeding at these times, as reported in other studies (e.g. Smith 1970).

The following eight behaviors from McMillan and Smith (1974) and/or Cole and Smith (1987) were monitored during trials:

1. Approach behavior - male approaches test fish.
2. Leading behavior - male swims from near the test fish directly to his territory and the other fish follows. This is not the same behavior as defined

by Cole and Smith (1987). Difficulty was encountered determining whether the territorial male was simply returning to the territory or "leading" a possible partner.

3. Lateral display - the male moves in front of, or at right angles to the test fish and hovers, extending dorsal, caudal, anal, and pectoral fins (same as Cole and Smith 1987).
4. Jumpswim - the male makes a quick upward swimming motion, turning onto his side, and then quickly swims downward (same as Cole and Smith 1987).
5. Tailbeating - the male moves the caudal fin toward another fish, which may direct a current toward the fish. (Lateral quiver of Cole and Smith seemed to be the same as tail-beating as defined by McMillan and Smith 1974).
6. Vibrating - The male and female come in close side-by-side contact and vibrate against each other. This was only observed when females were in territories and is part of spawning behavior (McMillan and Smith 1974).
7. Butting - the male moves toward the other fish and pushes at it with the snout. (same as McMillan and Smith 1987).
8. Chasing - the male rapidly moves toward and

pursues the other fish. (same as McMillan and Smith 1987).

The movements of male and female fatheads in and out of the territory were monitored as behaviors. Contact of the territory ceiling with the dorsal pad of the territorial male, i.e., rubbing behavior, was not included in my analyses. This action occurred during almost every entrance to the territory by the male and was difficult to quantify, because a male would make many contacts with the ceiling while in the territory. Also it was impossible to observe rubbing behavior in recorded sessions when the video camera was focused on the entire aquarium. No egg laying was witnessed as a result of spawning behavior.

Frequencies of behaviors were analyzed by comparing the territorial male responses to test female and test male fish via the Mann-Whitney two-sample U test. A preceding-following diad analysis was performed on male responses to female and male test fish and compared using the Mann-Whitney two-sample U test. Statistical analyses were performed by SAS (1985) with $\alpha = 0.05$.

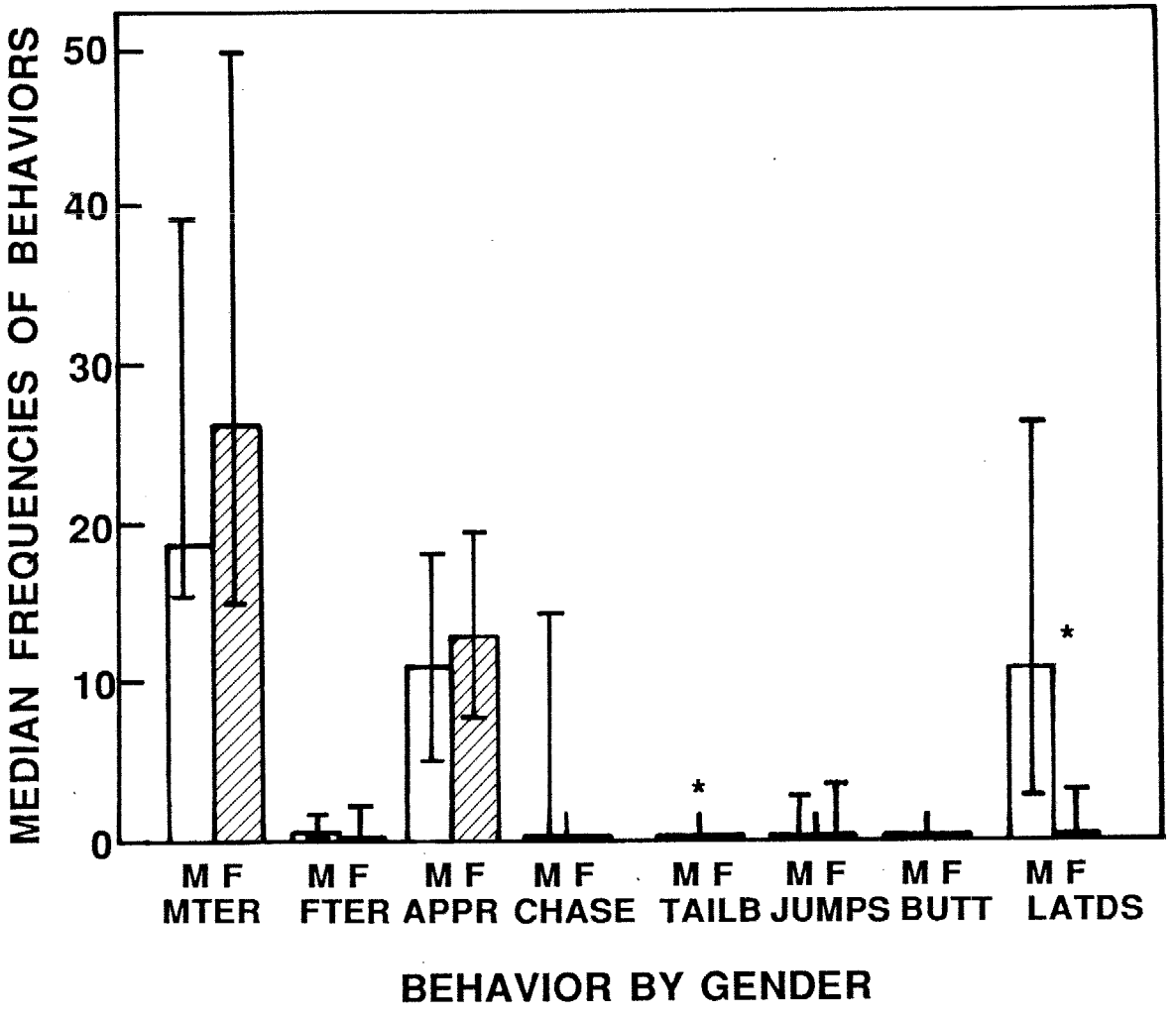
Results

The frequencies of most monitored behaviors of

territorial males (in and out of territory, approach, chase, jumpswim, butting, and leading) were not significantly different in the presence of male or female fish (Fig. 1). However, there was a significant difference (increase) in numbers of tailbeats by territorial males toward test males than test females. Females elicited little lateral display behavior from territorial males (median of 0) compared to a median of 10.5 when the test individual was a male. Mean ($\pm$SD) GSI's were 6.2% (3.1) and 1.3% (0.4) for females and males, respectively.

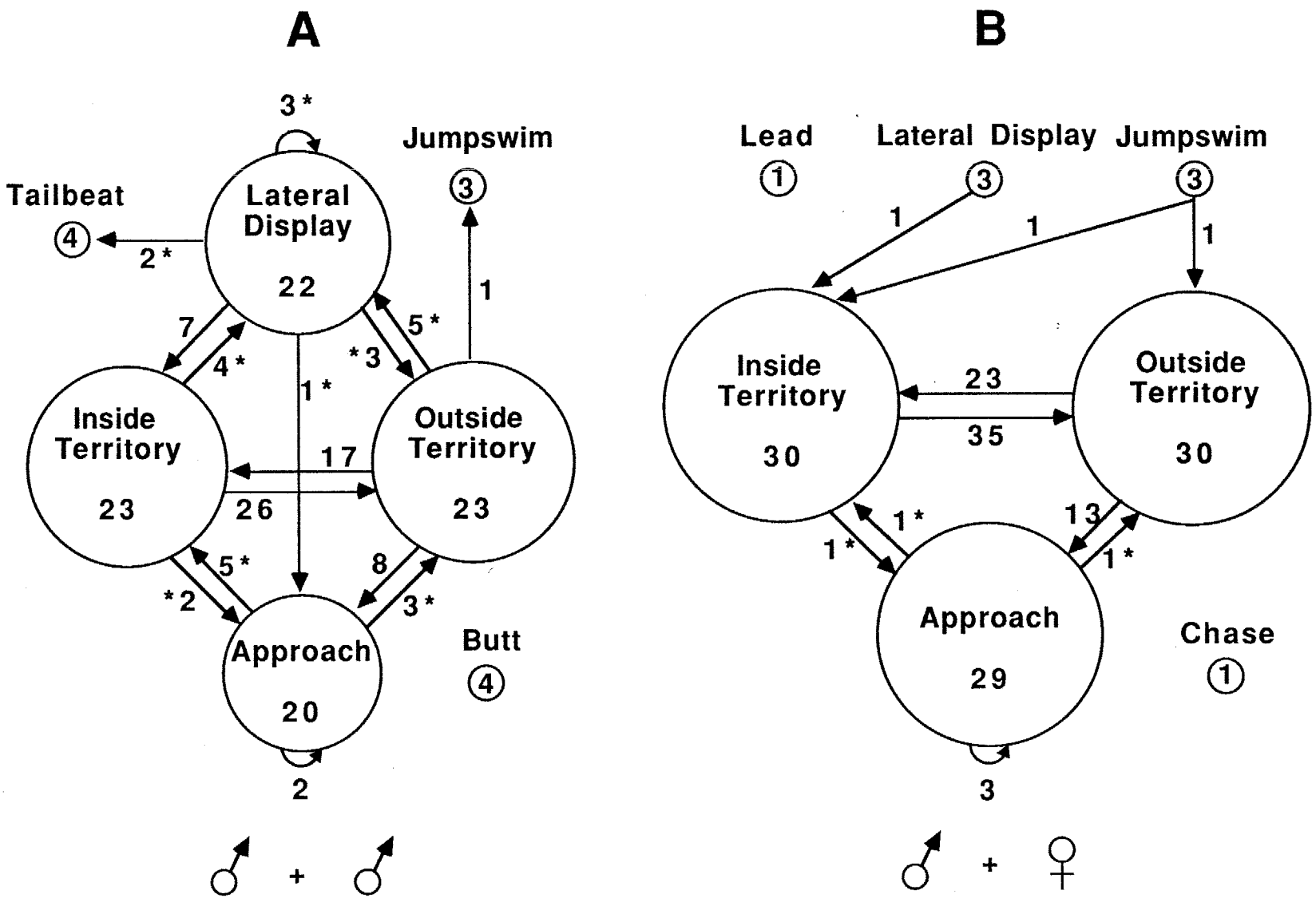
Behavioral sequences found to be significantly different using the Mann-Whitney U test by male or female addition (Figure 2) include inside territory to lateral display, lateral display to inside territory, outside territory to lateral display, lateral display to outside territory, approach to inside territory, inside territory to approach, lateral display to tailbeat, lateral display to lateral display, and lateral display to approach. Jumpswim occurred in similar frequencies in the presence of male and female test fish.

Figure 1.-Median frequencies of observed behaviors during 1000-second periods. Interquartile ranges are indicated by vertical lines. MTER and FTER refer to frequencies of male and female fish entering the territory, respectively. Other behaviors depicted are approach, chasing, tailbeat, jumpswim, butting, and lateral display.



