

THE EFFECTS OF GONADECTOMY AND METHYL TESTOSTERONE
ON THE REPRODUCTIVE BEHAVIOR OF THE BLUE GOURAMI
{TRICHOGASTER TRICHOPTERUS}

by

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ABSTRACT

The blue gourami (Trichogaster trichopterus), does not appear to go through a ritualized prespawning behavior sequence. The female is apparently brought into a state of sexual activity as a result of the presence of a dark, nest building male. Spawning is initiated by the female and it consists of a stereotyped cycle of behavioral events.

In the majority of cases, male castration results in the cessation of nest building, a reduction of colour change, a partial atrophy of the morphological secondary sexual characteristics (S.S.C.), and the absence of spawning. Treatment with methyl testosterone brings back all of these characteristics.

In a few cases, castration resulted in only a partial reduction of nest building, colour change and morphological secondary sexual characteristics and the retention of spawning. It is tentatively suggested that there may be an extragonadal source of androgen.

It seems the physical act of spawning is

necessary to trigger full parental behavior regardless of gonadal condition. Agonistic behavior is not apparently affected by castration. Methyl testosterone given to unoperated females resulted in male-like agonistic behavior, coloration, secondary sexual characteristics and some evidence of nest building.

TABLE OF CONTENTS

	Page
ABSTRACT	i
TABLE OF CONTENTS	iii
LIST OF TABLES	v
LIST OF FIGURES	vi
ACKNOWLEDGEMENTS	viii
 Chapter 1. Introduction	 1
Chapter 2. Materials and Methods	6
Chapter 3. The Reproductive Behavior of the Blue Gourami	14
Chapter 4. An Analysis of the Effects of Gonadectomy on Male Reproductive Behavior	27
Chapter 5. An Analysis of the Effects of Methyl Testosterone on Male Reproductive Behavior	53
Chapter 6. Some Morphological Effects of Gonadectomy and Methyl Testos- terone Treatment on the Secondary Sexual Characteristics of the Male and Female.	69
Chapter 7. Parental Behavior seen in Sham Operated, Gonadectomized and Testosterone Treated Gonadec- tomized Males.	76

	Page
Chapter 8. An Analysis of the Agonistic Behavior between Gonadectomized and Sham Operated Males	85
Chapter 9. General Discussion and Conclusions.	93
LITERATURE CITED	111
APPENDIX	113

LIST OF TABLES

TABLE		Page
I	Results of reoperation at the conclusion of all experiments	49
II	Results of egg transfers	78
III	Fish combinations used to test agonistic behavior	86
IV	Sh and unoperated male fin ratios	114
V	Unoperated female fin ratios	114
VI	Gonadectomized (GII and GIII) male fin ratios	115
VII	Gonadectomized hormone treated male fin ratios	116
VIII	Gonadectomized Control (GII and GIII) male fin ratios	116
IX	Hormone treated unoperated female fin ratios	117

LIST OF FIGURES

FIGURE		Page
1.	A qualitative record of spawning times and nest building in sham operated and gonadectomized males.	30
2.	Approach.	34
3.	Display	34
4.	Butt	37
5.	Male Chase, Female Flee	37
6.	Nest Building	40
7.	Hiding.	40
8.	The percentage of male approaches resulting in female fleeing to Hiding	41
9.	The spawning cycle.	44
10.	Mean spawning cycles and nest building intervals for Sh and unoperated males	46
11.	Mean spawning sequences and nest building intervals for GII males	47
12.	A qualitative record of spawning times and nest building in G-H treated and G control males	55
13.	Approach.	58
14.	Display	58
15.	Butt	60

FIGURE	Page
16. Chase, Flee	60
17. Nest Building	62
18. Hiding.	62
19. The percentage of male Approaches resulting in female Flight into Hiding. .	64
20a. Mean spawning cycles and nest building intervals for G-H males showing overall mean and Sh overall mean.	66
20b. Effects of Gonadectomy and Methyl Testosterone on dorsal fin ratios	71
21. Mean frequencies of behavior patterns recorded for Sh-Sh, Sh-G, and G-G male encounters	89
22. Average bout length for each class. . . .	89

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CHAPTER 1

INTRODUCTION

During the past two decades much research has been directed towards the role of hormones in reproductive behavior among the vertebrates. The majority of this work however has involved the mammals. Until recently, the results seemed rather clear cut, i.e., gonadectomy resulted in a loss of sexual behavior and replacement therapy of exogenous hormones brought back sexual behavior to pre-operative levels. Recently however, as research has spread to include a wider range of species the situation has become less simple than was once thought. In the higher mammals, experience appears to play an increasing role. For example, copulation may persist for months or years in castrated male cats depending on the level of previous experience (Rosenblatt and Aronson, 1958). Harlow (1962) showed that monkeys raised on dummy mothers and deprived of play contacts with peers were unable to mate in adulthood. The recent work involving prenatal or postnatal hormonal sex determination of the brain has opened a whole new area of research.

The results of these recent mammalian findings may help to explain the apparently contradictory results being

reported from the lower vertebrates, fish in particular. No one has investigated the role of experience or developmental factors in fish thus far.

Aronson (1959) reviewed the work involving fish. He reports that among females, spayed jewel fish (Hemichromis bimaculatus) and Siamese fighting fish (Betta splendens) exhibited no sexual activities when placed with ripe males. Similar results were obtained from the female West African mouth breeding cichlid (Tilapia macrocephala). Spayed swordtails (Xiphophorus helleri) on the other hand remained sexually attractive to males.

Among males he noted that several authors have found nearly a complete cessation of nest building behavior following castration of the three spined stickleback (Gasterosteus aculeatus). Castrated Salmo salar showed no interest in females. Castrated male jewel fish and fighting fish were reported to show typical courtship, spawning, fertilization and brooding. There is some doubt as to how fertilization could occur if castration was complete! One and a half months from castration, the male blue acara (Aquidens latifrons) still displayed spawning behavior with little change in the frequency of occurrence or duration from preoperative levels. The only difference

S

noted was an increase in nest passing behavior and a decrease in nest building. In male platyfish following gonadectomy, the only change in sexual behavior was a decrease in gonapodial thrusting. In goby males (Bathygobius soporator) spawning behavior is not affected by castration but operated males became indiscriminate and courted males and females in the same manner.

Aronson drew the following conclusions:

- 1) Removal of testes or ovary causes eventual decline in some or all sexual behavior.
- 2) In all species studied, the decline is greater in the female than in the male.
- 3) In all species, certain elements are affected more rapidly than others.
- 4) In males, parts of the sexual pattern remain for long periods after castration.

Pickford (1952, 54) and Aronson (1957) state there is some evidence showing that certain components of reproductive behavior may be controlled directly by pituitary action. Aronson suggested this may be a primitive condition in vertebrates. Wai and Hoar (1962) confirmed that the testes was necessary for nest building in sticklebacks but that a high level of pituitary gonadatrophin was essential for the normal expression of the behavior. Liley (1965)

concluded that gonads were not essential for sexual behavior in the female guppy. On the other hand the gonad appears to exert a regulatory influence superimposed on a more direct neural and/or pituitary control of receptive behavior.

The present research was initiated on the basis of this rather confusing and conflicting background of findings. The choice of an Anabantid fish, the blue gourami, (Trichogaster trichopterus) as an experimental animal was made largely as a result of reading an excellent description of its reproductive behavior by Miller (1964). It was realized from Miller's work that this was a species with an elaborate set of behavior patterns, easily bred and hardy under laboratory conditions and about which very little was known in terms of behavioral regulatory mechanisms. It was thought that if some preliminary studies along this line were undertaken and proved successful this would be an excellent experimental animal for future research.

The Anabantids are a group of highly specialized fishes native to much of South East Asia. There are some 16 genera containing 50 species. Forselius (1959) in a large monograph, gave a comprehensive review of the

literature dealing with the systematics, distribution and biology of these fishes. However, his observations were mainly concerned with the genus Colisa. Hodges and Behre (1953) were the first to publish an abbreviated description of the spawning behavior of T. trichopterus.

The present work is an exploratory study of the role of hormones in the reproductive behavior of the blue gourami. A "classical approach" has been adopted. This involved observing the effects of gonadectomy and replacement therapy. It was assumed that the gonads are the site of hormone production. As far as possible, all aspects of reproductive behavior were considered, i.e., sexual, agonistic and parental behavior. I was interested to learn whether the various components have a common causal factor or control mechanism or whether they are controlled independently.

CHAPTER 2

MATERIALS AND METHODS

A. Materials

All observations were carried out in twenty-four, glass and stainless steel aquaria measuring 51 cm x 27 cm x 31 cm and each holding approximately 43 liters. Numerous other tanks were utilized however, as recovery, breeding, stock and holding tanks. These ranged in size from 12 to 200 liters. The observation tanks were mounted on two stands each holding twelve tanks in two rows of six. Each was equipped with a sub-sand filter which was covered by a layer of course Del Monte (E.I. 16) white sand to a depth of about one inch. The temperature in all tanks varied between 25° and 29°C. The mean temperature in all tanks maintained throughout was 27°C. (80°F). The tanks were filled with tap water which was allowed to stand at least two days before fish were placed in it. About 3 gms of sea salt were added to each tank. Each tank had a layer of floating plants which were thinned about every 2 weeks. One plant was anchored in the sand at each end of every tank to provide cover. The tanks were illuminated by soft white fluorescent tubes mounted 23 cm above the centre line

of the tanks. All fish were kept on a twelve hour photoperiod from the time they were hatched.

Originally twelve pairs of Blue Gouramis were purchased from local aquarium dealers in Vancouver. These fish were utilized in preliminary familiarization observations and experiments. However, all fish used in the actual experiments were bred in the lab from these originals. In this way the age and history of all fish could be standardized. This also cut down on potential sources of disease.

The adult fish were fed once a day with frozen brine shrimp (Artemia) as their staple diet. This was supplemented with various forms of dried foods and liver and pabulum mixtures. The fry were raised initially with "Wardley's Small Fry" suspension until they were about 1 cm in length. They were then given fresh hatched living Artemia until they were large enough to handle the frozen form.

Recordings were made using an Esterline Angus, 20 channel, ink recorder which gave measures of duration and frequency. This was operated by remote control from a portable keyboard which could be taken to any tank desired.

Different codes were used on this keyboard depending on the type of sequence being recorded. Operations were performed under an Olympus binocular dissecting microscope.

B. Procedures

1) Behavioral testing

During the course of preliminary observations of the animal's normal reproductive behavior the following testing method gradually evolved. It was found that if a male and female were placed in a tank and separated by a loose fitting opaque partition, they became highly sexually stimulated after two or three days. Generally a breeding nest was constructed during this period. Upon removing the partition, courtship would begin and in about 70% of the cases proceed to spawning on the morning or afternoon of the second day. Behavior was recorded on the days following release until spawning occurred.

The following is an outline of the testing procedure in all the experiments to be described later:

Day 1 Male and female placed in a tank separated
 by a loose fitting black plexiglass
 partition.

Day 2 No change.

Day 3 No change.

Day 4 A.M. Partition removed.

Record I. Behavioral recording made immediately following release for 15 minutes.

P.M. Record II. 15 minute behavioral recording.

Day 5 A.M. Record III. 15 minute behavioral recording.

P.M. Record IV. Generally a continuous behavioral recording throughout spawning. (2-4 hours).

Day 6-10 Qualitative observations made as to nest size, state of eggs and/or fry.

Day 10 Female removed, test terminated.

Except for minor variations to be described later, this method was employed throughout to test normal males, gonadectomized males and gonadectomized hormone treated males.

2) Gonadectomy

A great deal of time and effort was expended in perfecting the following technique which may be broken down into 4 steps.

a) Anaesthetic

The fish was placed in 500 ml of a sol'n. of 1:2500 strength Tricaine Methanesulphonate (M.S. 222) in water. This sol'n was maintained at a temperature of 16-17°C giving the fish a ten degree temperature shock along with the anaesthetic. The fish was removed as soon as it began to lose balance and lie over and while it was still breathing. This varied between 2 to 3 minutes.

b) Operation

The partially anaesthetized fish was placed on its right side on a sponge pad in a wax filled operating tray designed for the fish; i.e. slightly concave to fit the contour of the fish. When in place on the pad it was totally immersed to a depth of 5 mm in a standard fresh water teleost saline at a temperature of 20°C.

The fish was then pinned down by means of 3 sponge rubber strips. One was placed across the mid region from just anterior to the dorsal fin to below the middle of the anal fin. The second crossed over the caudal peduncle. The third passed over the snout and eye region but did not cover the operculum. This latter strip was placed so the gills were free to move as the fish breathed slowly throughout the operations. It is believed

many fish were lost due to this head strip being too tight and thus causing death by suffocation.

A small glass nozzle was inserted into the mouth directing a flow of fresh aerated aquarium water over the gills.

A curved incision was made slightly anterior to and parallel with the natural posterior curve of the body cavity. On cutting two ribs dorsally in the incision the curved flap was folded back and held using a special clamp designed from a "bobby pin". This gave ample access to the body cavity. Following the removal of the testes, the flap was returned and held with one suture using surgical silk.

c) Revival

Often upon removal of the holding strips, the fish began swimming almost immediately. Usually, however, it was assisted with artificial respiration by means of squirting water into the gill chamber, on and off in time with its gill movements.

d) Recovery

Following initial revival, the fish was placed in one of four, all glass, three gallon recovery tanks

containing a 50% sol'n of the fish saline and maintained at 27 degrees C. These tanks were equipped with outside filters. All fish were kept in these tanks for three or four days before being replaced in their regular aquarium tanks where they remained for complete recovery. After 4 weeks they were retested. Sham operated control fish were given the exact treatment described above except the testes were not removed.

3) Hormone Administration

All fish were anaesthetized under the same conditions described. They were then held in moist cotton wool for injection.

Each fish received a series of 4 injections, one every two days. Each injection contained 1 mg of methyl testosterone suspension in 0.05 ml of distilled water and Tween 80 (one drop/25 ml). These were administered in the peritoneal cavity using a No. 26 hypodermic needle. On the day following the 4th injection, (8 days from the initial treatment) partitions were installed and females added. This led directly to the behavioral testing procedure described above.

4) Fish Measurements

Fish were placed on a moistened sponge pad and measurements were made of their length and fins using a pair of drafting dividers. Each fish was measured twice i.e. once following gonadectomy and once following hormone treatment. Two measurements were taken.

- a) the standard length (snout to caudal peduncle).
- b) the distance from the snout to the distal tip of the dorsal fin. The two resulting lengths gave a ratio as shown below.

$$\frac{\text{Snout to Dorsal Fin Length}}{\text{Standard Length}} = \text{Fin to Length Ratio}$$

5) Egg Removal, Transfers and Incubation

Eggs were removed from tanks by means of lowering a small petrie dish (submerged beneath the nest) and slowly lifting. Any plants caught in the dish were trimmed off with scissors as the dish was lifted. The eggs and bubbles were placed from the dish into other tanks by means of a teaspoon. Eggs to be incubated were left in the petrie dish and the whole dish plus eggs floated in a tank containing water at the desired temperature.