* Indicates significance between genders using a Mann-Whitney two-sample U test ($\alpha = 0.05$)

Figure 2.-Proportional frequencies of behavior sequences for territorial male fathead minnows in the presence of single males (A) or single females (B) of 2939 and 2449 total behaviors observed, respectively. Numbers in circles are scaled to the relative frequencies of each behavior. Arrows indicate sequences between behaviors by percentage. Frequencies of behaviors and sequences comprising less than one percent are not included. An * indicates significant differences of sequences by the presence of male or female minnows using the Mann-Whitney two-sample U Test, $\alpha = 0.05$.


A
3*
Jumpswim
3
Tailbeat
4
Lateral Display
22
2*
1
7
4*
*3
5*
1*
Inside Territory
23
Outside Territory
23
17
26
5*
*2
8
3*
Approach
20
Butt
4
2
+
B
Lead
1
Lateral Display
3
Jumpswim
3
1
1
1
Inside Territory
30
Outside Territory
30
23
35
1*
1*
13
1*
Approach
29
Chase
1
3
+

Discussion

Behaviors by male fathead minnows in the presence of females previously identified as courtship behavior (approach, lateral display, and leading) by Cole and Smith (1987) surprisingly occur commonly in the presence of another male. There are differences in the frequencies and sequences of these behaviors in the presence of male fish compared to females; however, the behaviors of the territorial male appear to be similar in the presence of introduced males and females. Leading behavior by a territorial male does occur in the presence of male and female additions.

As suggested by McMillan and Smith (1974), confining mature males in aquaria may invoke behaviors that do not occur in nature. An unaggressive male being harassed by a territorial male in an aquarium does not have the choice of fleeing. Yet, mutual butting has been witnessed in the field (McMillan and Smith 1974) where fleeing is an option. Males not returning aggressive actions were observed in this study to be repeatedly approached and had tailbeat and lateral display behaviors directed towards them. Seemingly aggressive behavior towards an intruding male did not necessarily reflect that the intruder was aggressive.

Lateral display behavior was observed significantly more

often in the presence of test male than test female fatheads. This seems to be a form of male aggression toward possible intruders. Lateral display behavior appeared to be a similar and less aggressive form of tailbeat behavior which sometimes was followed by tailbeat behavior. Both test males and females were approached frequently by territorial males.

Sequences differing significantly between the presence of male or female fish included aggressive behaviors, tailbeat and lateral display, with higher frequencies in the presence of male than female fish. Differences found among sequences involving lateral display may be entirely due to the higher proportional frequency of lateral display. A fish had to follow lateral display with one of the observed behaviors, so these sequences would increase in relation to increases in lateral display. It appears that lateral display behavior depicted in Figure 2 in the presence of a male is "replaced" partly by approach behavior in the presence of a female.

The occurrence of the same courtship behaviors in the presence of females or males has been observed previously in other species (Krebs and Davies 1981). Perhaps some of these behaviors (by fathead minnows) serve to repel males and attract females at the same time as in the mating call of the pacific tree frog (Whitney and Krebs 1975a,b),

although this may not be a good analogy, as all of the female fatheads in this study may not have been in a reproductive state as indicated by low mean GSI. Breeding female fatheads in nature have GSI's several times those in our study (Smith 1978). Smith (pers. comm.) suggests that territorial males may treat nonreproductive fish of either gender in similar ways since they are neither competitors nor potential mates but are probably egg predators. These observations contribute to our knowledge of basic fathead minnow behavior. Territorial males demonstrate similar behaviors in the presence of non-reproductive males and females. Low GSI's of the test fish may minimize the gender differences expressed by individuals in reproductive condition. These behavior and behavior sequence data do indicate gender recogniton by territorial males.

CHAPTER IV

BEHAVIOR OF MALE FATHEAD MINNOWS WITH THE ADDITION OF FATHEAD MINNOWS EXPOSED TO ESTRADIOL-17β

In contrast to mammals where gender is genetically determined, the gender of fish is under the influence of various environmental factors including hormones such as estrogens and androgens (Yamamoto 1969). Yamazaki (1983) lists sex steroid hormones as "primarily inducers of various reproductive phenomena including differentiation of gonads, gametogenesis, ovulation, spermiation, spawning or courtship behavior, secondary sex characteristics, morphological and physiological changes at the spawning seasons, or sex pheromone production." Hormones which cause fish to spawn, grow quicker, and change sex have become useful tools in research and in the culture of fish (Schreck 1974). Sex changes, both feminization and masculinization, have been produced by administration of steroid hormones (Donaldson and Hunter 1981; Yamazaki 1983). Two specific hormones which have been shown to be effective for gender-reversal in fish are estradiol-17β and 17α-methyltestosterone (Yamazaki 1983). With these hormones, monosex cultures of some fish species are possible. Chemical manipulation of gender leads to some interesting research opportunities because fish can be

phenotypically one sex and genotypically the opposite sex. Monosex cultures have been useful tools in management for population control, weed eradication (Schreck 1974), and preventing reproduction and spreading of introduced species. The ability to produce a group of the same sex eliminates the chore of sexing by morphological sex differences and may be desirable for aquaculturists (Yamazaki 1983).

The effects of sex hormones depend on the exposure method and species of fish. Hormones have been successfully administered to fish in three ways: incorporation into food (Yamamoto 1958), injection (Boney et al. 1984), and immersion in water containing the hormone (Goetz et al. 1979). Hormone exposure protocols have not been developed for many species and need to be refined to be more practical and economical. Although commonly used in salmonids, estradiol-17β exposures have not been attempted in cyprinids (Hunter and Donaldson 1983). Concentrations of estradiol-17β in diets of fish to induce feminization have ranged from 5 mg/kg diet (Oncorhynchus kisutch, with two and six, two-hour immersions) to 120 mg/kg diet (Salmo gairdneri, without immersion), with varying degrees of success up to 100% production of females (Donaldson and Hunter 1981). Successful (100% female production) immersion concentrations and durations have varied from

0.18 ug/l water for 18 days with Oncorhynchus masou under static conditions (Nakamura 1984) to 400 ug/l water for two hours with O. kisutch (Hunter et al. 1982).

This study will attempt to identify concentrations of estradiol-17β by immersion and diet separately and in combination which will induce gender change in the fathead minnow, Pimephales promelas. The behavioral interactions of both male and female fathead minnows exposed to estradiol-17β in the presence of non-treated males will be examined and compared to the behaviors of unexposed controls in the presence of non-treated males. Alternate hypotheses to be tested will be: 1) frequencies and/or durations of male behaviors are different in the presence of fathead minnows exposed to estradiol-17β from fathead minnows not exposed; and 2) sequences of behaviors by males are different in the presence of minnows exposed to estradiol-17β than minnows not exposed.

Materials and Methods

Observation aquaria were either 38 (51 x 32 x 26 cm) or 190 (92 x 52 x 41 cm) liters with corner filters containing activated charcoal and filter floss. Temperatures were maintained at 24-26!C with electric hanging heater units. Lighting was flourescent with LD 18:6. Frequency and

temporal observations were made of the following behaviors described by McMillan and Smith (1974):

1. Male, Agonistic Ethogram: Fighting and all behavior associated with fighting (Morris 1955)
    a. Butting (moves toward other fish and pushes with snout)
    b. Charging (swimming directly at opponent, often precedes chasing, biting, or butting)
    c. Chasing (rapidly swimming after other fish)
    d. Tailbeating (locating itself alongside another fish and propelling water against the other fish with caudal fin and posterior of body)
    e. Assumption of lateral banding (a dark banding pattern of two stripes provides a measure of aggressiveness) by assigning a number, either 1) light, 2) faintly banded, 3) dark gray body with pale bands, 4) black body with distinct light bands (Unger 1983)
2. Female, Location - in or out of territory

Two untreated territorial males (breeding colors, dorsal tubercles) were added to each test aquarium. Five of the treated fish were added to each test aquarium and at least one hour passed prior to behavior observation. Behavior of fish were recorded on a portable computer with a program written to record frequency and duration of entered

behavioral data (see previous chapter for description).

Exposure Methods

Eggs were collected from a fathead minnow culture at North Texas State University Department of Biological Sciences and placed in 20-cm long, 7-cm (ID) PVC tubing sections covered with 1-mm plastic mesh. These were immersed in 2-liter plastic containers with an airstone in each. Water temperatures were maintained at 22-25!C by controlling the temperature of the room. Estradiol-17ß (Sigma Chemical Company, St. Louis, Missouri) was delivered by dissolving it in a carrier, 100% ethanol, and then placed in the appropriate concentrations (see below) in 1 liter of dechlorinated tap water in the plastic containers. Immersions were conducted in unlighted cabinets as the hormone undergoes photolysis (Sigma Chemical Company). The concentration of ethanol never exceeded 0.04% to minimize any effects of ethanol on fish. Eggs and fry were immersed 2 hours, twice, in the hormone solution or in a solution of ethanol without the hormone. Hormone concentrations were 0, 25, 50, 100, and 400 ug/l water. PVC tubing with nylon mesh on one end was used to transfer eggs and fry between solutions. Containers were rinsed with dechlorinated tap water to keep them free of the hormone. Eggs and fry were

maintained in dechlorinated tap water with daily changes (after noticing much fungus growth on eggs with less frequent changes). All equipment coming into contact with fry was sterilized by soaking in water with sodium hypochlorite. Fry were fed newly hatched brine shrimp (Artemia sp.) for the first week, thereafter TetraMin flake fish food was used. The hormone was added to the TetraMin diet for one group of fish receiving 50 ug/l estradiol-17β immersions. Estradiol-17β was added to the food at a concentration of 10 mg/kg. Commercial flake fish-food (TetrMin) was sprayed with the hormone in a 100% ethanol carrier, and the ethanol was evaporated. This was stored at -60 C until presentation to fish.

Radioimmunoassay (RIA) was used to attempt to quantify concentrations of estradiol-17β in fish exposed to 0, 50, 100, and 400 estradiol ug/l. Seven days after the last immersion, groups of ten fry were removed and ground in 400 ul of water. In this method a known amount of a radiolabelled hormone combines with a known amount of the antibody. Added samples of hormone cause a correspondingly lower amount of the labeled hormone to be bound to the antibody. The bound labeled hormone is separated from the free hormone and radioactivity is measured to calculate a standard curve. The detailed assay procedures are described by Radio Systems Laboratories (Carson,

California) and were modified by diluting the samples to include the concentration range of the assay. Dilutions were made by factors of 50, 100, 100, and 500 for exposure group concentrations of 0, 50, 100, and 400 ug/l estradiol, respectively.

Gender of fish was determined by presence or absence of an enlarged urogenital papillae (Lewis 1977) or body coloration and compared to expected gender (one male: one female of the null hypothesis) using the chi-square statistic with Yates continuity correction ($\alpha = 0.05$). Each fish was weighed (to 0.1 g) and measured (total length to 0.1 cm.).

Frequency and duration behavioral data were analyzed using Dunnett's multiple range test on ranked data ($\alpha = 0.05$ for all statistical comparisons). Assumption of lateral banding data were analyzed using chi-square. Diad sequences of behaviors were summed, and percentages were calculated for each behavior for comparison of unexposed and ethanol exposed fish with treated fish using Dunnett's multiple range test.

Results

Frequencies of male fatheads entering territories when compared by treatments to the control fish showed

significant differences only in the fish treated with 25 ug/l estradiol (Figure 3). Only the 400-ug/l estradiol treatment group showed significant differences in frequencies of males charging or chasing females from the control fish (Figure 4). When compared by durations of males in territories, significant differences from the control fish were seen for the treatment group exposed to 25-ug/l and the group exposed to 50-ug/l with dietary estradiol (Figure 5). Comparison of the durations of females in territories (Figure 6) showed no significant differences from the control fish.

Analyses of behavior sequence diads by treatment (Figures 7-12) showed many significant differences from the control fish. The ethanol-exposed fish showed significant differences from the control fish only for the sequence from outside territory to male chasing male (Figure 8). Fatheads exposed to 25-ug/l estradiol showed significant differences in male behavior sequences from controls for 6 of 48 (12.5%) sequences compared (Figure 9). The 50-ug/l estradiol-exposed fish showed significant differences from controls for 5 of 48 (10.4%) sequences analyzed (Figure 10). Fish exposed to 400-ug/l of estradiol had significant differences from the control fish for 8 of 48 (16.6%) sequences (Figure 11). The fish receiving a 50-ug/l exposure and dietary estradiol showed significant

Figure 3.-Median frequencies of male fatheads in territories during 100-second periods by the treatment regime. Interquartile ranges are indicated by vertical lines and sample sizes are in parentheses. Treatment regimes are control (C), ethanol (N), 25, 50, 400 ug/1 estradiol (F25, F50, F400, respectively) and 50 ug/1 plus 10 mg/1 dietary estradiol (F50D10). An * indicates significance from the controls at $p = 0.05$ with Dunnetts multiple range test on ranked data.

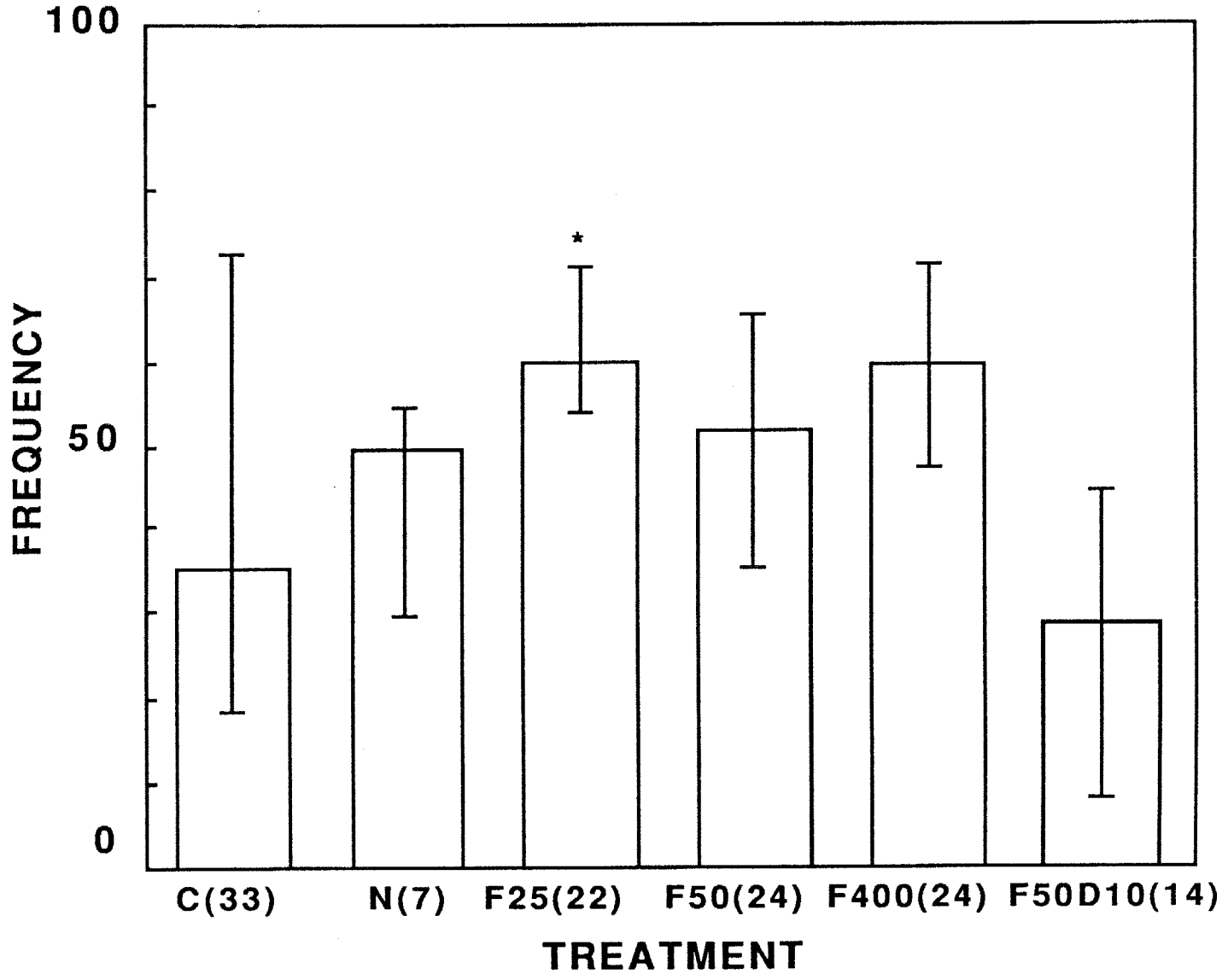

100
50
0
FREQUENCY
*
C(33)
N(7)
F25(22)
F50(24)
F400(24)
F50D10(14)
TREATMENT

Figure 4.-Median frequencies of male fatheads chasing or charging females during 1000-second periods by the treatment regime. Interquartile ranges are indicated by vertical lines and sample sizes are in parentheses. Treatment regimes are control (C), ethanol (N), 25, 50, 400 ug/1 estradiol (F25, F50, F400, respectively) and 50 ug/1 plus 10 mg/1 dietary estradiol (F50D10). An * indicates significance from the controls at $p = 0.05$ with Dunnetts multiple range test on ranked data.

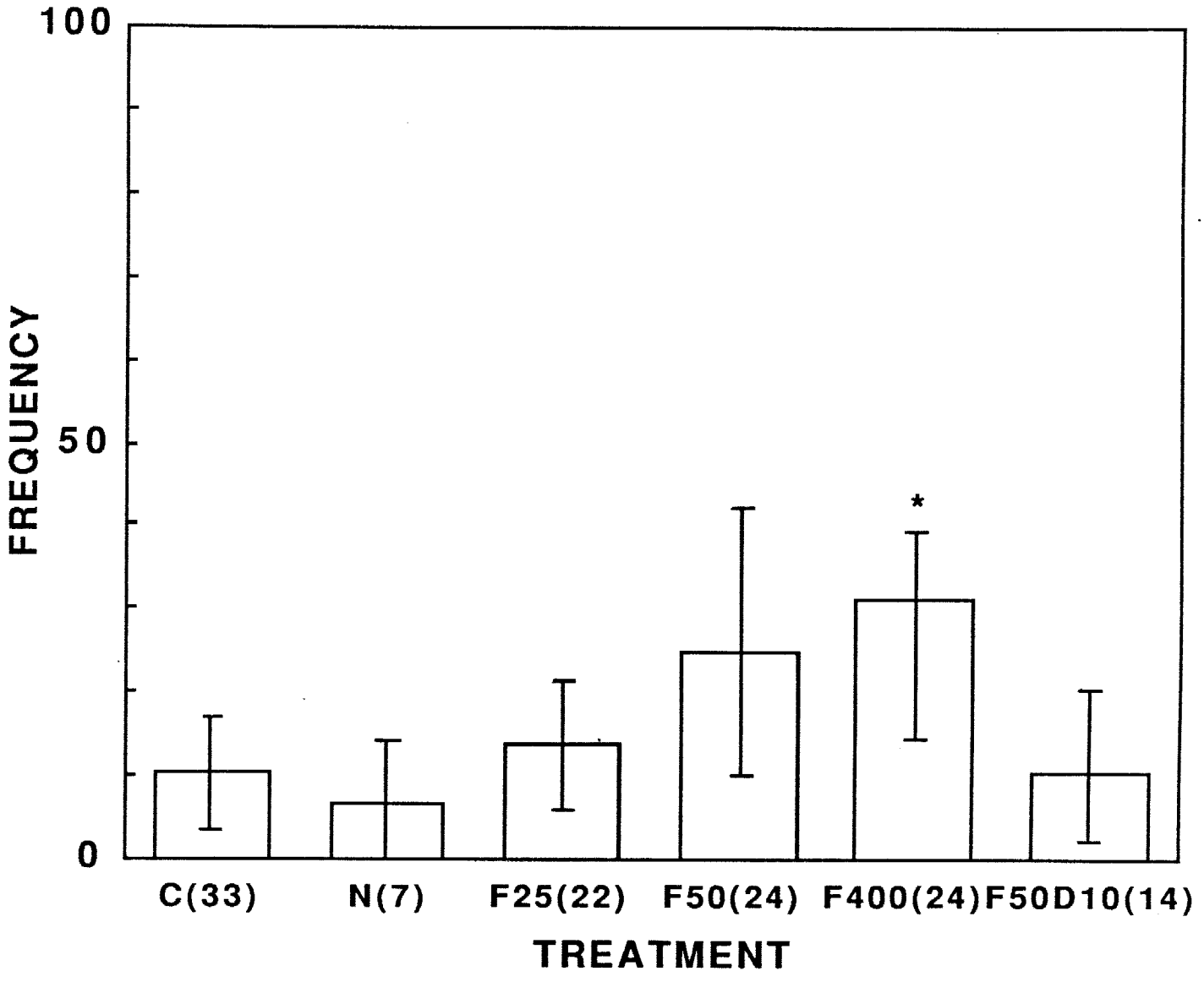

100
50
0
FREQUENCY
*
C(33)
N(7)
F25(22)
F50(24)
F400(24)
F50D10(14)
TREATMENT

Figure 5.-Median durations of male fatheads in territories during 1000-second periods by the treatment regime. Interquartile ranges are indicated by vertical lines and sample sizes are in parentheses. Treatment regimes are control (C), ethanol (N), 25, 50, 400 ug/1 estradiol (F25, F50, F400, respectively) and 50 ug/1 plus 10 mg/1 dietary estradiol (F50D10). An * indicates significance from the controls at $p = 0.05$ with Dunnetts multiple range test on ranked data.