CHAPTER 3

THE REPRODUCTIVE BEHAVIOR OF THE BLUE GOURAMI

Introduction

A long series of preliminary studies enabled the observer to become familiar with the various behavior patterns involved in the total reproductive behavior. The individual behavior patterns recorded were described in detail by Miller (1964) and will be presented only briefly here in the first section. A general qualitative account of the reproductive and agonistic behavior is included in the second section to provide a better understanding of the experimental work to follow.

A. List of behavior patterns recorded

The following is a brief description of the behavior patterns recorded during this research. The records were taken in terms of frequency or duration of behavior or both. The symbols given beside the names indicate the behavior pattern code letters and will appear again in this thesis.

I. Male Behavior

- 1) Approach (A) The act of purposely swimming towards the female in any way from any direction.
- 2) Pelvic Fin Ray Contact (P) The act of reaching out and contacting the body of the female at any point with the pelvic fin rays.
- 3) Partial Display (Fin Erection) (E) The act of erecting the fins in what is regarded as a low intensity display. This means a display lacking sigmoidal body torsion.
- 4) Full Display (D) A high intensity display which involves full fin erection plus lateral sigmoidal body torsion. This display may take place in front of the other fish (frontal), or parallel to it (lateral). No distinction was made since one phase may lead into the other and back again depending on the motor component.
- 5) Butting or Biting (B) The act of butting or biting at the female generally at the anal fin or caudal peduncle area.
- 6) Chasing (Ch) The act of pursuing the female around the tank rapidly. It is generally accompanied by Butting.

7) Colour Change (Co) The darkening of the male through the reproductive period was recorded in terms of intensity on a scale of three: 1 = blanched; 2 = vertical dark bars giving a grey mottled appearance; 3 = inky black all over.

8) Nest Building (N) The act of gulping air at the surface and spewing out bubbles into and around the nest, collecting, mouthing and placement of eggs.

9) Rubbing (R) The act of moving back and forth under the female rubbing her ventral surface just prior to spawning.

10) Spawning (S) This includes five separate behavior patterns and only the inclusion of all five in succession on a record indicates a complete spawning act.

- a) Encircling:- The act of curving around the female until the clasp is established.
- b) Clasping:- The actual rigid grip of the female by the male.
- c) Rollover:- The act of inverting the female at the end of the clasp.
- d) Oviposition:- The release of eggs and presumably spermatazoa.

- e) Swimming Inhibition:- The period of time from the breakup after rollover until the female recovers and the male begins to chase her away.

II. Female Behavior

Certain behavior patterns of the female were recorded simultaneously. Many of these are identical to the male patterns and therefore only new patterns will be elaborated on.

- 11) Approach (A)
- 12) Pelvic Contact (P)
- 13) Partial Display (E)
- 14) Full Display (D)
- 15) Biting or Butting (B)
- 16) Fleeing (F) The act of rapid retreat from the male, usually simultaneously with the male Chasing.
- 17) Colour Change (Co)
- 18) Hiding (H) The act of remaining inconspicuous behind or below substrate plants or occasionally among the surface plants.
- 19) Appeasement (Ap) The act of rolling over laterally when the male approaches exposing to him the flanks and belly. This act appears to inhibit male attack.

20) Fin Tugging (Ft) The act of grasping a fin (usually the dorsal or caudal) in the mouth and rocking backwards and forwards or sideways. This appears to be the most aggressive act employed by this species and usually occurs following a long series of Butts.

The behavior patterns described will be referred to throughout this thesis. To clarify, they will be "capitalized" whenever used.

B. A Qualitative Description of Reproductive Behavior

Trichogaster trichopterus, the Blue Gourami, is a bubble nest breeder. Like the other Anabantids, it possesses a labyrinth chamber dorsal to the pharynx which enables it to store air and utilize aerial oxygen to some extent. Indeed, some members of the group can remain out of water for long periods of time using this apparatus. With a secretion of mucus from within the pharyngeal chamber, the male gourami can, on gulping in air, issue a stream of bubbles at the air-water interface. By continuous gulping and blowing, the animal builds a mound of bubbles which may reach 15 cm in diameter and 1 cm deep. It is generally anchored to the floating plants or in their

absence, spreads out along the sides of the tank.

This nest becomes the focal point of the reproductive behavior. The nest is generally begun during the initial phase under the conditions of the testing procedure outlined in Chapter I i.e. during the time when the male and female are separated by the partition. In fact the presence of a nest at this time is a good indication that spawning will take place shortly after release.

Sexual dimorphism in T. trichopterus is less distinct than that found in other closely related species. In the non-reproductive phase, it is limited mainly to two characteristics.

- 1) The normal male possesses a large, elongated and pointed dorsal fin, whereas the female has a much shorter, rounded dorsal fin.

- 2) The abdominal region of the male is flat or smoothly curved laterally whereas the female possesses a distinct abdominal bulge laterally caused by pressure from the ovaries. This abdominal bulge was used as a rough indication of the physiological state of readiness to spawn in the female.

When in the reproductive phase of behavior the male

becomes increasingly darker until, during spawning, he is inky black. The female rarely darkens as much, but is more of a grey-black at this time. When not spawning both sexes are a silvery powder blue with very little distinction between them.

The actual reproductive behavior can be subdivided into three phases.

I Prespawning Courtship

II Spawning

III Post Spawning Parental

I Prespawning Courtship

Initially, upon release the two fish Approach each other extending the Pelvic fin rays by which they contact each other. Then follows a series of lateral or frontal Displays with or without Colour darkening. The Display consists of a full erection of the median fins plus a rigid body tortion which is best described as sigmoid in shape. The extent of Display and darkening appear to depend on the relative size of the fish. The larger the female is, the more intense the male Displays. The Display sequence is usually terminated by a Butt or Bite

by the male aimed at the region of the caudal preduncle or anal fin tip of the female. She may return the Display and Butt to the male and if so the bout may continue. Usually, the male asserts his dominance after one or two exchanges, particularly if he has a nest in the tank. The male Butt is usually followed by the female either Appeasing or Fleeing with the male pursuing in a Chase. The female then seeks refuge behind one of the bottom plants. However, she soon will emerge and the whole sequence is repeated. Gradually over the course of this first day the male becomes more aggressive. His Butts and Chases increase in vigour until the female is nearly completely confined to Hiding. Between the intervals of Chasing the male spends more and more time Nest Building. During the morning of the second day he is occupied at Nest Building almost constantly, except to defend the nest from the female. During this period of intense Nest Building the colour of the male darkens and remains dark unless he is disturbed. A sudden noise or movement will cause blanching in about 3 seconds. In about 70% of the trials spawning began around noon on the second day.

II Spawning

The spawning sequence appears to be a stereotyped reaction chain. It may begin in one of two ways:

1) The male may Approach the Hiding female, Display to her and lead her to the nest. This behavior was, however, rarely observed.

2) The female leaves her spot of refuge on her own initiative and rapidly Approaches the Black, Nest Building male and Butts him smartly two or three times in rapid succession on the side just posterior to the central lateral spot. This gesture appears to inhibit the male's aggression, but in extreme cases he will turn on the female and a violent Chase results following which the female seeks refuge and the male returns to Nest Building. Normally, however, the Butt elicits a male Display which proceeds to Rubbing. The Rubbing behavior is unique to T. trichopterus (Miller 1964). It consists of the following actions. The male orients himself directly under the nest at an angle of 30 degrees to the water surface, head up. The female approaches from above and behind the male. She moves into a position

parallel to the surface so that the male's dorsum contacts her belly and genital areas. Then the male moves backwards and forwards about once a second and may continue from 20 to 120 seconds. He then draws forward in a curving Display. The female moves into the curve and places her head above his dorsum. The male continues to tighten the curve until he wraps completely around the female who is now oriented almost vertically to the water surface. If both the fish are oriented properly, this results in the Clasp by which the male grips the female tightly and begins to vibrate. Sexual ducts are brought very close during the Clasp. The Clasp lasts about 35 seconds and is terminated by Roll Over. The caudal fins of both fish cause this which results in a complete inversion of the female so that her genital opening is directed upward. It is at this point that the gametes of both sexes are released. The eggs and sperm float to the surface to become caught up in the sticky bubbles. The male now releases the female and both begin to sink slowly with no other movement. The male recovers first and gently begins to nudge the female down and away from the nest site. The female

recovers almost immediately and begins to swim with the male in rapid pursuit. The Chase is of short duration. The female Hides and the male returns to the nest. He now begins collecting any stray eggs and spews them into the bubble nest. He works continuously, Nest Building and egg placing and mouthing. Generally he clumps the eggs in the center of the nest.

The whole spawning cycle may be repeated many times over a period of up to four hours. Quite often the initial spawning attempts do not result in oviposition. The same occurs near the end of the spawning when the last clasp produces only three or four eggs or none after which the male will not tolerate the female under the nest again. During the course of the spawning up to 1000 eggs may be laid.

III Parental Behavior

The fry hatch in about twenty four hours. The male shows parental behavior which consists of collecting, mouthing and spewing the fry into the bubbles. After about two days, however, the fry become active and spread out through the tank. After this, the time spent nest tending gradually diminishes over a period of about five

to six days. Males have been left with growing fry for upwards of four weeks during which time they were apparently completely inhibited from eating them. Fry or eggs placed with a male not recently spawned are immediately eaten. No accurate measure of the time length between individual spawning cycles was obtained since the female was removed one week after spawning. Indications are that a new cycle might start in about ten days to two weeks. However, on three occasions this observer noted pairs spawn on successive days.

C. A Description of Agonistic Behavior

When two males are released together the behavior which results is quite different from that which occurs between a male and female. The males Approach each other initially and exchange a number of Pelvic Ray Contacts and then begin to circle each other with a series of Full Lateral Displays. Sooner or later one of the males will turn in and begin Butting the other on the flank and caudal peduncle regions. The fish receiving the Butts remains in the Display flexure. Then it will suddenly turn and begin Butting. The original Butting fish now assumes the Display posture.

Thus a series of mutual, alternate Display and Butting sequences begin. During this time both fish become inky black in colour. As Displaying and Butting progresses alternate Fin Tugging begins to appear following long Butting sessions. This behavior continues until one of the fish suddenly stops Butting, blanches and either swims away or Flees. The victor rarely pursues the loser, instead both simply part company and return to their original ends of the tank. Once dominance has been established there is little further recourse to such hostilities. If the dominant fish happens to have a nest in his half of the tank, he will defend it by a rushing Approach, a Display or a Chase.

CHAPTER 4

AN ANALYSIS OF THE EFFECTS OF GONADECTOMY ON MALE REPRODUCTIVE BEHAVIOR

Introduction

A comparison was made between the reproductive behavior recorded from a group of sham-operated, control males and a group of gonadectomized males. An analysis of the results also provides quantitative data to supplement the descriptive account given previously.

Procedure

Young males were isolated from a large growing tank as soon as sexing was possible and raised to maturity in all male tanks. Virgin males were then selected from these tanks as desired and paired with virgin females of the same age. The pairs were then put through the testing procedure described in Chapter 1 but no records were taken. All the fish, male or female, which spawned on the second or third day after release were selected for the experiment. All others were rejected. Thus all experimental fish used were capable of spawning

readily under the test situation and had had one spawning experience prior to testing. As mentioned earlier, the number of males responding in this way amounted to about seventy percent of those tested. The selected females were placed together until required. The selected males were either gonadectomized or sham-operated as controls and allowed to recover for one month. At this time they were again paired with the selected females and put through the test and recorded. Altogether over a period of two months 6 sham operated controls and 16 gonadectomized experimental males were tested.

Results

A Time to Spawning

Fig. 1 shows the day and time at which spawning occurred following the removal of the partition on Day 4. The "G" and "Sh" numbers indicate either gonadectomized or sham-operated and were assigned to the fish following the operation.

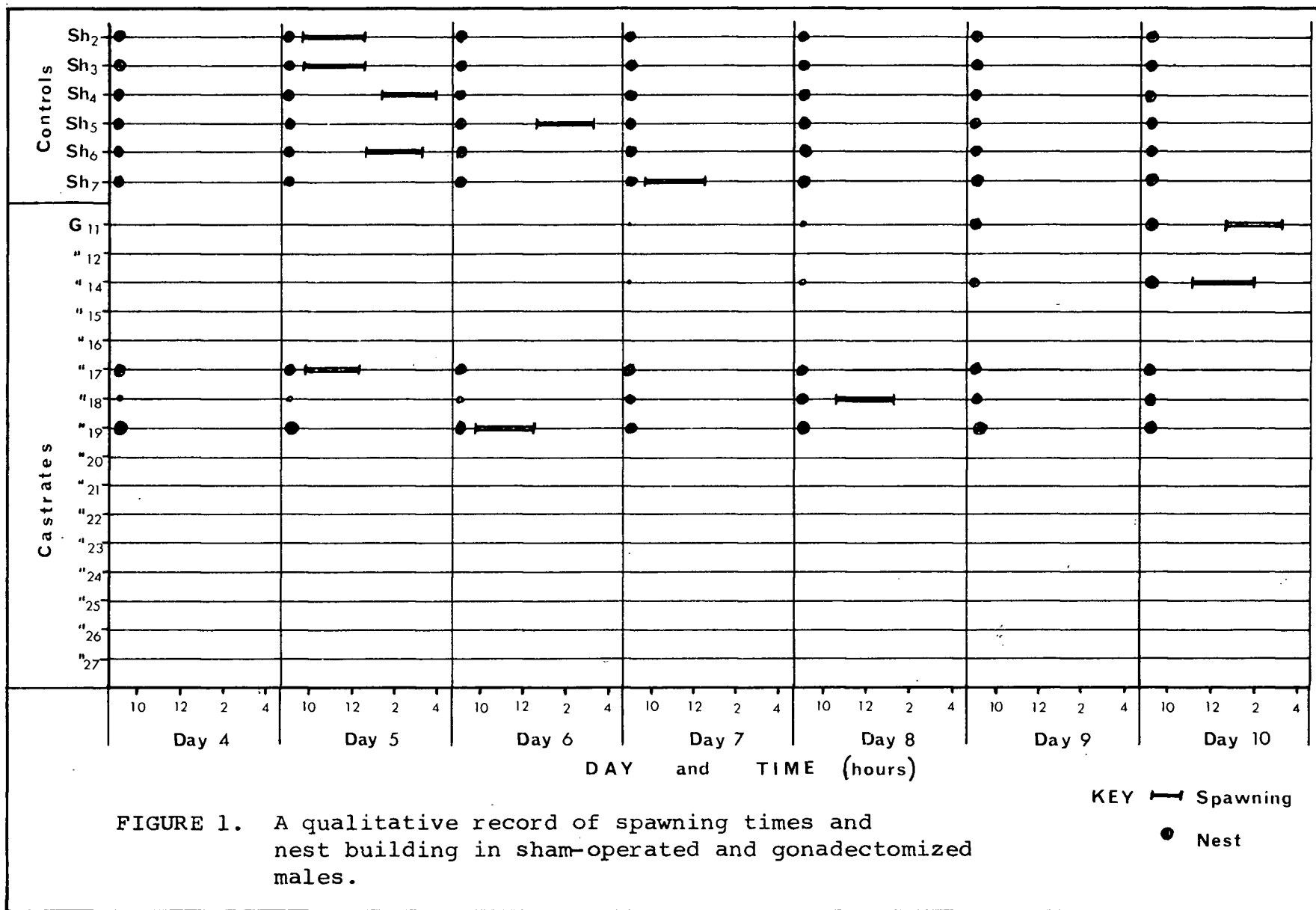
From this chart (Fig. 1), it can be seen that 100%

of the Sh males spawned under the test situation, although Sh₇ did not spawn until Day 7. The duration of the spawning varies between two to four hours although this chart is not intended to be accurate in this respect. It can be seen also that spawning may take place at any time of day but there is a tendency for it to occur during the first half of the day. All spawning fish had previously built a bubble nest during Days 1-3 and in all cases this was maintained following spawning until after Day 10. Only once during a year's observations was a spawning seen without the presence of a nest. It may be noted that no spawning has ever been known to take place at night. Further, when observed for short periods at night the fish were usually sitting quietly on the sand or among the plants and so it has been assumed that most activity takes place during daylight hours and therefore the dark hours have been eliminated from all charts and graphs.

The gonadectomized fish fall into three categories.

Category I:- Those G fish which spawned within seven days from release and produced viable or fertilized eggs (4 fish:- G₁₂, 13, 18, 19). These fish were

not shown in chart



reoperated and found to have regenerated testicular tissue, this was removed. During this process G₁₃ died. The remaining three were allowed a month's recovery and retested from whence they fell into either category II or III.

Category II:- Those fish which spawned within seven days of release but failed to fertilize any eggs (5 fish:- G_{11, 14, 17, 18, 19}). After a spawning of a G fish, about 50% of the eggs were removed and incubated separately to check for viability. Fig. 1 also shows a much greater variability in the spawning time from release among the Cat. II fish than among the controls. Only two fifths spawned within the first three days compared to five sixths of the controls.

Category III:- Those which failed to show any sexual behavior (11 fish).

The categories II and III will be referred to throughout. From this point on they will be abbreviated as GII and GIII.

B. Prespawning Behavior

The three 15 minute records for each pair sampled the behavior from the time of release (a.m. Day 4) to

the middle of Day 5 when the majority of Sh spawnings began. The results have been presented as a series of mean frequencies, durations or percentages. Although this research is primarily concerned with male behavior, a good deal of female behavior was recorded as well. Some of this has been included as it reflects the male behavior in that it is a response to male actions and thereby helps to illustrate male behavior.

All data presented in Figs. 2-8 were programmed through the University of British Columbia computing center. A calculation of the mean and standard error for every graph point was made. In addition, the following comparisons between the fish groups were made at each of the three Record points.

Sh males compared with GII males

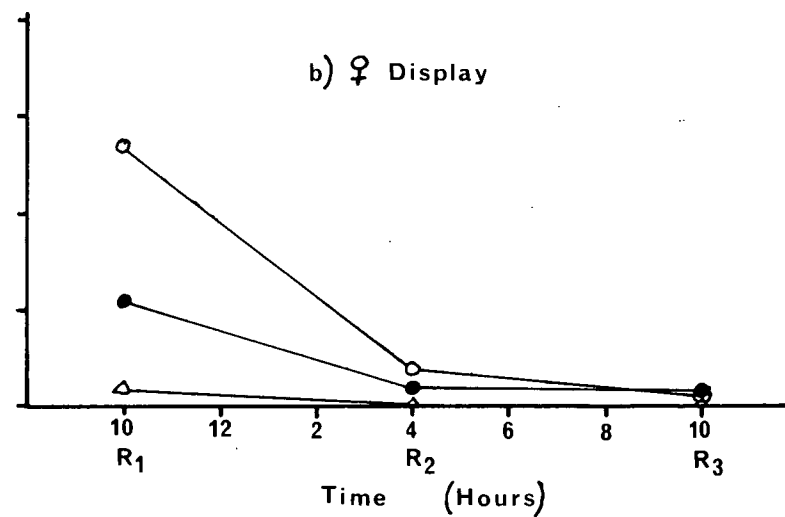
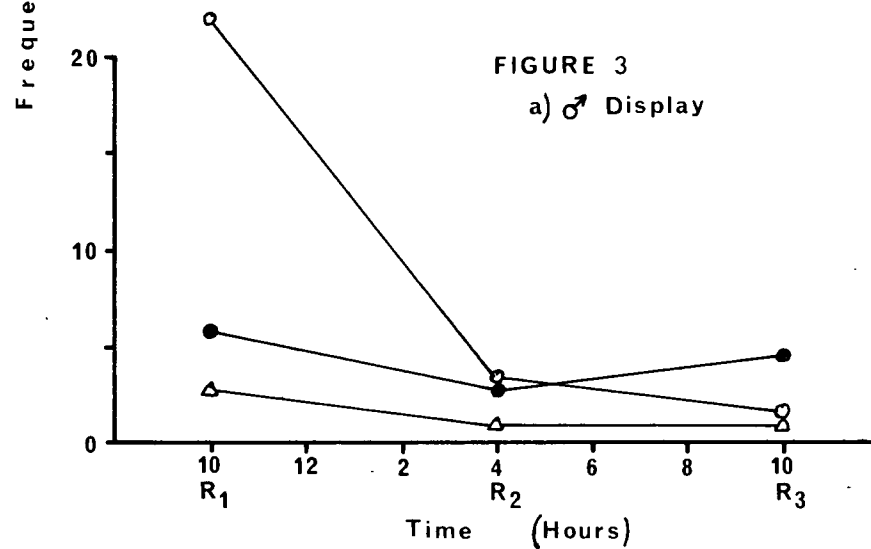
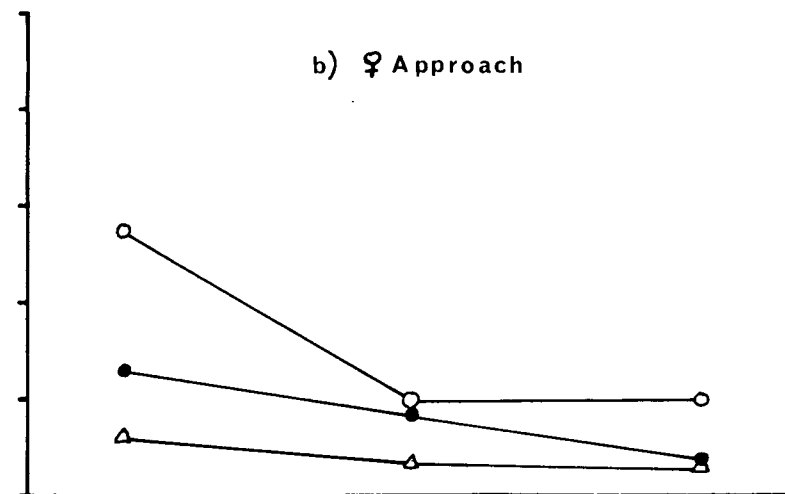
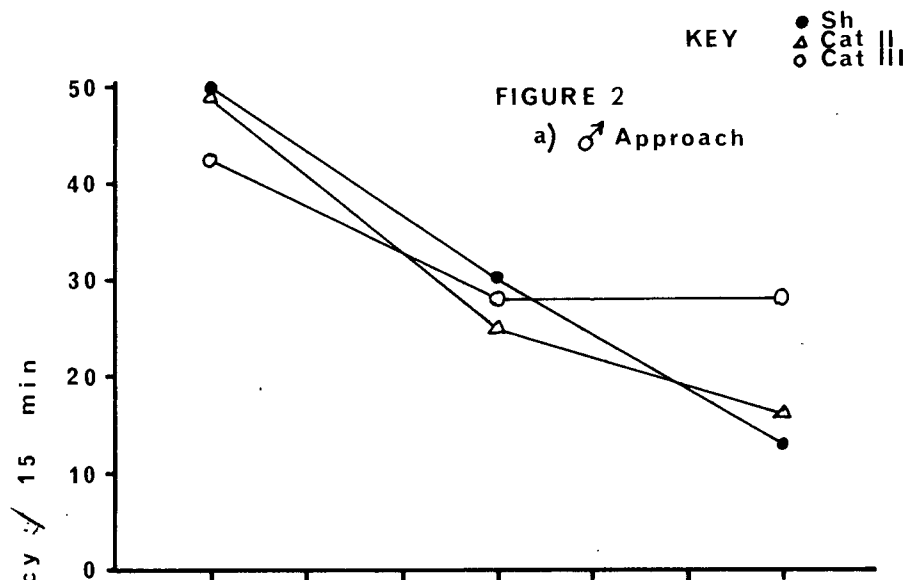
Sh males compared with GIII males

These comparisons were made using the Welch test. This is a "t test" which assumes independence and normality but does not assume equality of variances. It is a conservative test. Values for p were obtained in each case. Those values significant at or below the 5% level have been included in the text in every case. In

addition, larger values have been included to the 20% level to illustrate trends. For all points of comparison where no "p" value appears it may be assumed there is very little significant difference.

Approach Fig. 2

The mean frequencies of male and female Approach are given in Fig. 2. R1 in each case is marked by a high frequency of Approach as this is the initial "meeting period" recorded immediately after removal of the partition. The males and females generally Approach each other and frequently contact each other with the pelvic fin rays during any initial encounter regardless of their physiological condition or sex. As time progresses, the frequency of Approach decreases steadily in both sexes. It is generally always higher in the males, however. GII and III males follow a very similar pattern to the Sh males during R1 and 2 but in R3 the frequency of Approach tends to level off for GIII males (R3: Sh to GIII $p = 0.0540$). Correspondingly, the frequency of Approach for GIII females is slightly higher than the Sh females during all three Records (R1: Sh to GIII females $p = 0.0494$); (R3: Sh to GIII female $p = 0.1278$).



It will be seen later that this is a result of a lower aggressive level on the part of the GIII males.

Displaying and Butting (Figs. 3-4)

These figures indicate trends in the male and female Displaying and Butting. Examining the Sh males first it can be seen that initially there is an equal amount of Display between males and females and that with time, the frequency of D falls to a very low level in the female. In the male, it is maintained and may even rise in R3 since spawning has either started or is imminent. GII males and females show parallel trends but at a lower level. Only once did a GII male spawn at this time, hence the very low level of Display. In R3, the p value for the difference between the Sh and GII males is 0.1070. In R2 and R3 for female Display, comparing Sh and GII females p values are 0.1747 and 0.1073 respectively.

A very different picture was seen in the case of the GIII males and their females. The difference between the Sh and GIII males for R1 of Displaying and Butting is highly significant ($p = 0.0037$ and 0.0212

respectively). Similarly a significant difference appears between the Sh and GIII females in R1 for Butting ($p = 0.0280$). Mutual Displaying and Butting and some Fin Tugging was regularly observed in the majority of cases. During these observations it became very obvious to this observer that these fish were behaving in an agonistic manner almost identical to that of two normal males released together (Chapter 7) or for that matter two females. A casual observation made about 3-4 weeks after the operation began now to clarify itself. Many of the GIII males had considerably shortened and more rounded female-like dorsal fins. Indeed it was difficult to distinguish them from the females. This observer suggests the GIII males were initially mistaken for females by the females. It may be further noted that female Butting of the male was never observed under normal heterosexual contact except during spawning.

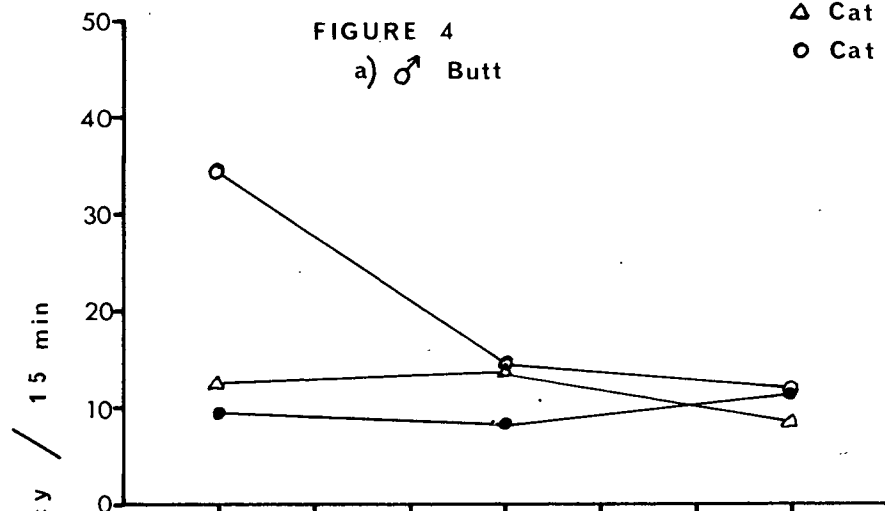
Chasing and Fleeing (Fig. 5a-b)

The trend in male Chasing is illustrated in Fig. 5a. The amount of actual Chasing by Sh males is very slight initially but tends to increase as spawning time nears. GII males show a similar trend initially which levels

KEY ● Sh
 ▲ Cat II
 ○ Cat III

FIGURE 4

a) ♂ Butt



b) ♀ Butt

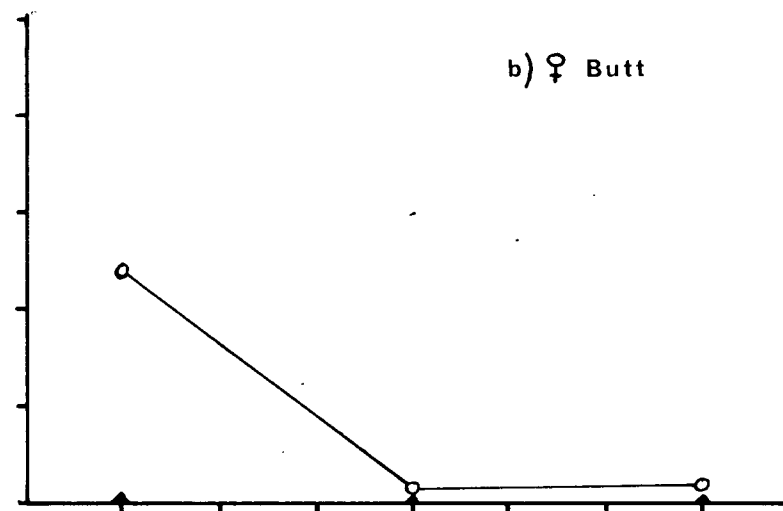
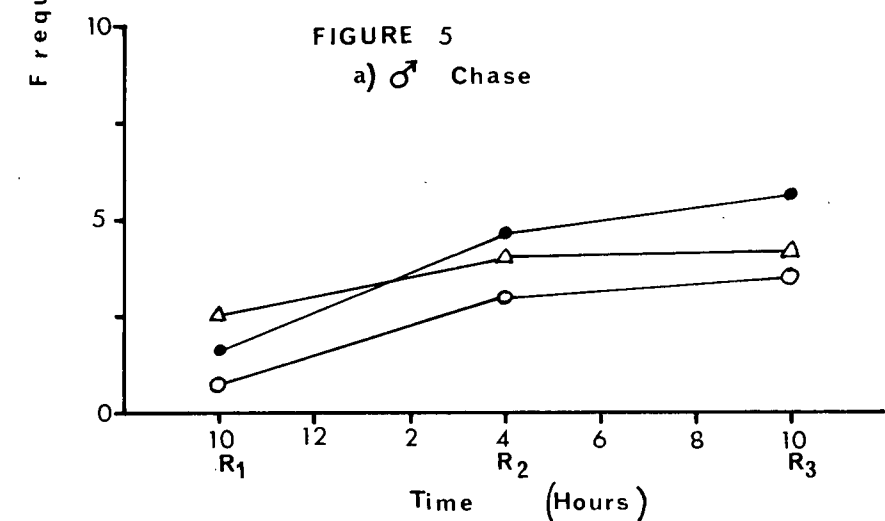
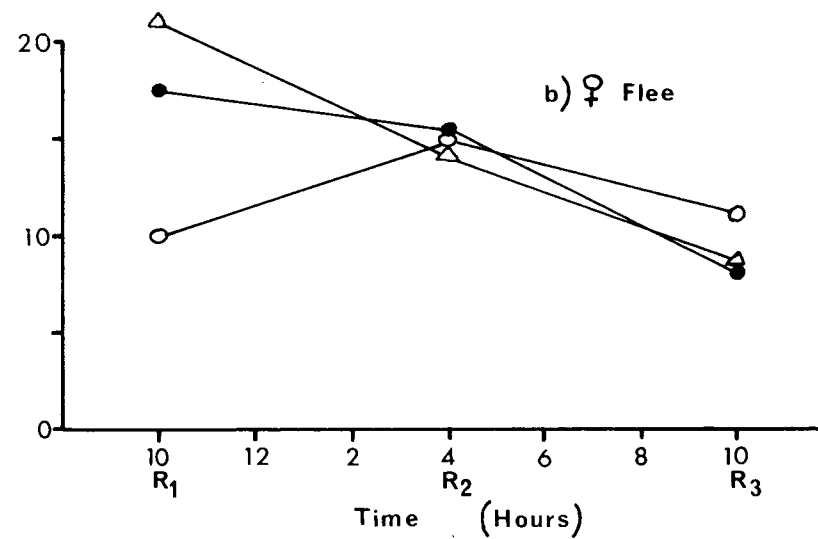


FIGURE 5

a) ♂ Chase



b) ♀ Flee



off in R3 as spawning is not imminent. GIII males follow a similar trend but at a lower level.