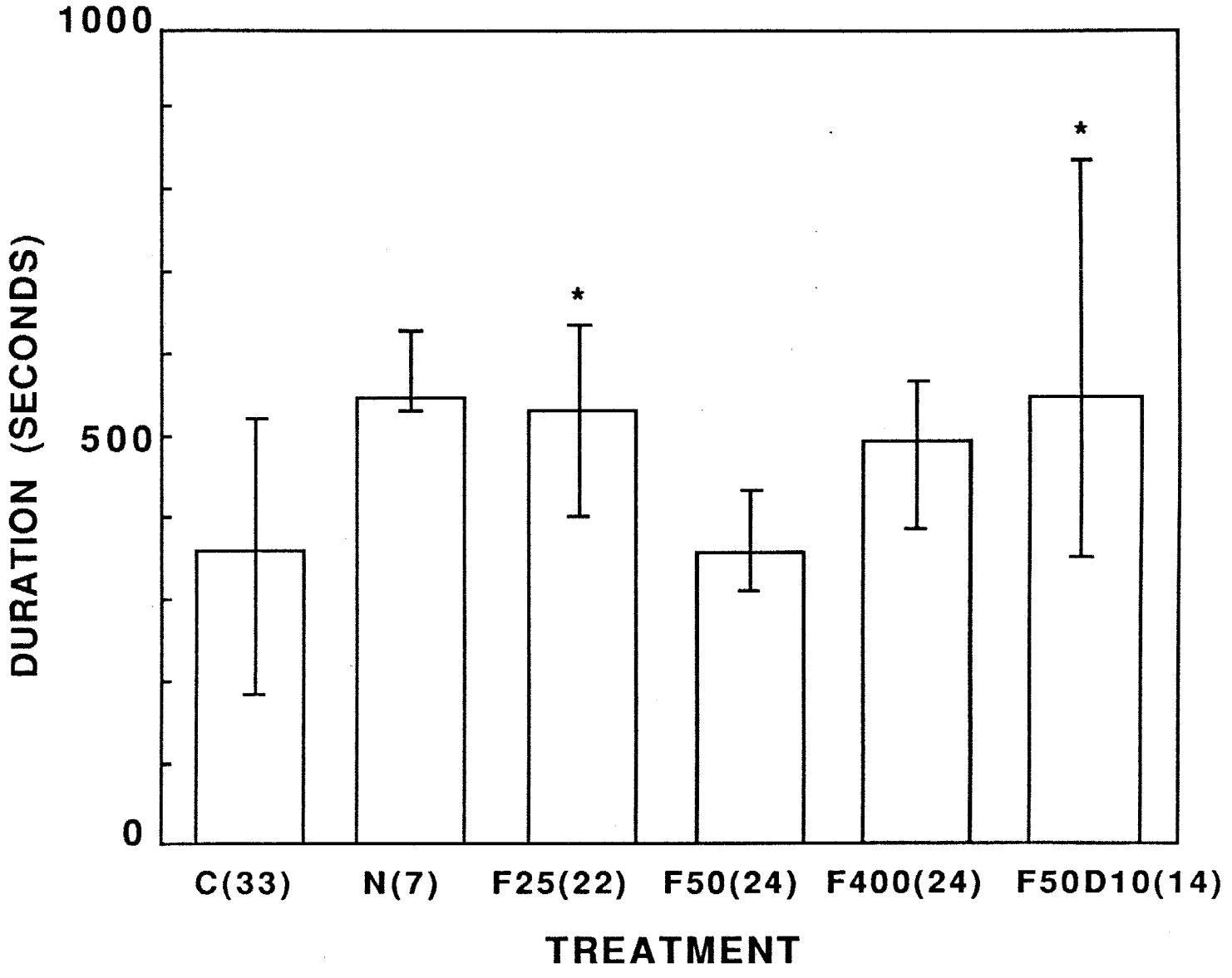

1000
500
0
DURATION (SECONDS)
*
*
C(33)
N(7)
F25(22)
F50(24)
F400(24)
F50D10(14)
TREATMENT

Figure 6.-Median durations of female fatheads in territories during 1000-second periods by the treatment regime. Interquartile ranges are indicated by vertical lines and sample sizes are in parentheses. Treatment regimes are control (C), ethanol (N), 25, 50, 400 ug/1 estradiol (F25, F50, F400, respectively) and 50 ug/1 plus 10 mg/1 dietary estradiol (F50D10). An * indicates significance from the controls at $p = 0.05$ with Dunnetts multiple range test on ranked data.

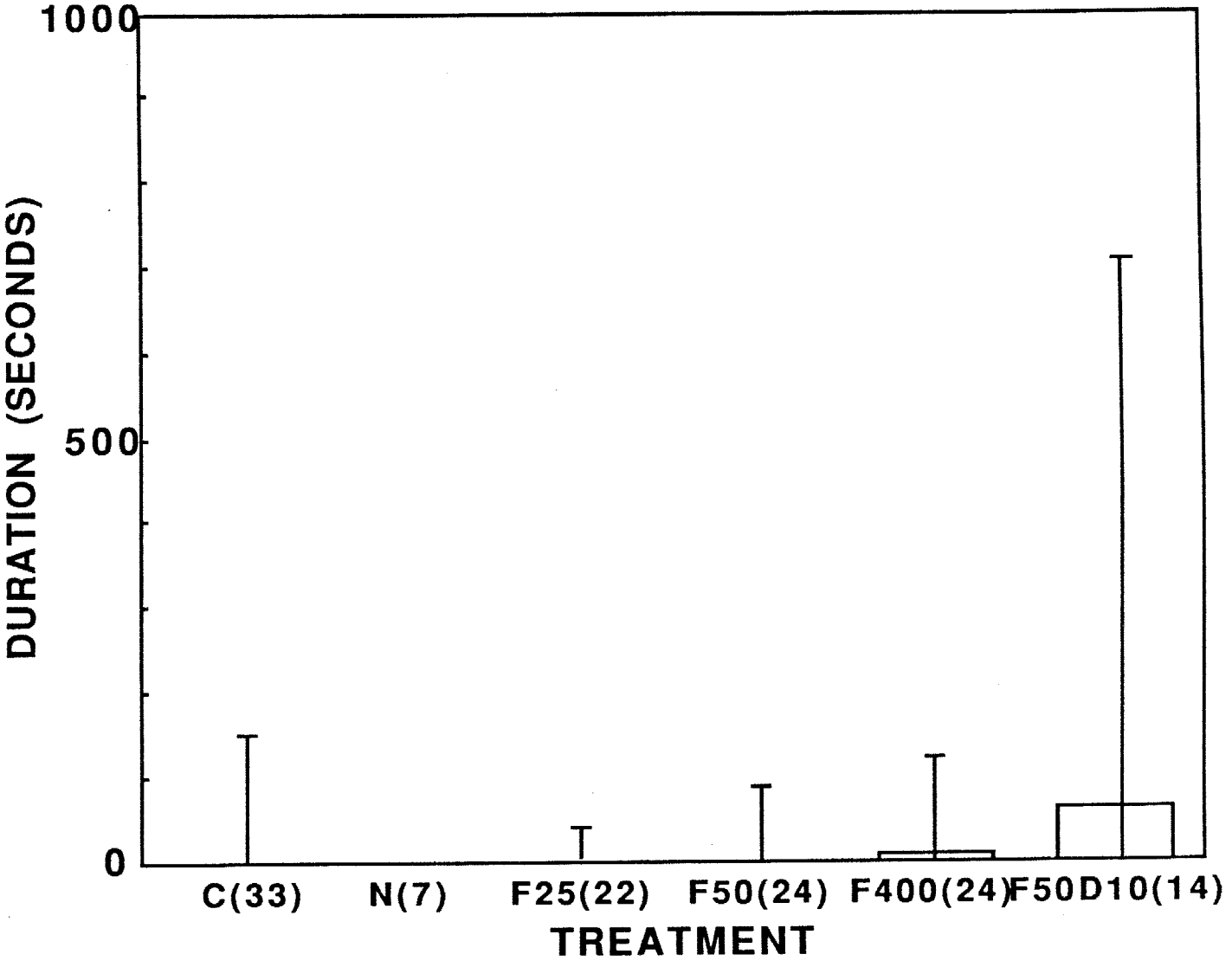

1000
500
0
DURATION (SECONDS)
C(33)
N(7)
F25(22)
F50(24)
F400(24)
F50D10(14)
TREATMENT

Figure 7.-Proportional frequencies of behavior sequences for male fathead minnows in the presence of females not treated (control) of 8115 total behaviors observed. Arrows indicate sequences between behaviors by percentages. Frequencies of behaviors and sequences comprising less than one percent are not included.

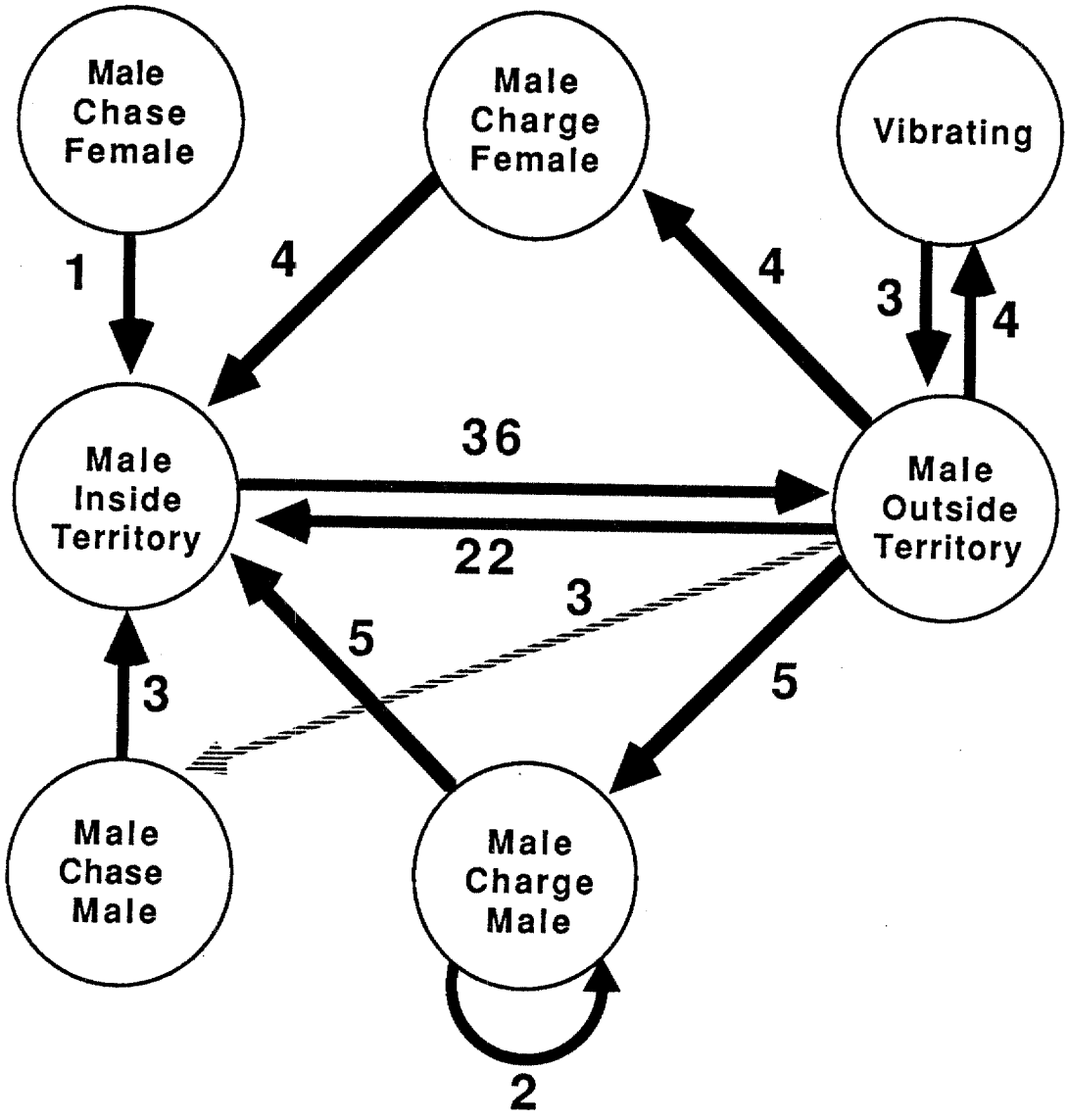

Male Chase Female
Male Charge Female
Vibrating
1
4
4
3
4
Male Inside Territory
36
22
Male Outside Territory
3
5
5
3
Male Chase Male
Male Charge Male
2