Fig. 5b indicates the trend in female Fleeing. Once again there is a close parallel between the Sh and GII females. Both show a high frequency of Fleeing initially which indicates male dominance has been established immediately. GIII females initially Flee much less since the males at this time have not established dominance. In one case, the female remained dominant throughout the test period and Butted and Chased the male into Hiding which is a complete reversal of the normal trend. The difference between R1 Sh female Fleeing and GIII female Fleeing is indicated by $p = 0.1664$. The general decrease in Fleeing frequency noted in R2 and R3 is perhaps misleading. It does not indicate a lowering of male aggressive tendencies but simply that the females Flee less frequently because they are spending more and more time Hiding.

Nest Building (N) and Hiding (H) (Fig. 6-7)

In Sh males, as spawning time nears there is a dramatic increase in the amount of N. Fig. 6a shows

the mean number of actual nest building trips made to the nest. It can be easily seen that GIII males did not Nest Build. GII males show no N at R2 and a mean frequency of one trip per 15 minutes in R3. The differences in R3 between Sh and GII males and between Sh and GIII males are significant ($p = 0.0215$ and 0.0106 respectively). The average time spent in N per Nest Building trip is illustrated in Fig. 6b (Total N duration divided by frequency of N). It appears as though GII males are spending more time Nest Building in R3 but this figure results since they average only one trip. In actual fact, only two of the five GII fish were Nest Building at this time since the majority did not spawn until later (Fig. 1).

The frequency and mean duration per trip of female Hiding behavior is indicated in Fig. 7a-b. In the Sh females, the frequency of H drops in R3 but the mean time per trip increases correspondingly, indicating a much larger total duration. Once again a similar trend is set by the GII females but at a lower level. The occurrence of H appears to be a direct result of male aggressiveness and therefore these graphs are a

FIGURE 6
a) ♂ Nest Building

KEY ● Sh
Δ Cat II
○ Cat III

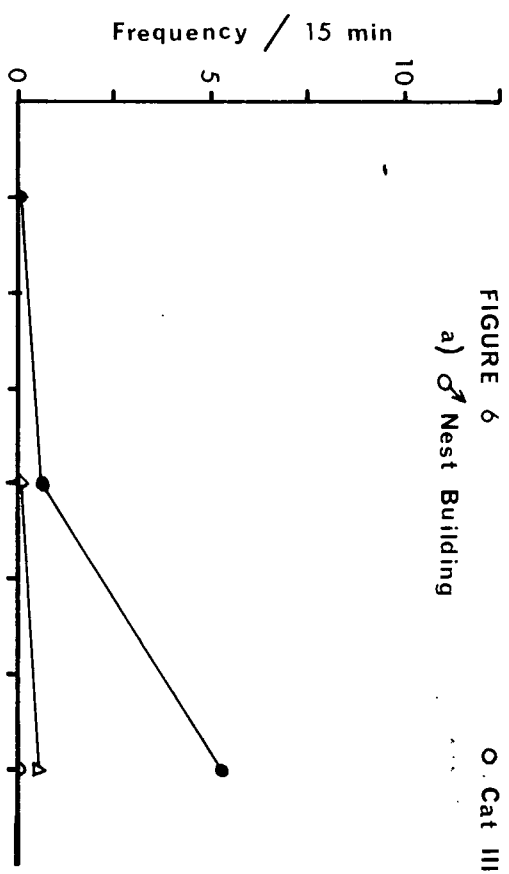
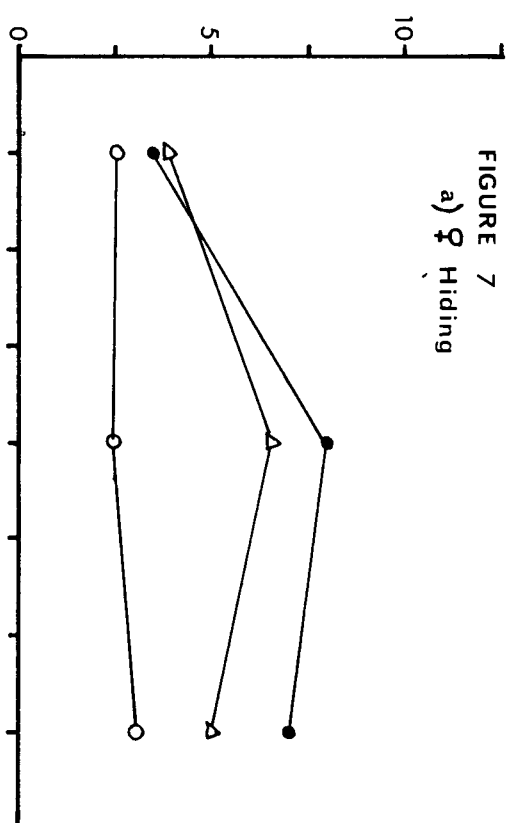
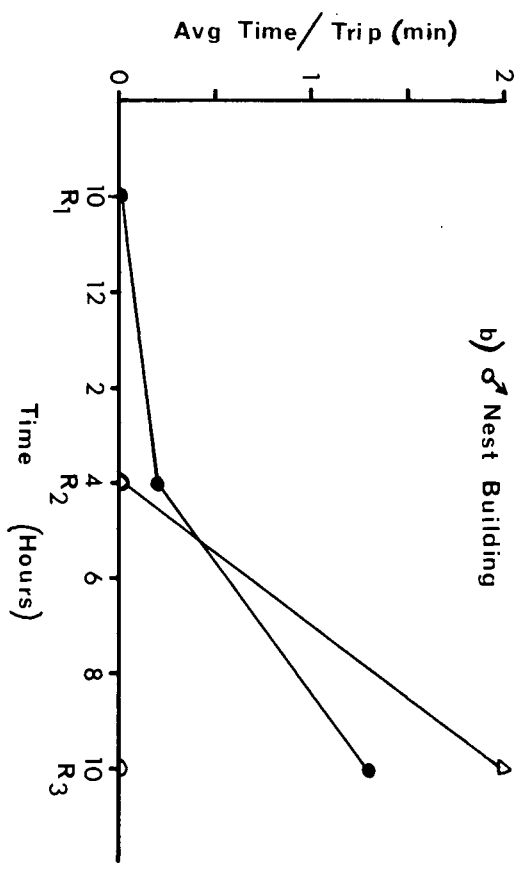


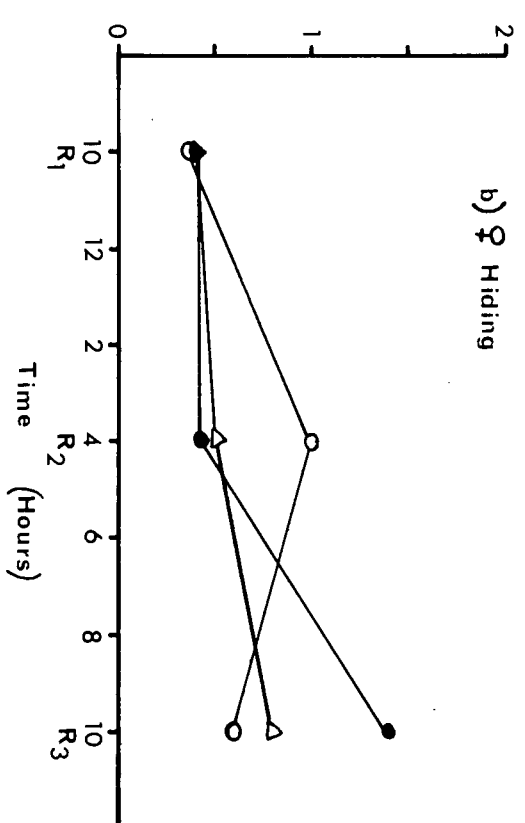
FIGURE 7
a) ♀ Hiding



b) ♂ Nest Building



b) ♀ Hiding



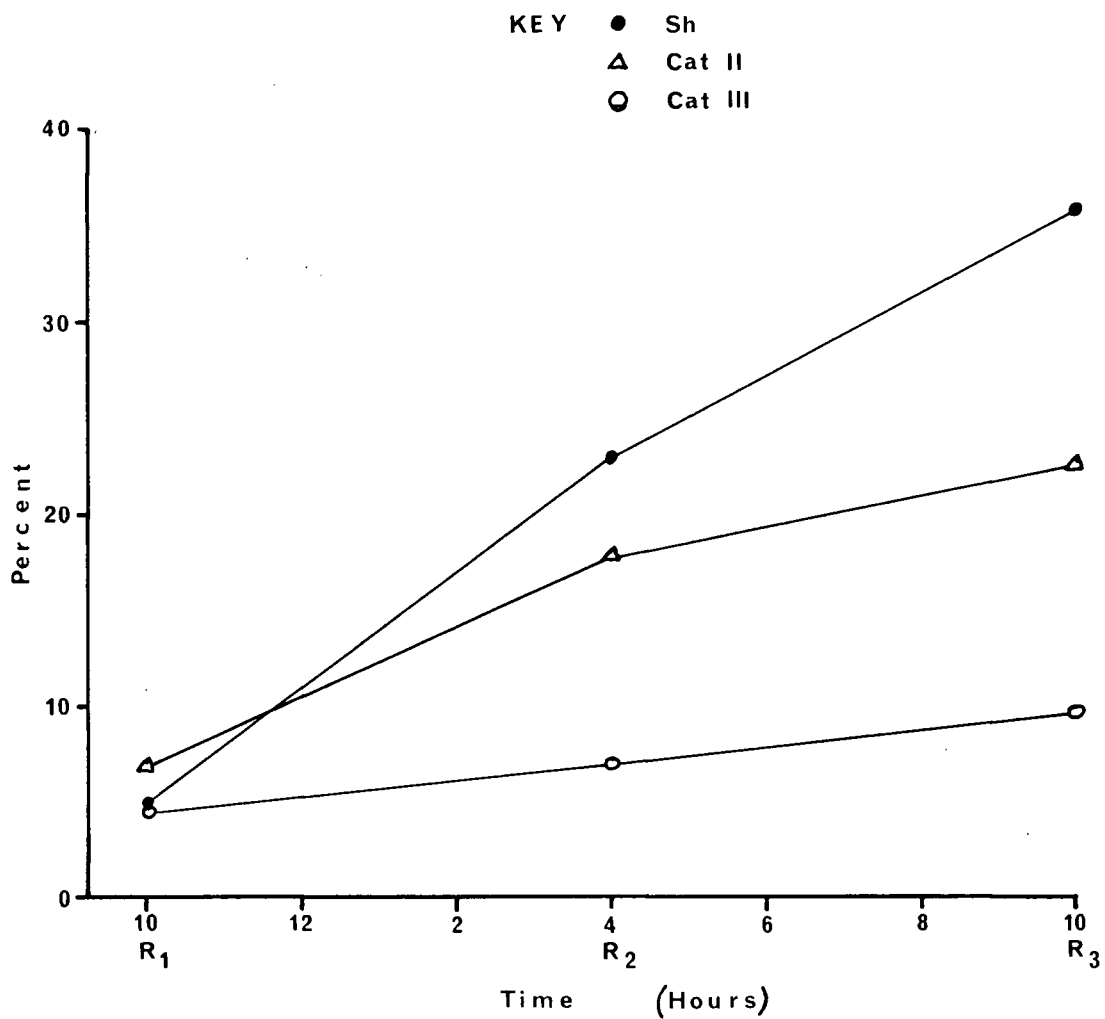


FIGURE 8. The percentage of male Approaches resulting in female Fleeing to Hiding.

good indication of the male aggression levels at the time. As might be expected therefore, the GIII females show the lowest frequency of H and furthermore this varies little throughout. The difference between GII and Sh females in R2 is significant ($p = 0.0385$).

In Fig. 7b another indication of the lack of female intimidation by the GIII males may be seen by the fact that the mean time spent in H per trip actually drops in R3 for GIII females.

In summary of the prespawning behavior trends observed, it appears that the Sh males tend to establish dominance over the females very soon after meeting and continue to exert this dominance by being increasingly aggressive as spawning time nears. This results in females Fleeing into Hiding. This aggressive tendency was evident in all males tested but at different levels. This is clearly illustrated in Fig. 8. Here the percentage of male Approaches which result in female Flight into Hiding has been plotted for each category. Note once again the GII males achieve a level between the Sh and GIII males. Values of p were not obtainable

for this graph due to the manner of its makeup i.e. percentages computed from means. Therefore it can only be taken as an indication of trends.

C. The Spawning Cycle

The behavior involved in the spawning cycle was described qualitatively in Chapter 2 and will therefore not be elaborated here. The names of the events and their order of occurrence are indicated in Fig. 9. Also shown are points where the cycle may be broken or shortened. Incomplete cycles generally occur at the beginning and at the end of a spawning. The actual number of cycles performed by any mating pair varies tremendously. This observer over a period of one year recorded as few as four cycles and as many as thirty two. The latter includes all incomplete attempts i.e., a breakup before successful termination in oviposition. Actually, in this case, only fourteen of the thirty two attempts were carried to successful termination in oviposition.

For each complete spawning cycle a record of the duration of each event comprising it was obtained. After recording a number of such cycles, the durations

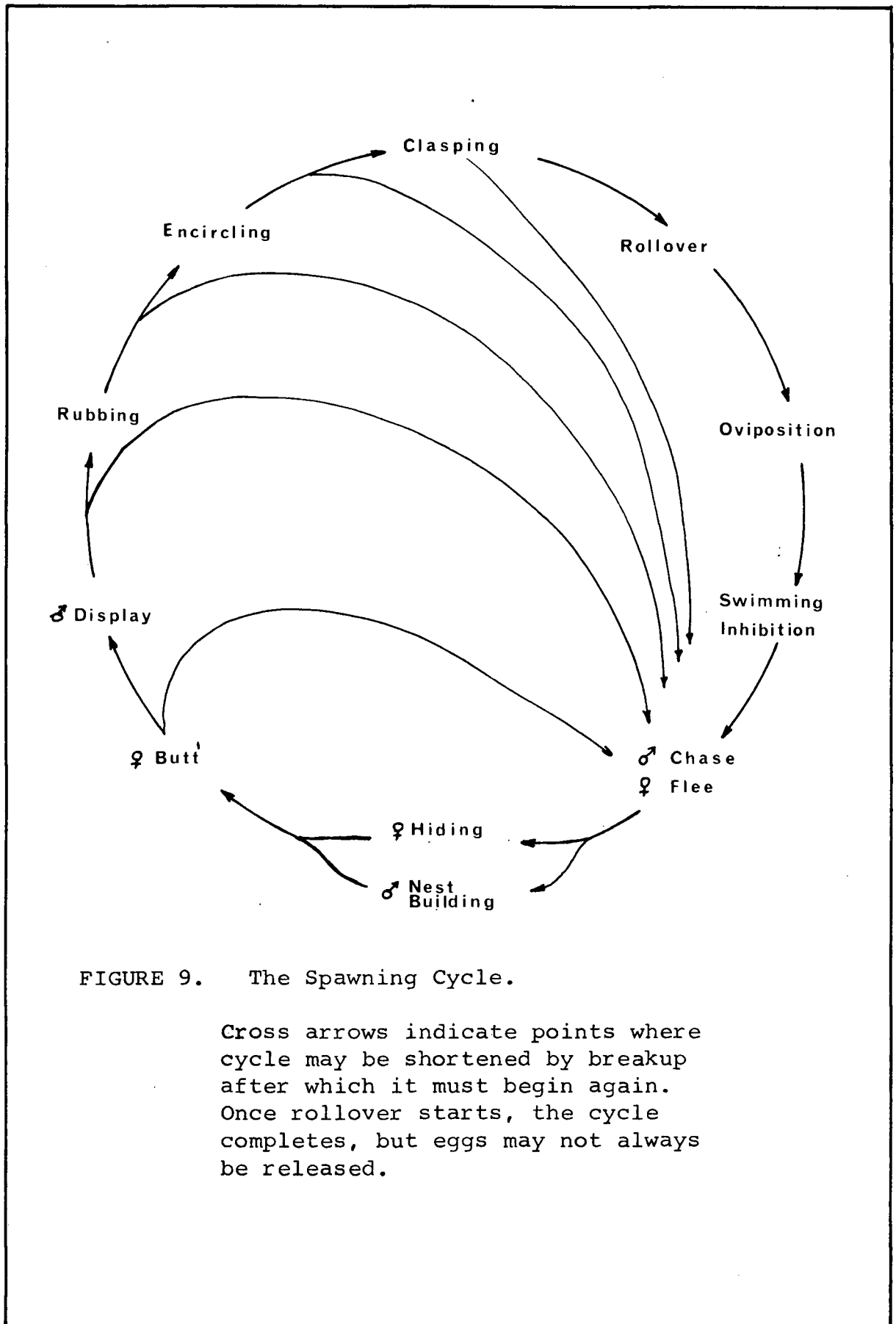


FIGURE 9. The Spawning Cycle.

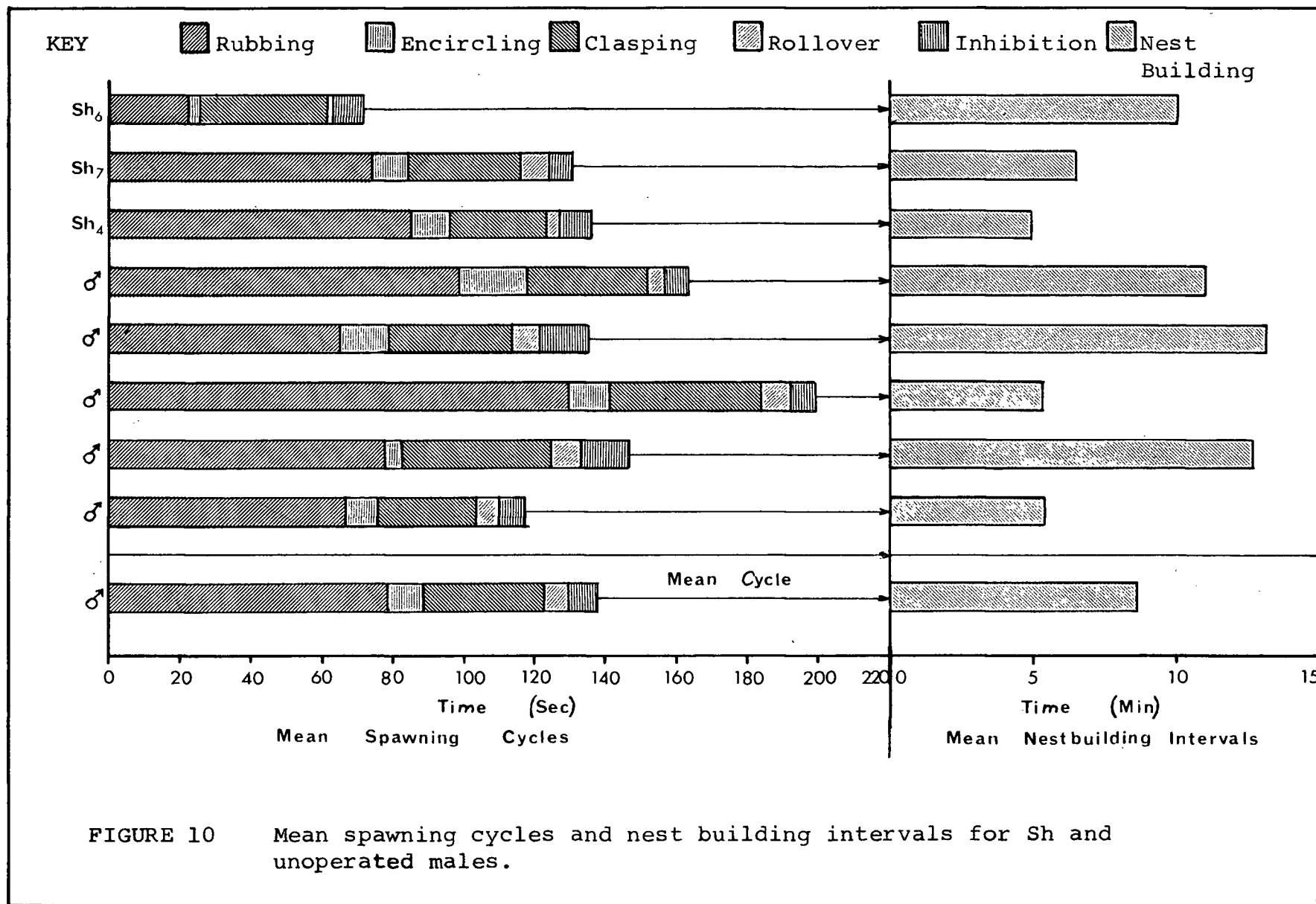
Cross arrows indicate points where cycle may be shortened by breakup after which it must begin again. Once rollover starts, the cycle completes, but eggs may not always be released.

of each repeating event were averaged to give a mean spawning cycle and nest building interval for one pair of fish.

Figure 10 indicates the mean cycles for Sh₄, Sh₅, and Sh₇ plus previous records of five other normal spawnings to give more complete picture. The latter were not involved in this experiment. The eight mean spawning cycles were averaged among themselves to give an overall mean spawning cycle and nest building interval.

A similar treatment of the GII spawnings was made (Fig. 11). It was only possible to record three of these spawnings.

In comparing Figs. 10 and 11, it can be seen that there is practically no measurable difference. From the two overall average readings the only difference is in a slightly longer average Rubbing period and a shorter average Nest Building interval for the GII males. The latter would indicate a slightly higher frequency of spawning cycles. An interesting point to note is that in all cases (Sh and G males) the duration of the actual Clasp varies the least. A successful



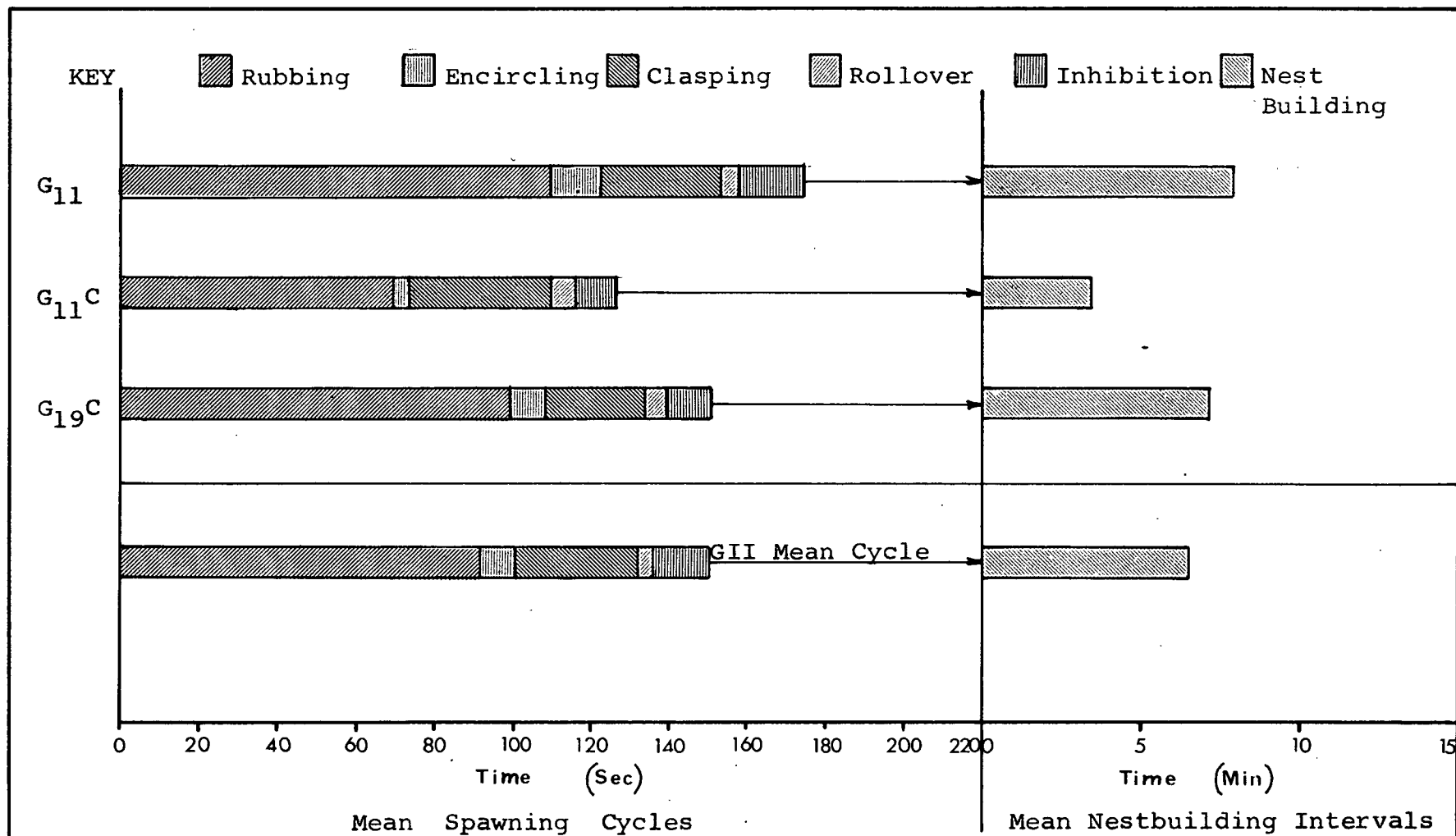


FIGURE 11 Mean Spawning Sequences and Nestbuilding Intervals for GII males.

Clasp i.e. one leading to Oviposition, was never observed to be less than twenty four seconds or more than forty five seconds. Further, the length of Clasp remained fairly constant for each fish. If the average clasp time was e.g. 26 seconds for Sh_4 , then the time for all Sh_4 Clasps varied little from 26 seconds.

All the behavior patterns occurred in the GII males that occurred in the controls; further, they occurred in the same order and at the same intensity and for similar duration so as to coordinate perfectly with the females and achieve oviposition. In no case did any egg hatch. The eggs left in the nest with the GII males in attendance had invariably disappeared by the next morning. These were either eaten by the male or disintegrated in the water. The former was assumed to be the case since the incubated eggs were still present the next morning though white and bloated.

Evidence of Regeneration

Nearly a month following the termination of experiments all the gonadectomized males were reoperated to determine if in fact any testicular regeneration had

CATEGORY	FISH	ORIGINAL OPERATION DATE	DAYS TO FINAL OPERATION	EVIDENCE OF REGENERATION AND DORSAL FIN DESCRIPTION	
III					
CONTROLS	G 15	16/3/66	109	Yes-2-3 mm Testis Tissue	Fin long
	G 16	16/3/66	109	Yes- 1 mm Testis Tissue	Fin long
	G 23	1/4/66	94	No trace	Fin short
	G 26	26/4/66	68	No trace	Fin short
II					
CONTROLS	G 11	11/3/66	114	No trace	Fin long
	G 17	24/3/66	100	No trace	Fin long
	G 19	3/5/66	62	Yes-2-3 mm Testis Tissue	Fin long
III					
HORMONE	G 20	24/3/66	115	No trace	Fin med.
	G 21	1/4/66	95	No trace	Fin med.
	G 22	1/4/66	95	No trace	Fin med.
	G 25	16/4/66	79	No trace	Fin long
	G 12	15/4/66	80	No trace	Fin long
II					
HORMONE	G 14	16/3/66	110	No trace	Fin long

TABLE I A summary of reoperation findings at
conclusion of all experiments.

taken place during the three to four months since the original operations. The findings have been summarized in Table I along with a qualitative description of the dorsal fin at the time.

Discussion

On examination of the results it can be readily seen that all Sh males spawned under the test situation. Further, these males and all G males had previously spawned under the same conditions prior to operation. Therefore it may be assumed that the differences noted for the G males on the second testing are due to the effects of gonadectomy and presumably the lack of gonadal hormones. It was noted that four G males did spawn and fertilize eggs. On reoperating a large easily distinguishable regrowth of testicular material was seen in the ventral region of the abdominal cavity immediately posterior to the rectum. This was presumably caused from an incomplete removal of the duct during the original operation.