Figure 8.-Proportional frequencies of behavior sequences for male fathead minnows in the presence of females treated with ethanol, of 919 total behaviors observed. Arrows indicate sequences between behaviors by percentage. Frequencies of behaviors and sequences comprising less than one percent are not included. An * indicates significant differences from the control fish using Dunnetts multiple range test ($\alpha = 0.05$).

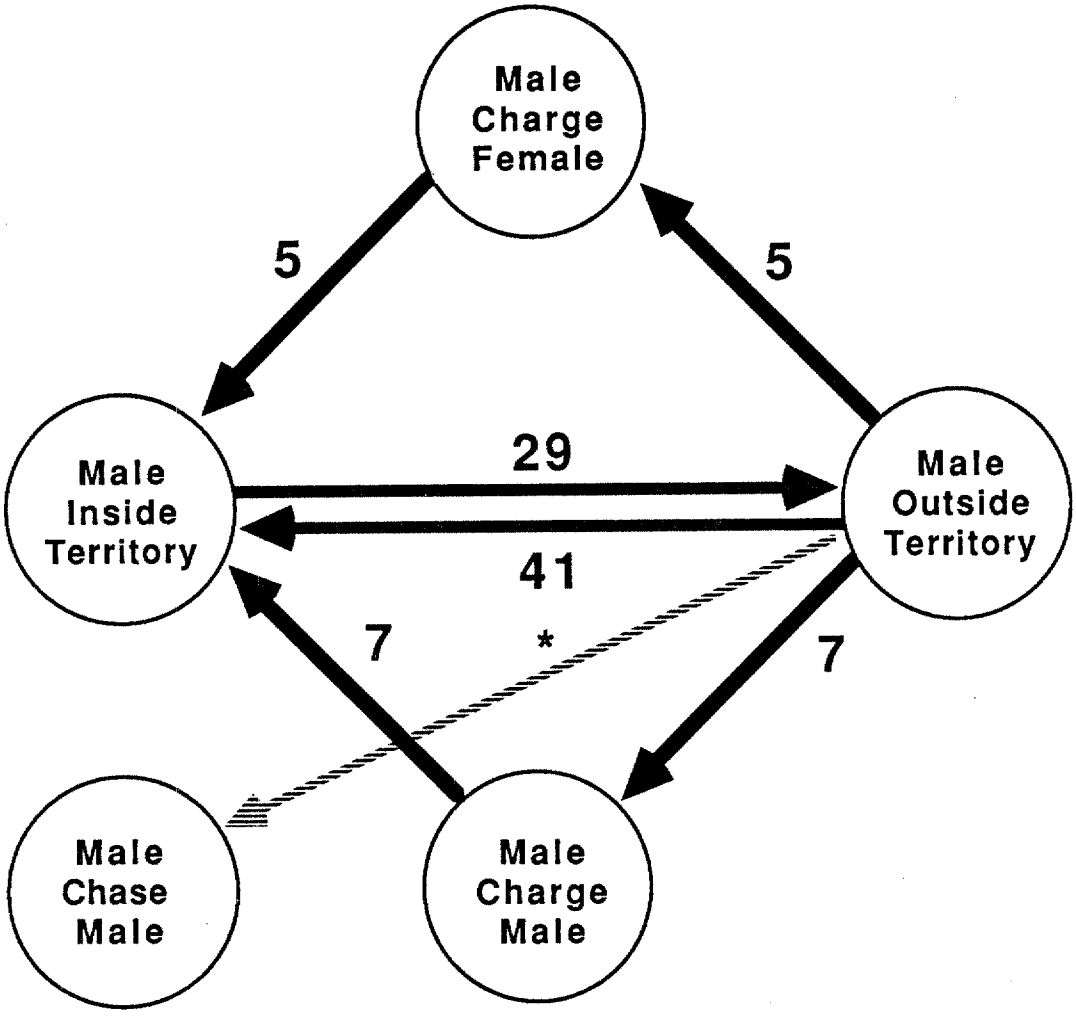

Male
Charge
Female
5
5
Male
Inside
Territory
29
Male
Outside
Territory
41
7
*
7
Male
Chase
Male
Male
Charge
Male

Figure 9.-Proportional frequencies of behavior sequences for male fathead minnows in the presence of females treated with 25 ug/1 estradiol, of 3057 total behaviors observed. Arrows indicate sequences between behaviors by percentage. Frequencies of behaviors and sequences comprising less than one percent are are not included. An * indicates significant differences from the control fish using Dunnetts multiple range test ($\alpha = 0.05$).

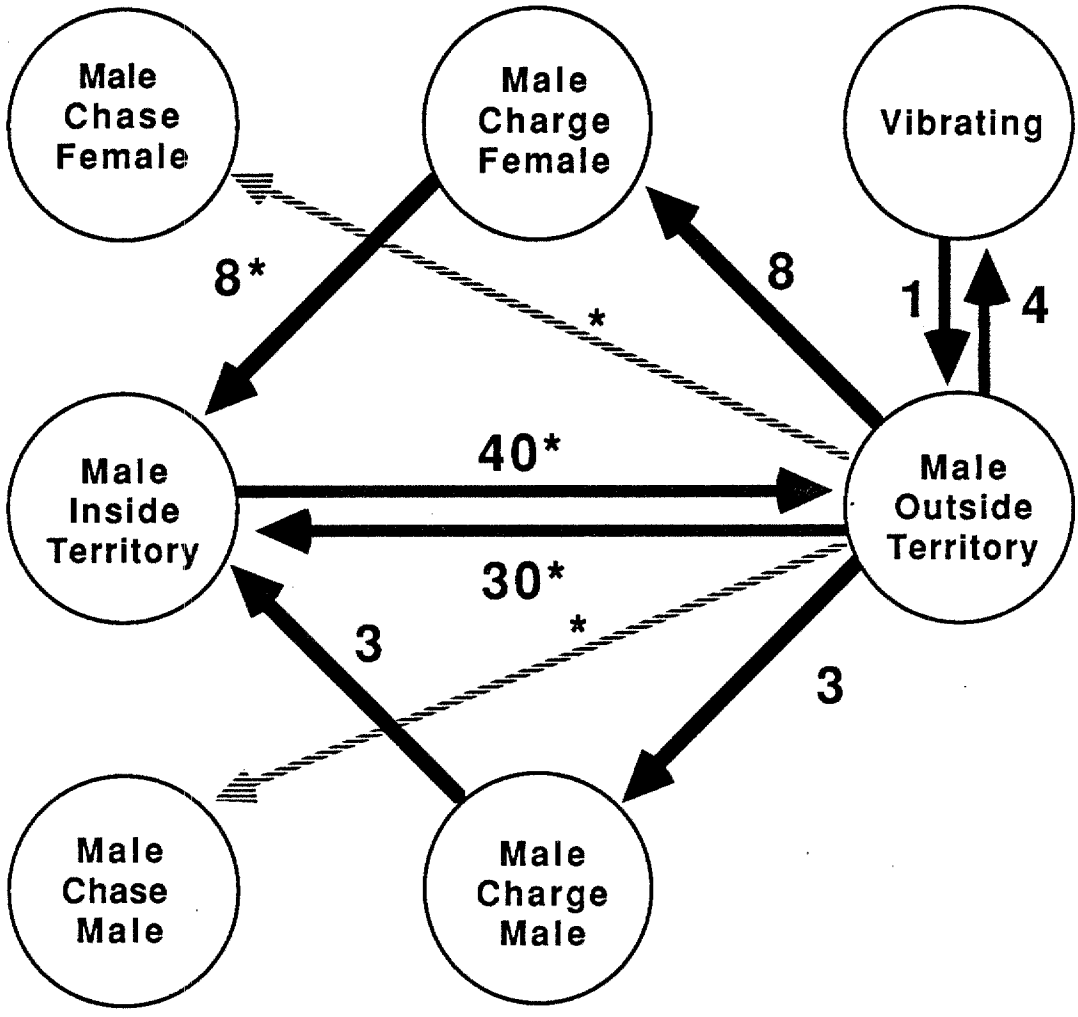

Male Chase Female
Male Charge Female
Vibrating
8*
*
8
1
4
Male Inside Territory
40*
Male Outside Territory
30*
*
3
3
Male Chase Male
Male Charge Male

Figure 10.-Proportional frequencies of behavior sequences for male fathead minnows in the presence of females treated with 50 ug/1 estradiol, of 2997 total behaviors observed. Arrows indicate sequences between behaviors by percentage. Frequencies of behaviors and sequences comprising less than one percent are are not included. An * indicates significant differences from the control fish using Dunnetts multiple range test ($\alpha = 0.05$).