Since the majority of G males i.e., GIII males, showed very little reproductive behavior in an organized

form it would appear that gonadectomy was complete and the resulting lack of hormones was responsible. It is interesting to note however, that the GIII males still showed all the behavioral elements of the Sh males but at a much lower intensity level. There was no build up of aggressions to a level necessary to intimidate the female into Flight and Hiding for increasing periods. Neither was there Nest Building. However, five G males (GII) did spawn. These fish displayed all the normal behavior but at a level which fell between the controls and GIII (Fig. 9). This resulted in a delayed spawning and one in which no eggs were fertilized. It was suspected that regeneration had occurred. If so, it must have been of a nature or in a position not allowing the release of sperm.

It appears also that once the male aggression level reaches a certain point whether rapidly (Sh) or over a longer period (GII), spawning is possible providing the female is ready and cooperative. Once spawning started, the behavior shown by the GII males was virtually indistinguishable from the Sh males.

It is suggested that the main role of gonadal hormone is in the prespawning organizational period and not in the actual spawning itself.

The results of reoperation show that in fact by the end of three to four months some fish had regenerated small amounts of testicular tissue. However, at the time of the present experiment, these results were not known. It is the author's intent, therefore, to reserve further discussion of these results to Chapter 9 after all data have been presented.

CHAPTER 5

AN ANALYSIS OF THE EFFECTS OF METHYL TESTOSTERONE ON THE MALE REPRODUCTIVE BEHAVIOR

Introduction

A comparison was made between the reproductive behavior recorded from a group of gonadectomized control males and a group of gonadectomized males treated with methyl testosterone. The results are also compared with the behavior displayed by a group of sham operated males described in Chapter 4.

Procedure

Fourteen gonadectomized males made up of GII and GIII from the previous experiment were utilized here. These were divided roughly in half to permit one group of seven to receive testosterone and one group of seven to act as controls. The groups were composed of:

Group I Hormone treated:-

 GII G₁₄ G₁₈

 GIII G₂₀ G₂₂ G₂₅ G₂₁ G₁₂

Group II Controls:-

GII	G ₁₁	G ₁₇	G ₁₉
GI	G ₂₆	G ₂₃	G ₁₅ G ₁₆

Group I males were treated with methyl testosterone by the method described in Chapter 2. The control group received exactly the same treatment but with no hormone added, i.e. they received injections of distilled water containing "Tween80" only. Following one week's treatment all fish were paired with females and tested in the same manner as before.

From this point on, gonadectomized fish receiving hormone treatment will be referred to as G-H males.

Results

A. Time to Spawning

A record of the number, the time and the day of spawning for each fish has been summarized on Fig. 12. Looking first at Group I it can be seen that five sixths out of the fish tested, spawned within the testing period. Unfortunately the second GII male, G₁₈, died just prior to

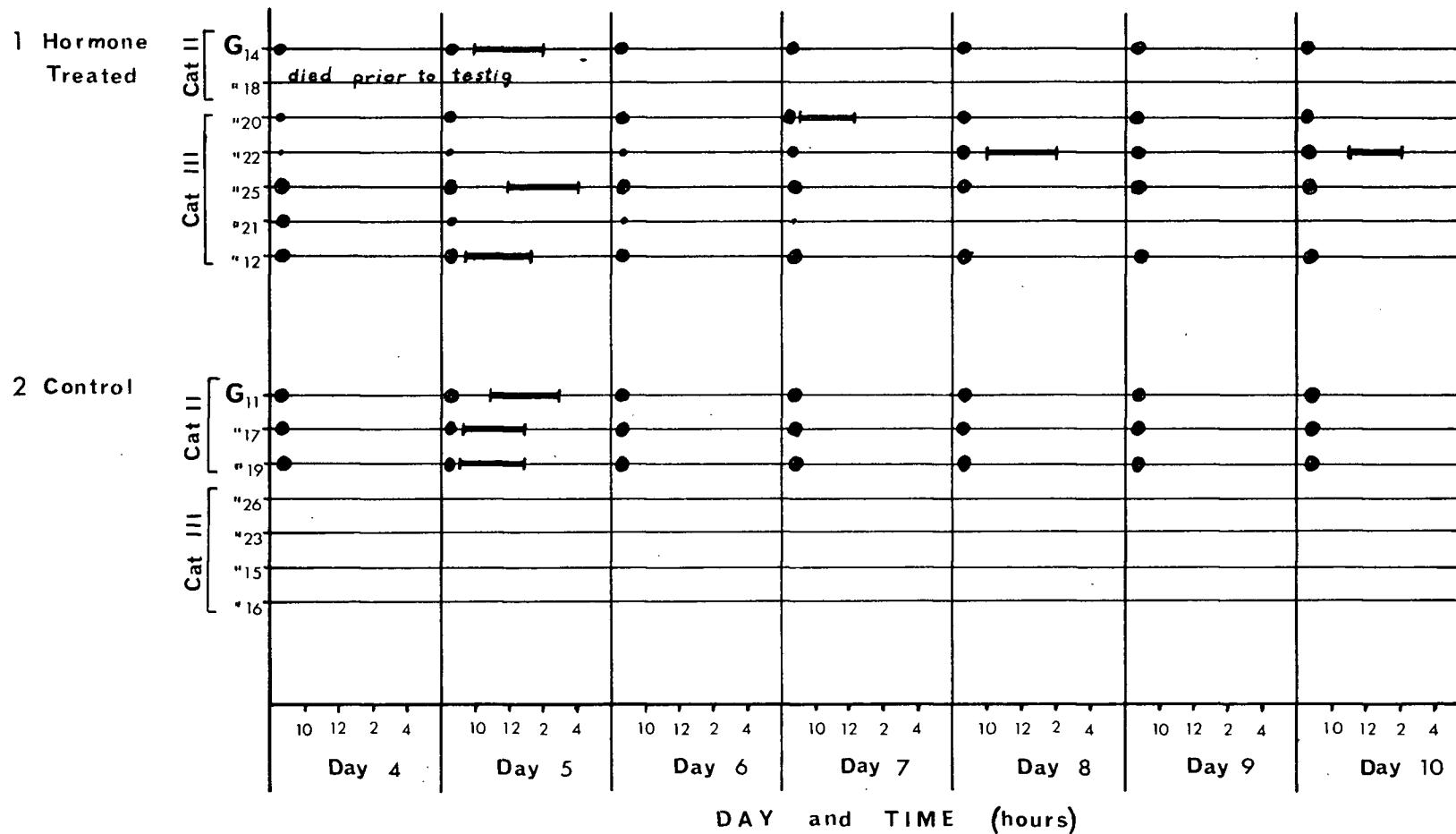


FIGURE 12. A qualitative record of spawning times and nest building in G-H treated and G control males.

KEY — Spawning
● Nest

testing, probably as a result of injury through injection. Therefore, of the five GIII males given the hormone treatment, four spawned with G_{22} spawning twice (Day 8 and 10). G_{21} built a large nest initially but it tapered off during the week and no spawning occurred. None of these fish spawned prior to hormone treatment (Fig. 1). Quite a variable picture as to spawning day can be seen which is quite reminiscent of GII males previously (Fig. 1).

The Group II controls show an even more interesting pattern. Firstly the GIII males did not spawn nor did they undertake nest building. The three GII males all spawned as before but this time, all on Day 5. This behavior follows closely that of the Sh males (Fig. 1). It might also be noted that nearly a month had passed in most cases since the previous testing and two months since the original operation.

B. Pre Spawning Behavior

The histograms (Figs. 13-19) indicate the pre-spawning activities of the G-H treated males and the GII and III controls in the same manner as was presented in

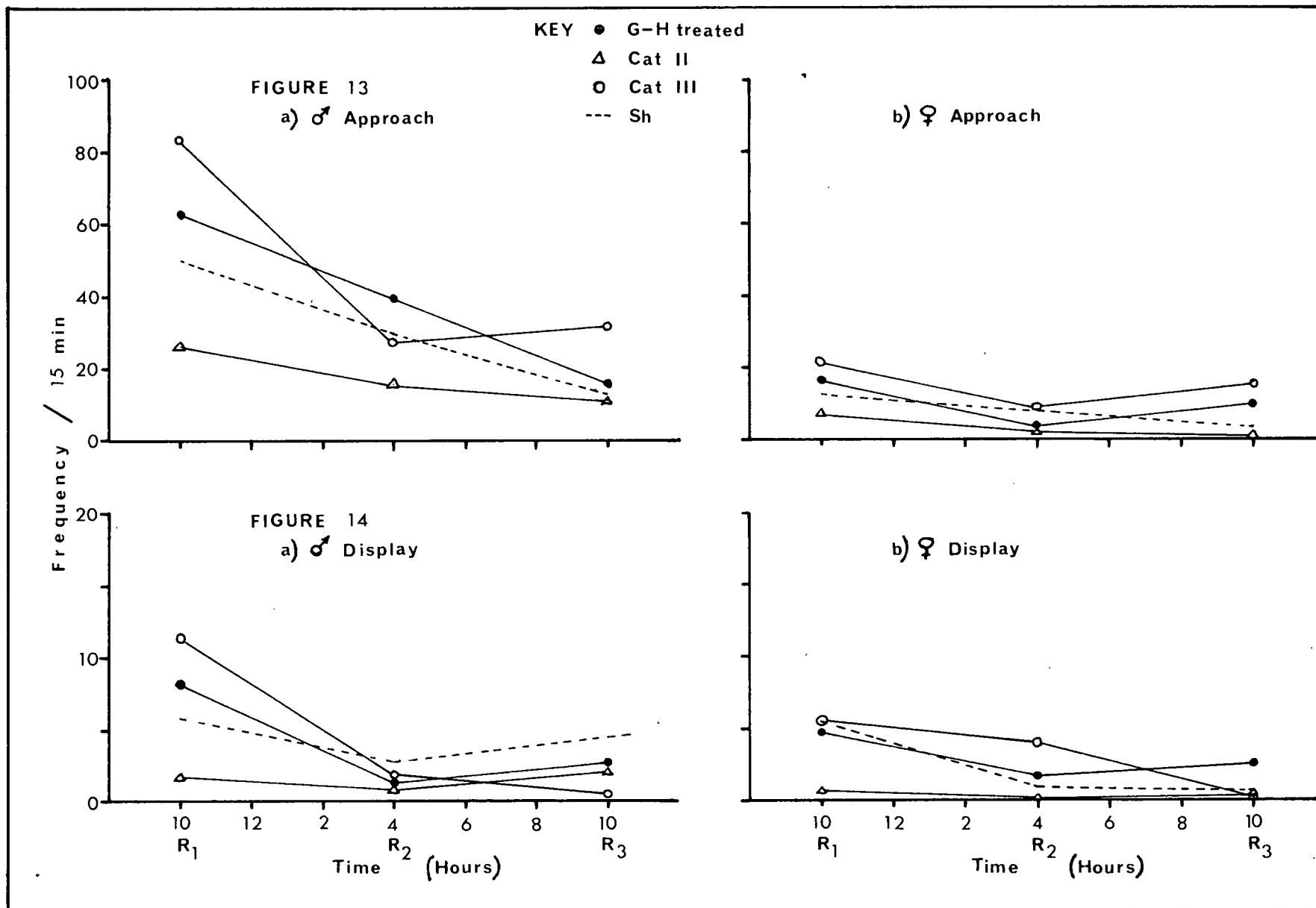
Chapter 4. In addition the original Sh male trends have been included for comparison.

Once again the data appearing in Figs. 13-19 have been treated statistically in the same manner as those presented in Chapter 4. Here also the "p" values have been included where applicable for the following comparisons:

H treated G	to	Cat. II Controls
H treated G	to	Cat. III Controls
H treated G	to	Original Sh Controls

Approach (Fig. 13)

The mean frequencies of male and female Approach are given in Fig. 13a-b. Once again male A is highest in R1 and diminishes through R3 except the GIII males who show a leveling off at R2 (G-H males to GIII males, $p = 0.1153$). There is however, a significant difference between GII males and G-H males in R1 ($p = 0.0396$). The G-H and Sh males follow a parallel trend in Approach. GII males are similar but at a lower level. The GIII females and G-H females show a parallel trend in Approach.