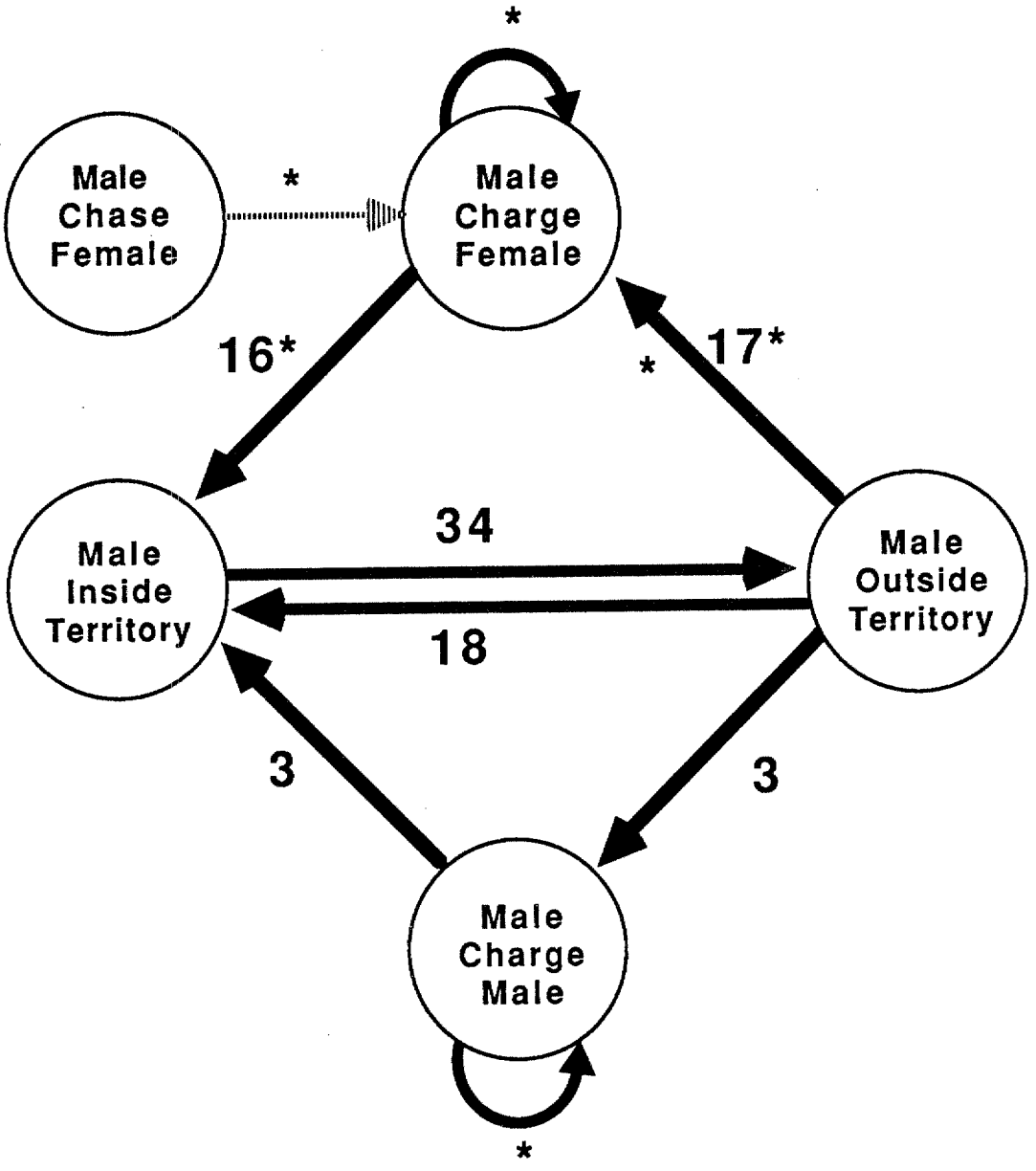

*
Male Chase Female
*
Male Charge Female
16*
*
17*
Male Inside Territory
34
18
Male Outside Territory
3
3
Male Charge Male
*

Figure 11.-Proportional frequencies of behavior sequences for male fathead minnows in the presence of females treated with 500 ug/1 estradiol, of 4473 total behaviors observed. Arrows indicate sequences between behaviors by percentage. Frequencies of behaviors and sequences comprising less than one percent are are not included. An * indicates significant differences from the control fish using Dunnetts multiple range test ($\alpha = 0.05$).

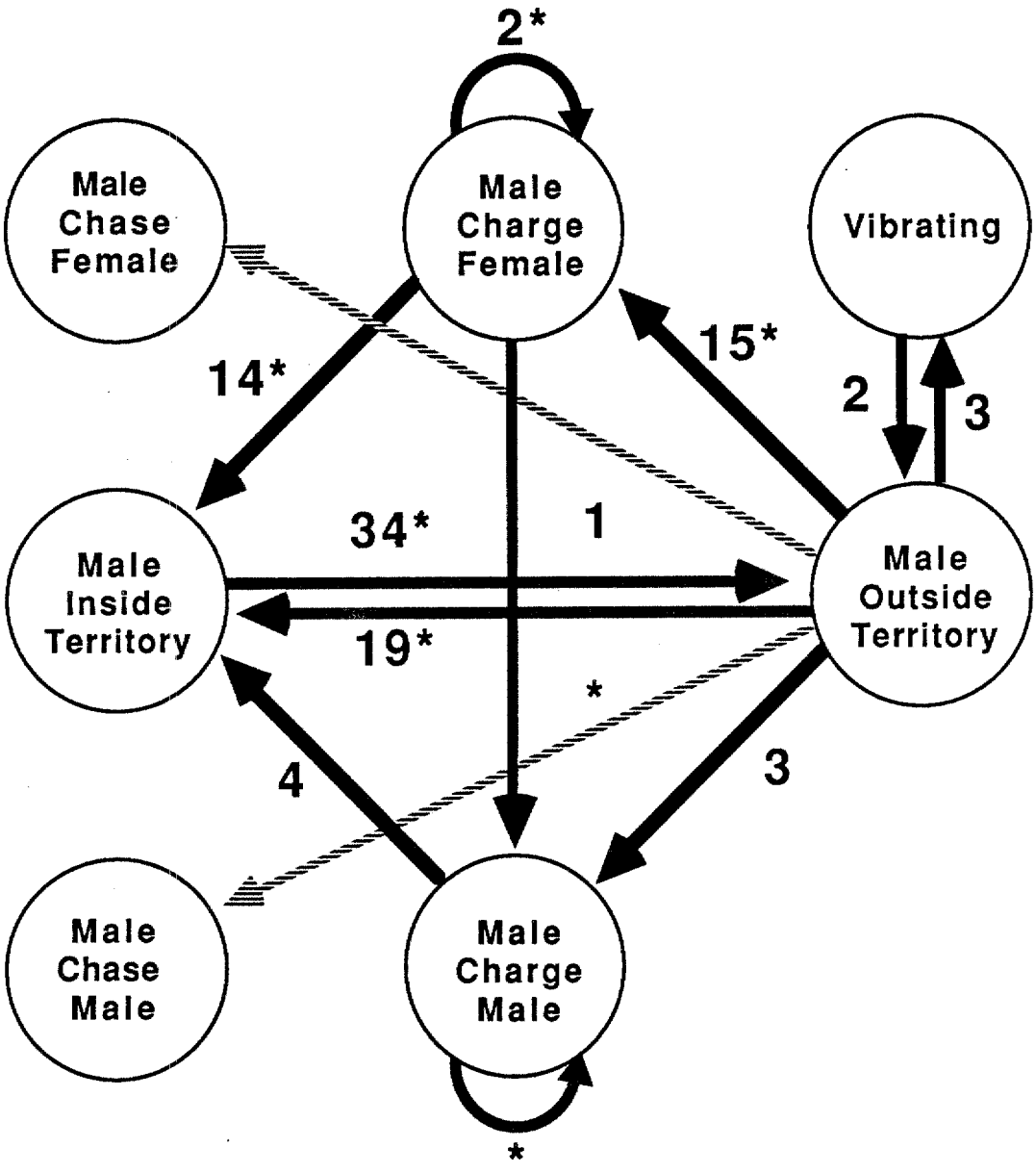
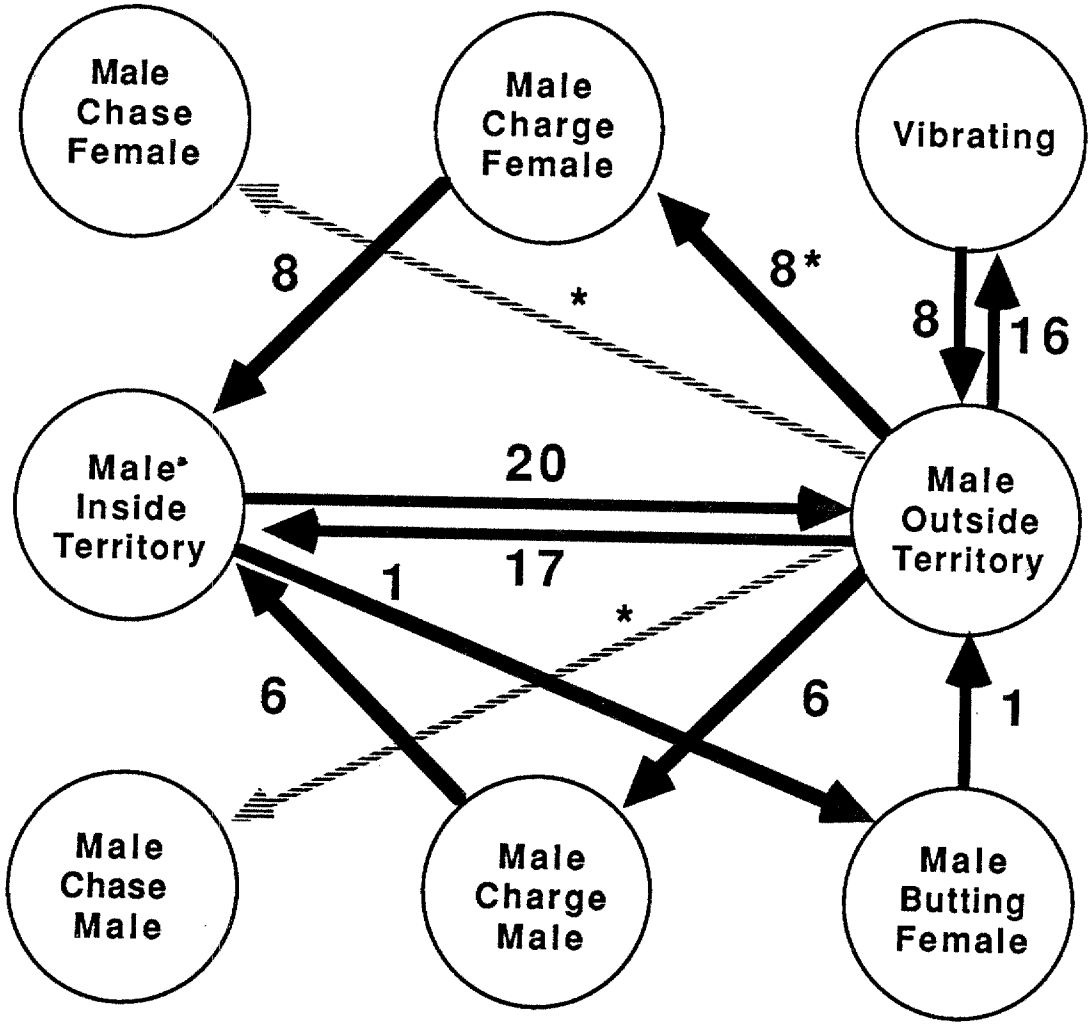

2*
Male Chase Female
Male Charge Female
Vibrating
14*
15*
2
3
34*
1
Male Inside Territory
Male Outside Territory
19*
*
4
3
Male Chase Male
Male Charge Male
*

Figure 12.-Proportional frequencies of behavior sequences for male fathead minnows in the presence of females treated with 50 ug/1 and dietary estradiol, of 1307 total behaviors observed. Arrows indicate sequences between behaviors by percentage. Frequencies of behaviors and sequences comprising less than one percent are not included. An * indicates significant differences from the control fish using Dunnetts multiple range test ($\alpha = 0.05$).


Male Chase Female
Male Charge Female
Vibrating
8
*
8*
8
16
Male* Inside Territory
20
17
1
Male Outside Territory
*
6
6
1
Male Chase Male
Male Charge Male
Male Butting Female

differences in behavior sequences from control fish for 3 of 48 (6.25%) sequences examined (Figure 12).

When assumption of banding of territorial males was compared, significant differences from the controls were found for all of the immersion treatment groups, 25 and 50, 400 ug/l, and the 50 ug/l immersion with dietary estradiol-17α (Table 2). Sample sizes for behavior of the 100 ug/l estradiol-17α exposure group were too small for statistical comparisons and were left out of this analysis. Ratios of genders were not significantly different than 50:50 in any of the seven treatment groups (Table 3). Radioimmunoassay results were extremely variable among treatments (Table 4).

Table 2.-Assigned frequencies for assumption of banding of territorial male fathead minnows by the treatment of the added fathead minnows. An * indicates a significant difference in coloration from the control fish using chi-square.

Treatment	Assumption of Banding			
Control	0	3	15	9
25 ug/l *	0	5	17	0
50 ug/l *	0	0	0	21
400 ug/l *	0	0	27	1
50 ug/l + diet *	0	8	6	1

Table 3.-Sample size (n), mean total length, and mean mass are presented by treatment and gender of the added fathead minnows. None of the treatment groups had genders significantly different from the expected 1:1 ratio (chi-square test with Yates continuity correction, $\alpha = 0.05$).

Treatment	n	%	Total Length±SD (cm)		Mass±SD (g)	
		MALES				
Control	5	55.5	5.9	0.5	2.3	0.7
Ethanol	30	61.2	4.3	0.8	0.8	0.8
25 ug/l	26	61.9	3.7	0.8	0.5	0.3
50 ug/l	2	18.1	5.5	0.7	2.4	1.0
50 + diet	2	28.5	4.5	0.0	1.2	0.2
100 ug/l	20	64.6	4.7	0.0	1.1	0.4
200 ug/l + diet	6	66.7	4.9	0.5	1.5	0.5
400 ug/l	3	21.4	4.3	0.2	1.5	0.2
		FEMALES				
Control	4	44.5	5.1	0.4	1.6	0.2
Ethanol	19	38.8	4.3	0.3	0.9	0.2
25 ug/l	16	38.1	4.1	0.2	0.6	0.1
50 ug/l	9	81.9	5.2	0.2	1.6	0.4
+ diet	5	71.5	4.1	0.2	0.8	0.2
100 ug/l	11	35.4	4.6	0.2	1.1	0.2
400 ug/l	3	33.3	4.3	0.3	0.9	0.4
+ diet	11	78.6	3.9	0.5	0.7	0.4

Table 4.-Concentration of estradiol-17β in treated and non-treated fathead minnow larvae, one week from hatching using radioimmunoassay.

Treatment	Estradiol-17β (ng/g)
Control	0.23
50 ug/l	60.3
100 ug/l	316.7
400 ug/l	2008.0

Discussion

No consistent trends in frequencies of males in territories, frequencies of male aggressive behavior (chasing or charging), or durations of males or females in territories were seen as the exposure of fish to estradiol increased (Figures 3-6) when compared to controls. A few differences were detected from controls, but these differences do not appear to be biologically important when median frequencies and durations are visually compared.

The analyses by behavior sequences showed many significant differences from the control fish sequences. The ethanol-treated fish did have one sequence with a significant difference from the control. This was the sequence from outside the territory to male chasing a male. This sequence was significantly different from the control fish sequence for every treatment group except the 50 ug/l.

This sequence may not be a good indicator for treated fish, or the control males may have been more aggressive than other groups.

A trend is seen for the number of sequences showing significant differences between controls and treated fish, with higher treatment concentrations exhibiting more differences (of the sequences observed). Significant differences in behavior sequences from the control fish were 6, 5, 8, and 3, for the treatment groups 25 ug/l, 50 ug/l, 400 ug/l, and 50 ug/l plus diet, respectively. It appears that the behaviors of the 400 ug/l exposure group were the most different from the control. The differences found for the 400 ug/l group indicated this treatment group received more aggressive behavior from the territoial males. Sequences showing increases were outside territory to charging female, outside territory to male chasing female, male chasing female to male chasing female, and male charging male to male charging male (Figure 11). The other differences from controls did not relate to aggressive behavior, with small differences in the percentage of entering and exiting the territory, and following male charging male with returning to the territory.

The assumption of banding by territorial males had high variation between treatments (Table II), although there was

statistical significance from the control fish. With so much variation, assumption of banding may not be a good indicator of treated fish.

The variation in the radioimmunoassay results were due to dilution errors. The procedure is accurate only for narrow concentration ranges and requires a different series of dilutions for each treatment analysis.

Fathead minnow exposure regimes to estradiol-17β in this study did not appear to induce gender change. Further, no gender change appeared to occur when fathead minnows were exposed to 1000-ug/l estradiol-17β under the same conditions (29 females and 30 males were identified to gender by presence or absence of urogenital papillae). Perhaps gender change would be induced by longer exposure durations or at different life-stages. Behavior differences from controls of territorial males to fish additions did not indicate that exposure to estradiol-17β produced consistent observable effects of the treatment. There was not a trend in effects from the low exposure concentrations to the high exposure concentrations. Behavior sequence diads compared to the control do not suggest clear possible effects. Further work in this area could address direct behavior of fish and not the behavior of territorial males in response to the addition of treated fish. Differences may exist in the behavior of the treated

fish but these differences are undetectable by observation of the untreated territorial males.

CHAPTER V

REPRODUCTIVE BEHAVIOR OF FATHEAD MINNOWS EXPOSED TO SELENIUM

Selenium is an anthropogenic pollutant known to have sublethal to lethal effects in organisms, depending upon dose and exposure (Adams and Johnson 1981). Selenium enters aquatic ecosystems from the disposal of solid wastes from burning of fossil fuels, particularly steam-electric generating plants (Gutenmann et al. 1976), and can eliminate sensitive fish from aquatic environments (Lemly 1985). Levels of 10 ug/l, selenium in a power plant cooling reservoir, Belews Lake, did not prevent recruitment of fathead minnows, Pimephales promelas, (Lemly 1985); although, previous laboratory studies have shown this level to be acutely (24 hour exposures) toxic to larval and juvenile fathead minnows (Cardwell et al. 1976). Watenpaugh and Beitinger (1985a) reported the 24-hour median lethal concentration for fathead minnows of unknown age exposed to water-borne selenate as 82-mg selenium/l in EPA hardwater. Exposure of fathead minnows to 60-mg selenium/l for 24 hours caused a significant decrease in thermal tolerance (critical thermal maxima) of 6°C (Watenpaugh and Beitinger 1985a); however, similar exposures did not affect metabolic rate (Watenpaugh and

Beitinger 1985b). Also, fathead minnows did not behaviorally avoid concentrations of selenate which would cause death within 24 hours (Watenpaugh and Beitinger 1985c).

The reproductive behavior of fathead minnows has been described in the field and the laboratory (McMillan and Smith 1974; Cole and Smith 1987). Little is known of the effects of sublethal selenium on reproductive behavior; however, this is an important criterion to the survival of a population (Rand and Petrocelli 1985). This study will attempt to identify whether exposure to sublethal concentrations of selenium-selenate will measurably affect reproductive behavior in the fathead minnow.

Materials and Methods

Fathead minnows, 6-months post hatching from a culture maintained at North Texas State University were held or placed in 38-liter aquaria containing aged tap water at 21°C. Water was filtered through activated charcoal and floss which were changed weekly. Fish were fed twice daily with newly-hatched, frozen brine shrimp (Artemia sp.), and supplemented with commercial flake food. Flourescent lighting provided a LD 18:6 photoperiod.

Ten fish per treatment group were exposed to selenium as

sodium selenate for 24 hours in 38-liter aquaria containing 25 liters of aerated, reconstituted hard water (U.S. EPA 1975). Nominal concentrations were 0, 20, 30, and 60 mg Se/l. Water samples were taken at the beginning and end of each exposure in two replicates. Actual concentrations of selenium were measured to an accuracy of 1 mg/l by flame atomic absorption spectrophotometry (Perkin-Elmer 1982).

Behavior was observed in 38-liter aquaria containing split-PVC tubing in lengths of 8 cm for nest sites. Test fish were removed from holding aquaria when they appeared to be in reproductive condition (banding and tubercles in males, enlarged urogenital papillae in females) and placed in observation aquaria in pairs of one male and one female. Five white cloud minnows, Tanichthys albonubes, were added to each aquarium to provide a dither effect (Barlow 1977). Observations (1000-second) were made between 0600 and 1000 hours as fish appeared to be most active at this time (Smith 1970; Pyron and Beitinger in review). Behaviors observed were from McMillan and Smith (1974) and/or Cole and Smith (1987):

1. Approach behavior - male approaches female
2. Leading behavior - male swims from near the female directly to his territory and the female follows
3. Lateral display - male moves in front of, or at right angles to the female and hovers, extending

dorsal, caudal, anal, and pectoral fins.

4. Tailbeating - the lateral quiver of Cole and Smith (1987) and tailbeating of McMillan and Smith (1974).
5. Vibrating - this was only observed when females were in territories and is part of spawning behavior (McMillan and Smith 1974). The male and female come in close side-by-side contact and vibrate against each other.
6. Butting - (same as McMillan and Smith 1974). The male moves toward the female and pushes at it with the snout.

The movements of male and female fish in and out of the male-defended territory were monitored as behaviors.

Research reported in chapter III demonstrated that differences in individual fish behavior appeared to be explained by differences in gonadal somatic indices (GSI). GSIs were calculated for females in this study by dividing the ovary weight by the weight of the fish, after behavioral observations. GSIs were not determined for males. Mean GSIs ($\pm$ standard deviation) were 6.9% ($\pm$ 2.7) for controls, 11.9% ($\pm$ 5.6) for the 20 mg/l selenium, and 11.0% ($\pm$ 1.6) for the 30 mg/l selenium exposed females.

Results

Five of the fish in the 60 mg/l selenium treatment group died before the 24 hour exposure was complete. The other five in this group died within 24 hours of exposure. One mortality occurred in the 30 mg/l selenium exposure group within 24 hours of the exposure. No mortalities were observed in the 20 mg/l selenium group. The actual concentrations of selenium in water samples were 66 mg/l selenium for the 60 mg/l group, 36 mg/l selenium for the 30 mg/l group, and 20 mg/l selenate for the 20 mg/l group. No variation greater than 1 mg/l was found in any of the samples.

In general, all behaviors were present in each treatment group. Control fish never spawned and hence vibrating behavior was not observed. This might be have been caused by the low GSIs in the control fish.