Display and Butting Fig. 14-15

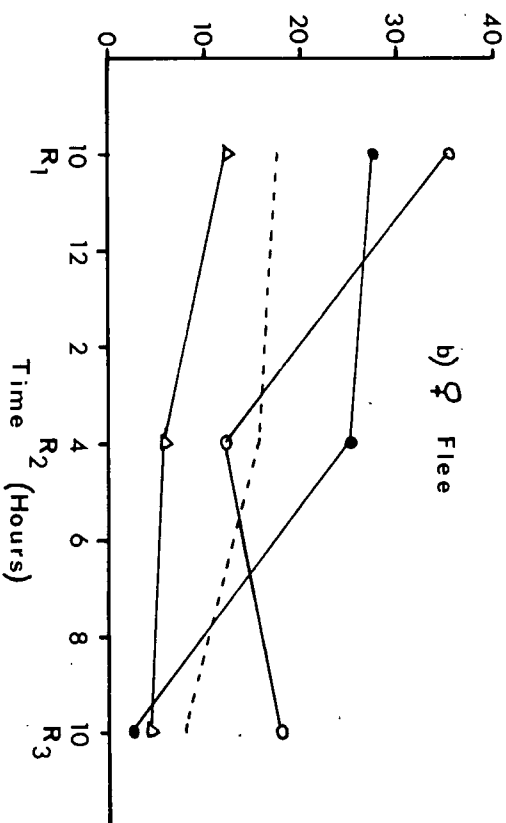
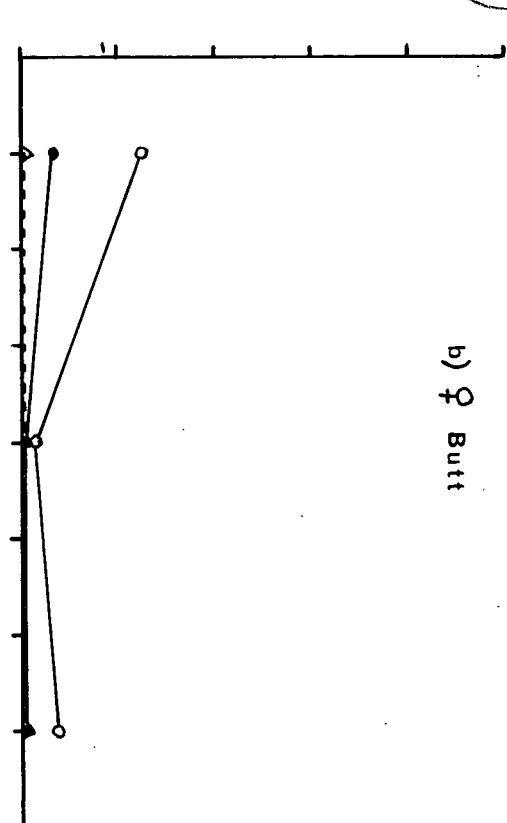
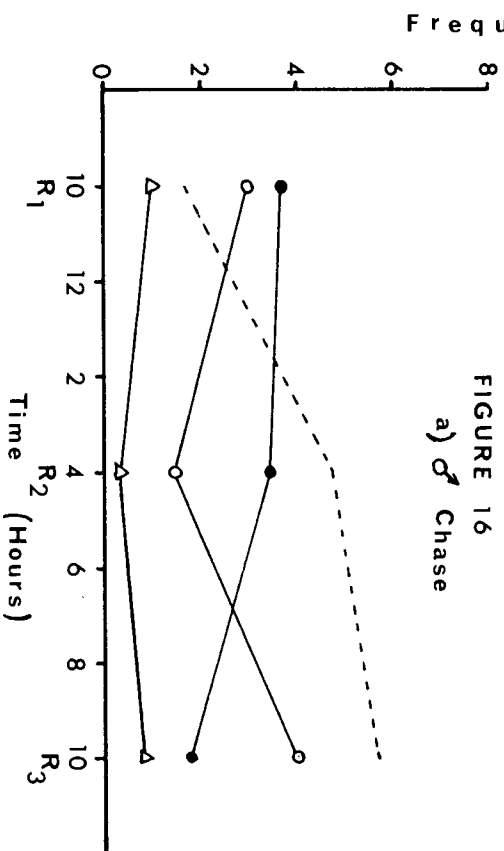
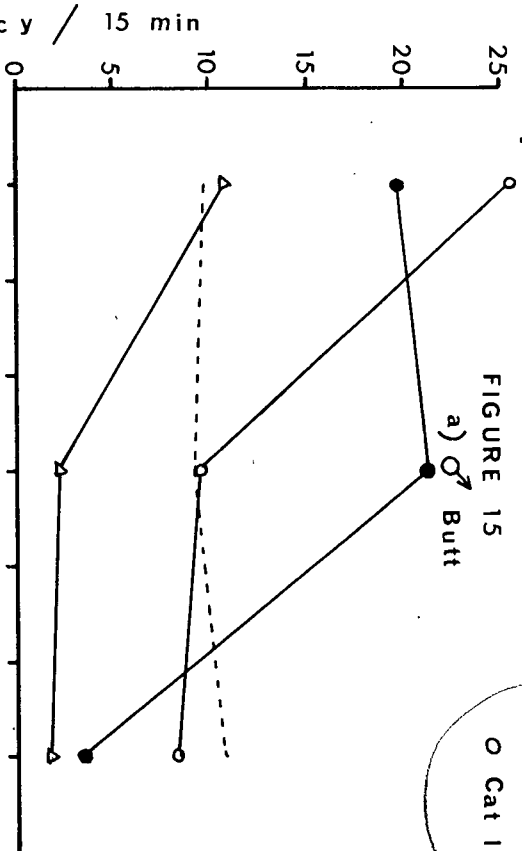
In both male and female Displaying D and Butting B there is a higher frequency in R1 for GIII than for any of the others. However, the differences here are not as significant as they were in the previous experiment. The G-H males and females are all very close to GIII in R1. GII males parallel the Sh males for D but at a lower level. In R2 and R3 there is a similar trend between GII, G-H and Sh males. Only GIII males continue to drop. For male B GII and G-H males drop to nearly zero in R2. There is no tendency to parallel the Sh males in B. None of these differences, however are significant. Female B did not occur for any fish after R2 except for GIII. Here GII and Sh females showed no B at all.

Chasing and Fleeing Fig. 16a-b.

The frequency of Chasing drops off for G-H males whereas it steadily increases for Sh males. GIII males show quite an upswing for Chasing after an initial drop off during Day 4. GII males showed very little chasing throughout.

Labeling

KEY ● Sh
 ▲ Cat II
 ○ Cat III



There is a parallel trend between the G-H male Chasing and Butting and GIII female Fleeing. GII females parallel Sh females for Fleeing tendency.

In these two graphs trends can be seen, but differences between the groups are not significant.

Nest Building Fig. 17

Turning to the trends for male Nest Building it is interesting to note the close parallel between GII males and Sh males for both frequency and mean duration per trip. The G-H males on the other hand show a much lower frequency of N but a correspondingly higher mean time per trip. When these values are computed in terms of total duration of N for a 15 minute observation it becomes apparent the G-H males and GII males are spending slightly less time than the Sh males. GIII males did not nest build (Comp. GIII males and G-H males: $p = 0.0948$).

Hiding Fig. 18

The trends in female Hiding appear to fluctuate greatly but actually each female is showing the same trend on a total duration basis i.e. that of an increase

KEY ● G-H treated

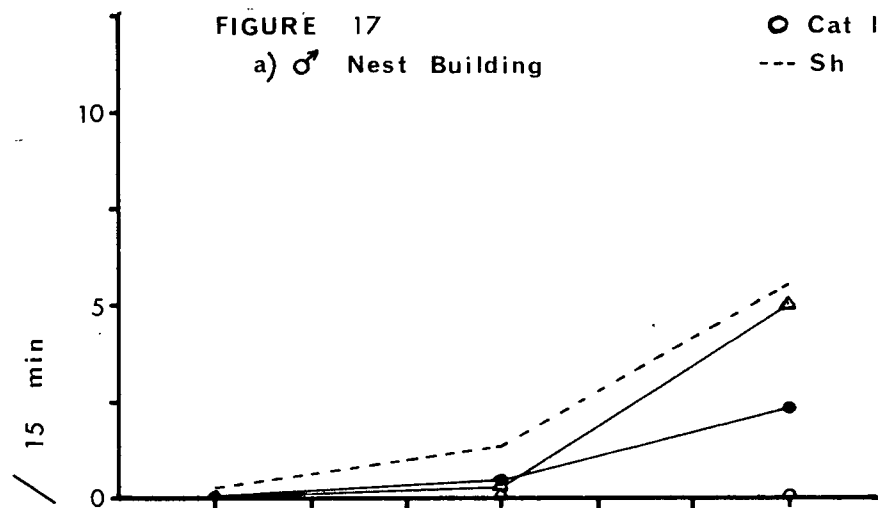
△ Cat II

○ Cat III

--- Sh

FIGURE 17

a) ♂ Nest Building



b) ♂ Nest Building

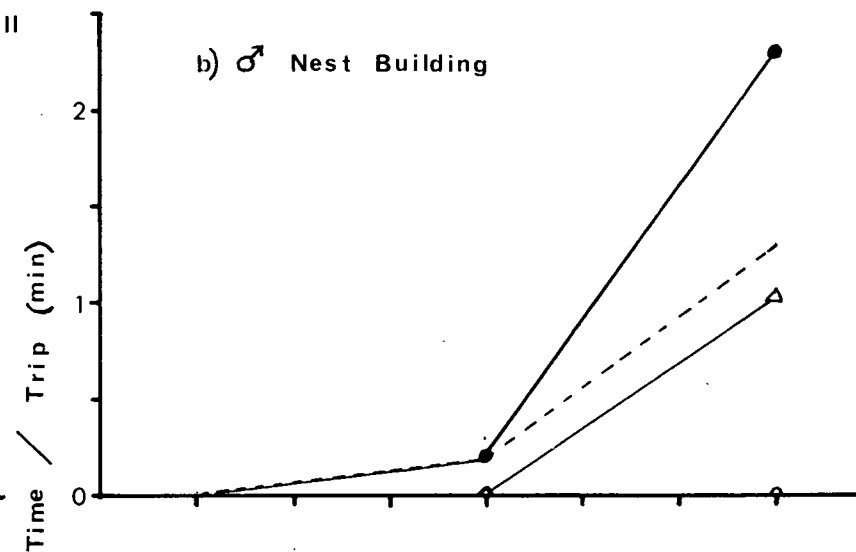
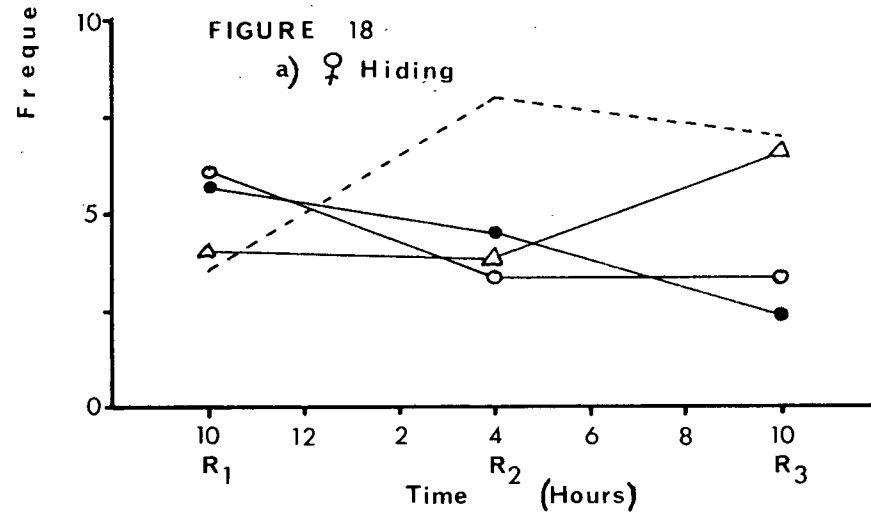
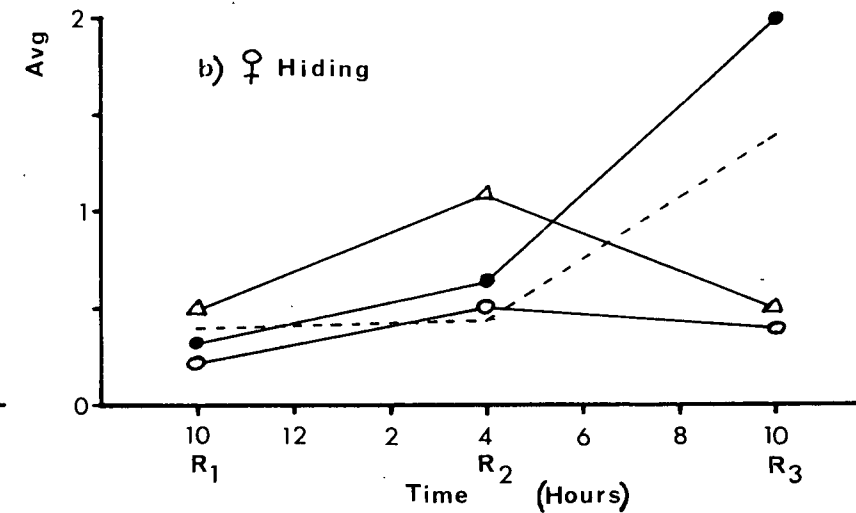


FIGURE 18

a) ♀ Hiding



b) ♀ Hiding



in the amount of time spent in Hiding. The only females not showing a net increase are those of GIII. If the two graphs are multiplied together it can be seen that Sh females are spending the most time in Hiding by R3, the G-H females are next, GII females next with GIII females the least.

In summary of the prespawning behavior, Fig. 19 illustrates the percentage of male Approaches resulting in female Fleeing into Hiding. Here can be seen a steady increase for GII males indicating a steadily increasing aggression level which might be expected since all three spawned on Day 5 (Fig. 12). However, the final level did not reach the height found for Sh males. The G-H males showed a parallel trend but at a much lower level. This is surprising since three fifths of these fish also spawned on Day 5 (Fig. 12), yet there is a large difference between these and the Sh males. The GIII males followed the G-H males exactly through Day 4 but then their dominance fell or aggression dropped right off to a very low level in R3. This drop is reflected in Fig. 13a-b by an upswing of A for both GIII males and

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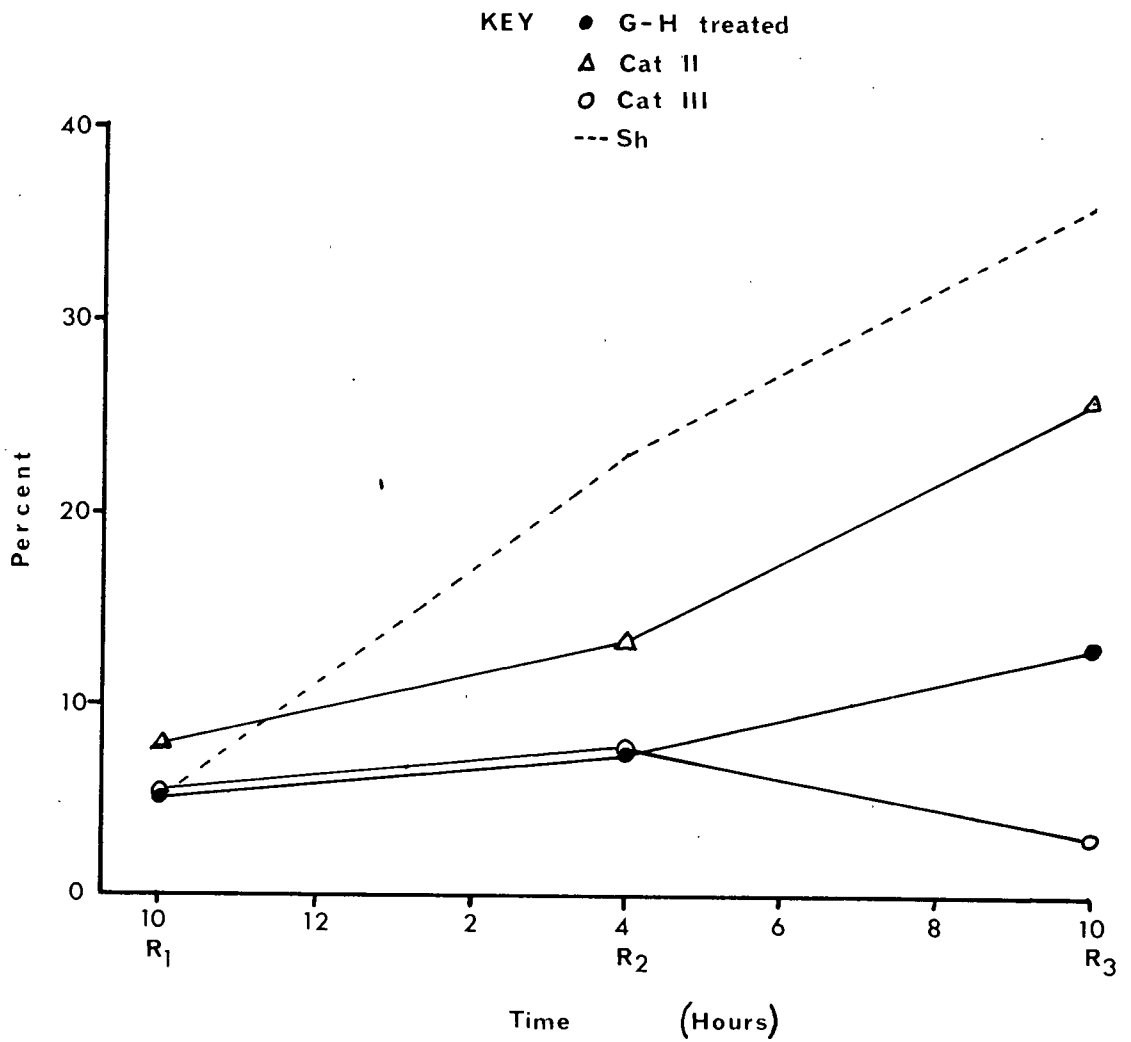


FIGURE 19. The percentage of male Approaches resulting in female Flight into Hiding.