Nearly all larvae produced from fish exposed to 20 and 30 mg/l selenium had gross abnormal morphology, characterized by general edema (Figures 14 and 15), similar to the edamatous bluegill larvae from adults exposed to 9 to 12 ug/l selenium within a lake population (Gillespie and Baumann 1986). The larvae from control fish (Figure 13) showed no anatomical effects.

Figure 13. Fathead minnow larva from control parents, four days from hatching.

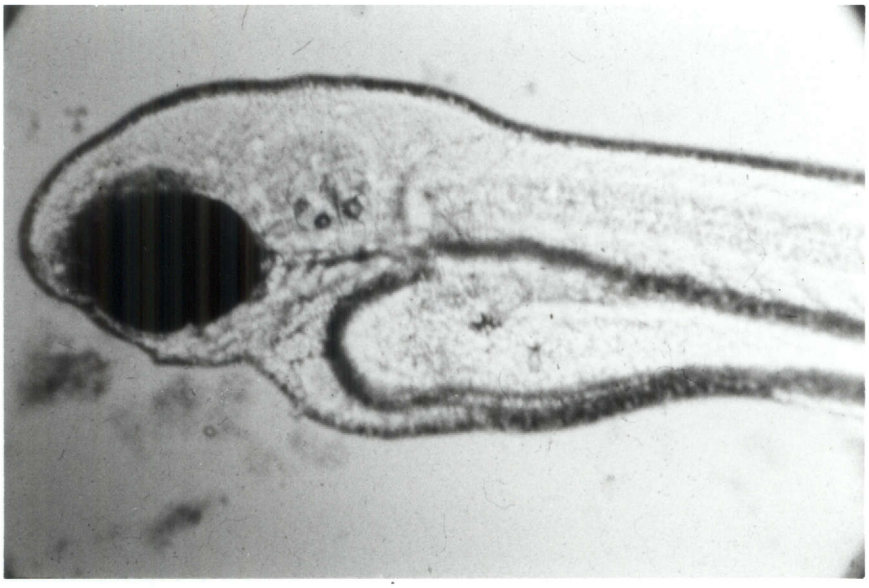


Figure 14. Fathead minnow larva four days from hatching, from parents exposed to 20 mg/l selenate.

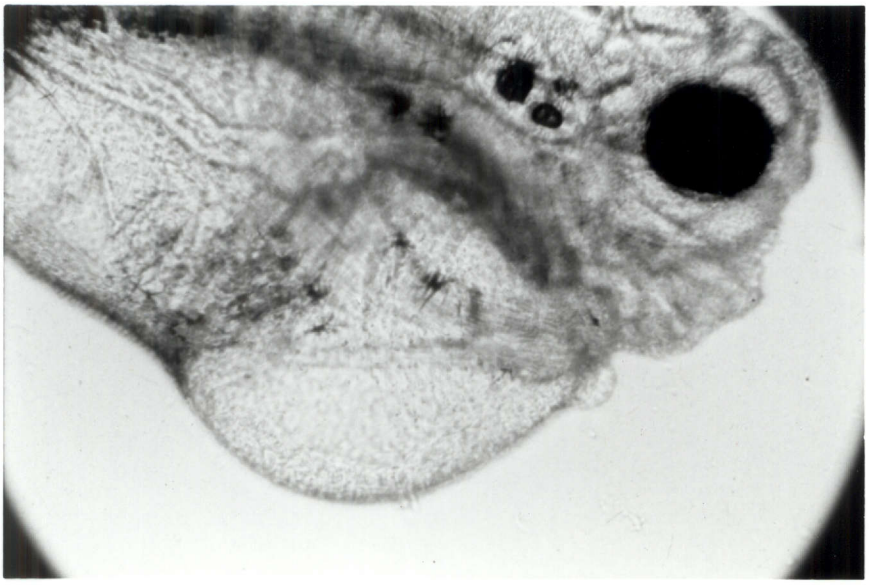
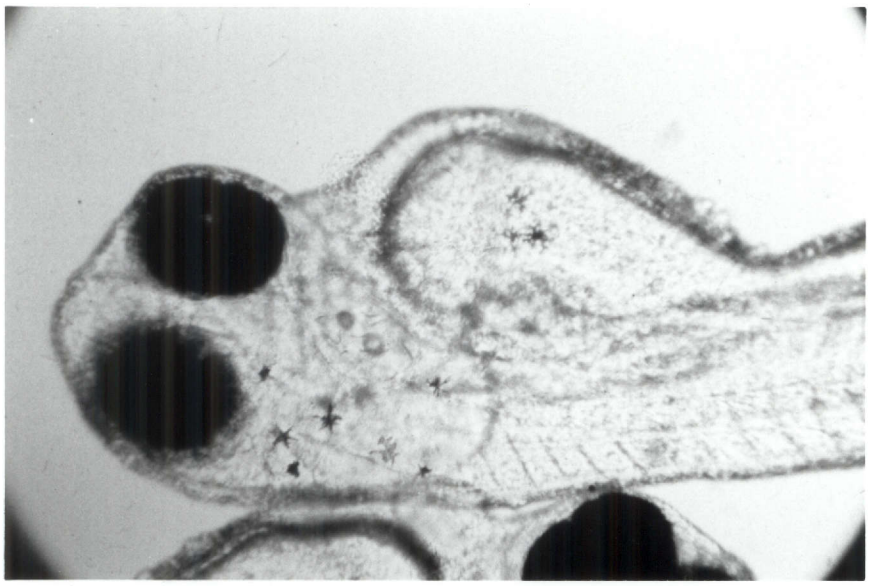


Figure 15. Fathead minnow larva four days from hatching, from parents exposed to 30 mg/l selenate.



Offspring of the 20 and 30 mg/l selenium fish had low survivability. None of the observed edematous larvae survived longer than 7 days post-hatching hatching and only a few of the others survived. The percent of larvae to hatch and survive the first seven days post-hatch was low for both treated groups.

Discussion

Selenium in the concentrations and exposure times used in this study did not have a noticeable affect on the reproductive behaviors monitored. Fathead minnows treated with 20 and 30 mg/l selenium both were successful in producing offspring, although a lower survivability of the offspring was seen. Body burdens of selenium were not measured. Gillespie and Baumann (1986) found that selenium in bluegill females (mean selenium in gonads and carcasses of 7.94 mg/l) caused edema in larvae, and even reproductive failure.

Further work may find that longer exposures to lower concentrations of selenium may have more effect upon reproduction and reproductive behavior of fathead minnows than found in this study. Eggs and sperm presumably were formed before the exposure so exposures probably did not affect the formation of gametes. Eggs may have been

affected as found by Gillespie and Baumann (1986) from uptake of selenium while in exposed females. The present study found that fathead minnow reproduction is successful with the concentrations of 20 and 30 mg/l selenium, but the offspring have a low survivability.

CHAPTER VI

CONCLUSIONS

This research has contributed to knowledge of fathead minnow, Pimephales promelas, reproductive biology (Chapters II, IV, and V) and behavior (Chapter III). Immature male and female fatheads minnows induce similar behaviors in territorial males although not in the same frequencies. Territorial males are able to recognize the gender of added fish. Perhaps territorial males do not invest as much in courtship behavior unless an added fish is female and sexually mature. Yet unknown are the mechanisms by which a territorial male recognizes the gender of an added fathead minnows. Cues may be behavioral and pheromonal as suggested by Cole and Smith (in press). Other hypotheses which could be addressed are: Do territorial fathead minnows behave similarly in the presence of mature males and females? Are the same behaviors present and are they in the same frequences or behavior sequences?

One reproductive effort by fathead minnow males or females does not lower the tolerance to CTM-level temperatures (Chapter II). If fish were tested after multiple spawnings for thermal tolerance or perhaps some other physiological changes, measurable changes in physiological well-being may emerge; or, some other

unexplained as of yet phenomena may be occurring. This research is the first attempt at using CTM methodology as a an assay method for determining physiological stress of a fish subsequent to a biotic activity.

Fathead minnows exposed to estradiol-17β in this study and added to aquaria containing a territorial male did not cause consistent obvious differences in the behavior of the territorial male. Differences that were observed did not seem to be of biological importance: no trends in behavior frequencies or sequences of the territorial males were observed from control fish to the highest exposure hormone concentration. Estradiol-17β did not cause gender-reversal in fathead minnows in the concentrations and durations used. Whether gender-reversal with the administration of steroid hormones will occur in fathead minnows is still unknown.

Further work with fathead minnows would benefit by the following suggestions: 1) Increase the time of observation sessions to include any behaviors or sequences which may take more than 1000 seconds to complete, 2) When analyzing behavior sequences analyze three, four, or if possible more behaviors in a sequence. I noticed that behaviors often occur in longer sequences than two and found myself anticipating these longer sequences while monitoring behavior. Sometimes I would correctly record

behaviors before they occurred. Many behaviors occur in the same repeated sequences (Dawkins 1976) or patterns (Eibl-Eibesfeldt 1970). 3) There seems to be much more occurring than I or previous observers have recorded. What I defined as one behavior is possibly several behaviors which have been difficult to isolate. For example, I used the McMillan and Smith (1974) definition of a spawning bout in a territory, with fish vibrating as the male and female come in close contact against against each other. After many observations, it appears some of what I was calling vibrating might have been the male attempting to "drive" the female from recently spawned eggs in the territory. I observed females preying on eggs, as has been reported by others (McMillan and Smith 1972; Unger 1983). Only females preying upon eggs deposited by other females would seem to gain from egg predation. Multiple spawning is common, e.g. Markus (1934). There are other examples. 4) Female fathead minnows do have behaviors as S. Vives has also observed (pers. comm.). They do not seem to be as numerous or obvious to an observer as the behavior of territorial males. I have witnessed frequent chasing of females by females, chasing of dither fish by females, and females butting males after receiving butts. I did not quantify these behaviors and their importance is unknown.

Selenium did not have an observable effect on

reproductive behavior in fathead minnows exposed for 24 hours to concentrations of 20 and 30 mg/l. Offspring of fathead minnows exposed to 20 and 30 mg/l selenium had low survivability. At the 60 mg/l level, death occurred in half of the exposed fish during the exposure and the rest died within 24 hours.

The behaviors monitored in these studies with the chemicals used do not appear to make a good bioassay for chemical effects. Differences found between treated and control fish may be due to normal variation present in these behaviors. If specific behaviors were absent in treated fish the results would be more conclusive. There may be chemicals which would have more drastic effects than those found in this study and further research could determine these.

This study has demonstrated that fathead minnow reproduction is not sensitive to a steroid hormone, selenate, and thermal stress. The behavior of this species of fish is probably not a good assay subject for these as environmental pollutants. The reproductive behavior of the fathead minnow shows much variation between fish, but it is also very stabile, resisting changes from external stimuli. Perhaps this stability is due to the extreme importance, evolutionarily and otherwise, for these behaviors in the survival of the species.

This research has furthered fundamental and applied knowledge of fathead minnow reproductive biology and behavior. In addition, it has raised other questions concerning the utility of this model system to assay for environmental influences on reproductive behaviors monitored in fathead minnows.

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