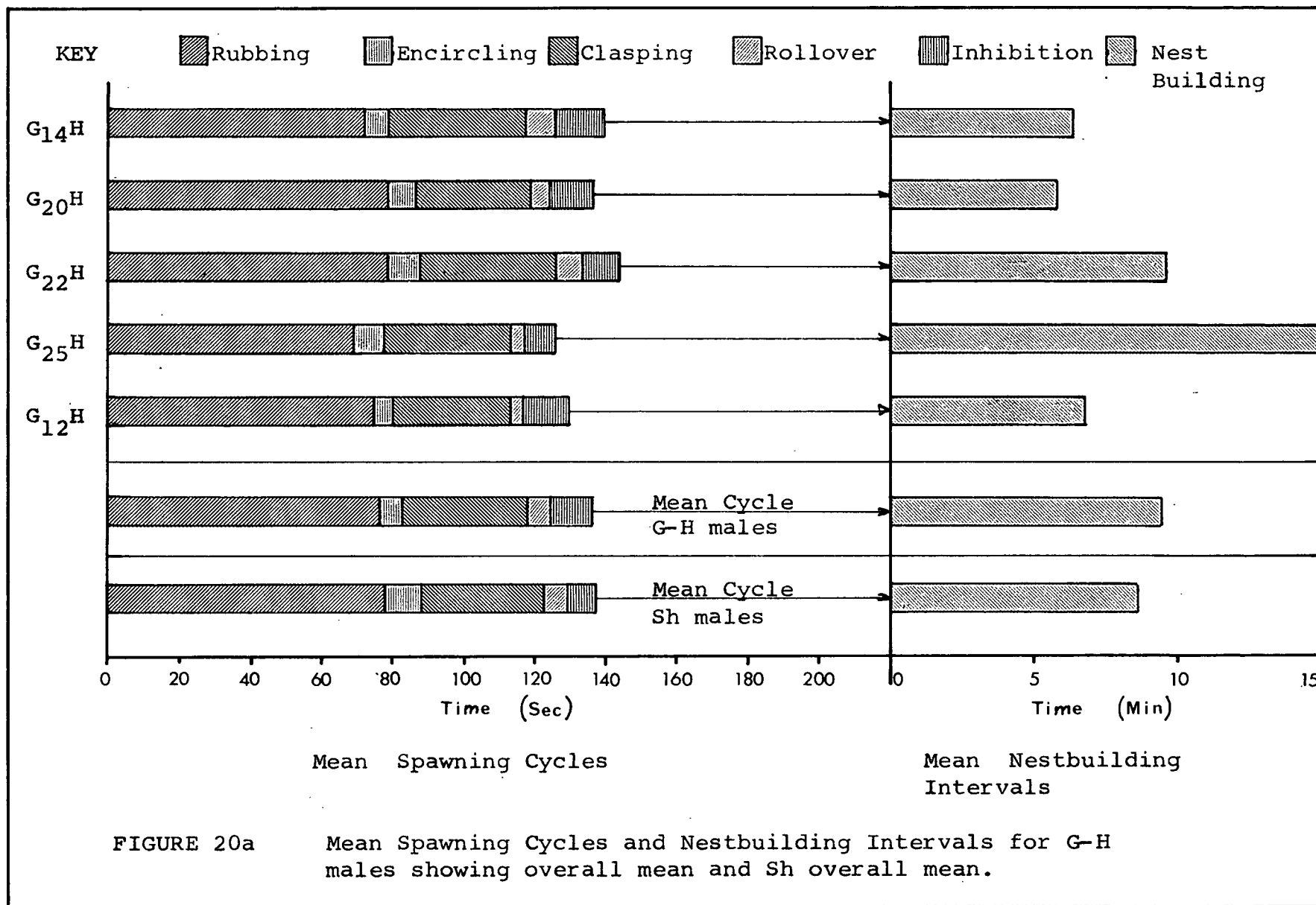
their females. Fig. 16a-b for G III show an increase in Chasing and Fleeing at R3 but this female Fleeing is not terminating in Hiding (Fig. 18). Thus the GIII females as in the previous experiment are not being intimidated into Hiding by dominant and increasingly aggressive, nest building males, and as before they did not spawn.

C. The Spawning sequence

Each G-H male which spawned was recorded for upwards of two hours. During this time approximately eight spawning cycles and nest building intervals were recorded for each pair. The behavior patterns involved followed exactly the same cycle as outlined in Fig. 9.

A mean spawning cycle and nest building interval for each G-H pair was calculated (Fig. 20). Then an overall mean calculated from the individual means was set up. Also in Fig. 20 the overall mean from the eight Sh and unoperated males (Fig. 10) was included for comparison.

Noteworthy here are the facts that in the case of the GIII males, four out of five did spawn with



hormone and the spawning pattern was completely organized in every respect. Further it can be seen there was very little variation of the means for each fish except for the nest building interval. The overall mean is practically identical to that for the Sh males. Fig. 12 shows far more variation of the means among the Sh and unoperated male controls.

Discussion

The results presented indicate quite conclusively that methyl testosterone treatment restored much of the reproductive behavior up to and including spawning among the GIII males. Indeed, the spawning cycles recorded were far more regular than those recorded for Sh males. It seems highly probable that the lone GII male, G₁₄H, which received hormone treatment would have spawned without it as did the three GII controls.

In this experiment GIII controls showed much the same behavior as they did previously (Chapter 4) in that they failed to intimidate the female into Hiding, did not Nest build and did not spawn. Yet on re-operating, two of the four showed some regeneration.

The GII controls on the other hand spawned again showing similar behavior to the previous testing. However, in this experiment they all spawned on Day 5 which is reminiscent of the original Sh control level.

CHAPTER 6

SOME MORPHOLOGICAL EFFECTS OF GONADECTOMY AND METHYL TESTOSTERONE TREATMENT ON THE SECONDARY SEXUAL CHARACTERISTICS OF THE MALE AND FEMALE

Introduction

About three weeks to one month following gonadectomy a pronounced shortening and rounding of the male dorsal fin was observed. Measurements were made of these and of unoperated males and females for comparison. Later, following hormone administration to certain males, further measurements were made to provide further evidence of the hormonal effects.

Procedure

The first set of measurements was made on May 31, 1966 or approximately five weeks following gonadectomy. The final set of measurements was made during the first week of July, 1966 or about ten days following the termination of hormone treatment.

The following groups of fish were measured:

- | | | |
|-----|---|---------------------|
| I | Unoperated and Sh males | |
| II | Unoperated females | |
| III | Gonadectomized males | (a) GII
(b) GIII |
| IV | Hormone treated Gonadectomized
males | (a) GII
(b) GIII |
| V | Hormone treated females | |

Results

The raw data (cm) and their resulting ratios together with the mean ratios calculated for each group have been included as a series of tables in Appendix I.

The mean ratios and their standard errors for each group have been plotted with respect to an approximate time scale (Fig. 20~~b~~). This scale is approximate since all final measurements were not in fact made the same day but spread over a week or more depending on testing time, day or period of certain groups.

Fig. 20b shows the range of fin to length ratios on the vertical axis. At an arbitrary point before

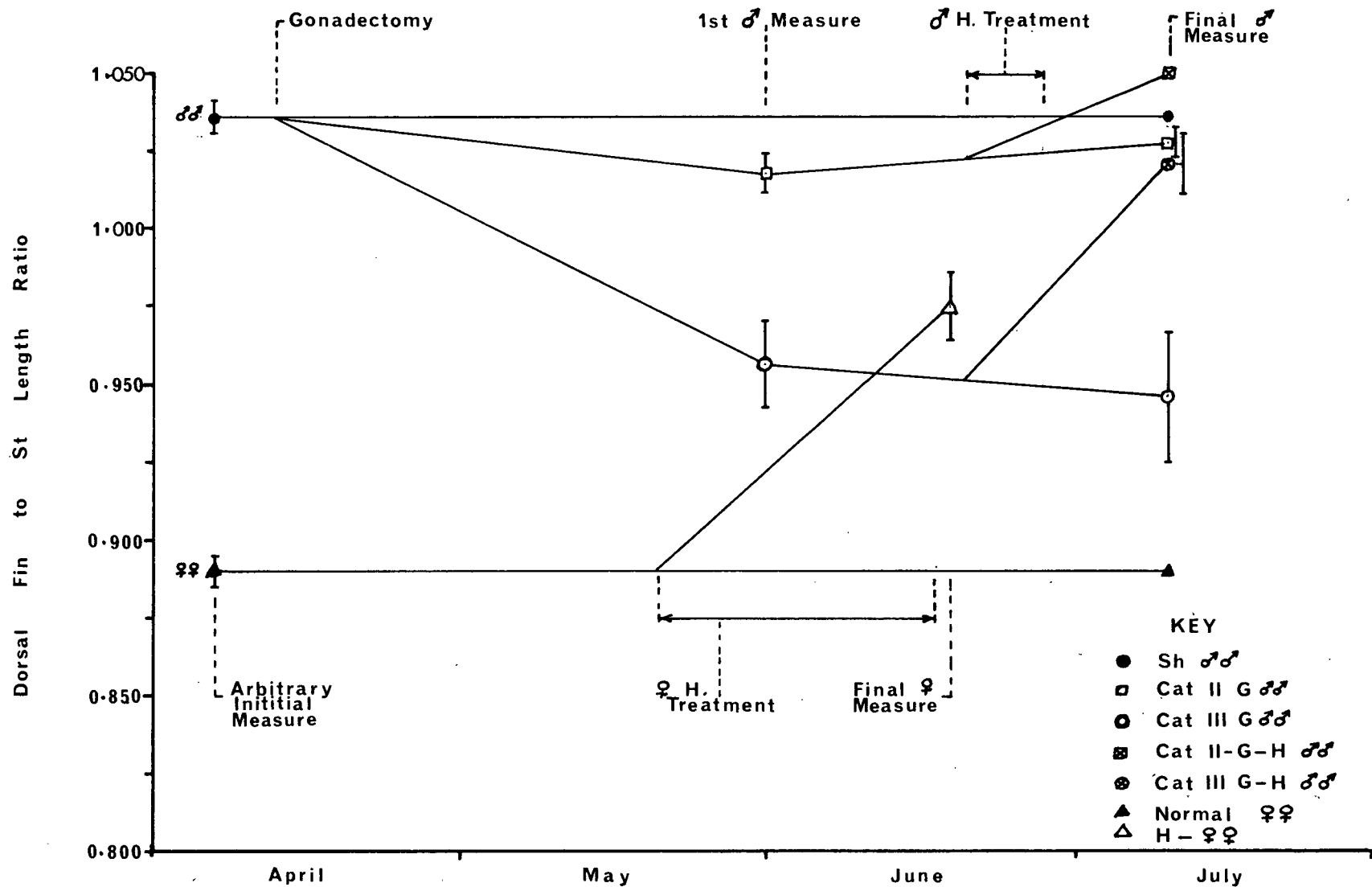


FIGURE 20b. Effects of gonadectomy and methyl testosterone on dorsal fin ratios.

gonadectomy a group of Sh males and normal females were measured and their mean ratios plotted to give a standard or base line for control males and females. It was assumed that these two values would not deviate much and hence they were carried across the time scale. The male value of 1.035 indicates the distance from snout to dorsal fin tip was 1.035 times the standard length. The mean female ratio was 0.891.

Following gonadectomy, males were classified according to their behavior into two groups (GII and GIII). On May 31, 1966, these two Categories of males were measured separately and the mean ratios for each group plotted. From this measure two things became apparent.

a) All males measured five to eight weeks after gonadectomy revealed a lower fin to length ratio than the control males.

b) There was a distinct difference in the amount of fin loss between the GII and GIII males. The GII males which displayed reproductive behavior

including spawning but at a lower level than Sh males had a correspondingly lower fin ratio of 1.017. The GIII males on the other hand which displayed much less reproductive behavior and did not spawn had a still lower ratio, i.e., 0.957 which was about mid way between control males and females.

Methyl testosterone was administered to 50% of the GII and GIII males and ten days following cessation of this treatment, all were remeasured including the 50% receiving control injections. The plotted results show a split in both G categories. It can be seen that testosterone treatment produced an increase in the fin ratio in both groups. Since there was only one GII-H male, the reading of 1.050 may not be reliable, however, hormone appears to cause it to become more male-like than the controls. The five GIII males receiving hormone showed a dramatic mean increase from 0.957 to 1.020. This measure correlates very closely with the pre-hormone measure of the GII males (1.017).

Of the G males receiving control injections the GIII group showed a further mean drop to 0.946.

The GII group showed a slight increase (1.017-1.028).

Finally, after treating a group of six unoperated females with methyl testosterone for an extended period (these were originally used to test dose levels and injection methods) they were measured in mid June. Here can be seen a dramatic increase in their fin to length ratios over their controls (0.891 - 0.975).

Discussion

The shape and size of the dorsal fin is the most obvious morphological difference between the male and female blue gourami. Apparently the larger, pointed shape of the male fin is under the control of gonadal secretions. In some cases following gonadectomy there was a drastic reduction in fin size to the point where it became almost indistinguishable from the female fin (GIII males). In other cases gonadectomy resulted in only a slight fin atrophy (GII males). Exogenous replacement by testosterone resulted in a regrowth of the fin to some extent in all cases. On injecting intact

females with testosterone their external morphology began to take on a male appearance. Therefore the dorsal fin has been classed as a morphological secondary sexual characteristic (S.S.C.) whose size and shape is controlled by androgens.

CHAPTER 7

PARENTAL BEHAVIOR SEEN IN SHAM OPERATED, GONADECTOMIZED AND TESTOSTERONE TREATED GONADECTOMIZED MALES

Introduction

Parental behavior as displayed by the male blue gourami consists of accepting the eggs at the time of spawning and maintaining a nest around them until they hatch. For two or three days following hatching, stray fry are collected in the mouth and spewed back into the nest. After this time the fry are too active and the male gives up collecting, but the nest may be maintained for another week to ten days. Certain aspects of this behavior were tested on Sh, GII and G-H treated males.

Procedure

Fertilized eggs were removed from the nests of normal and sham operated males by the method outlined in Chapter 2. These eggs were given to other Sh males as well as G males and G-H treated

males in various stages of the reproductive cycle for the purpose of determining when in the cycle the parental phase began.

Eggs were given at the following times.

- a) Before courtship i.e. before adding the female.
- b) During courtship but before spawning.
- c) Immediately after spawning i.e. same or next day.
- d) One week after spawning.
- e) Two weeks after spawning.

Eggs present in the tanks of the fish to be tested as a result of their own spawning, were removed prior to the addition of the test eggs.

Results

Table II summarizes the number of times the males of each group either accepted and cared for the eggs given, or rejected and ate them, at the points during the reproductive cycle described above.

No. of Trials	Time of Addition	Accepted	Rejected
7	Before courtship.	0	7
5	During courtship - before spawning.	1	4
7	Immediately after spawning.	7	0
4	1 week post spawning.	4	0
3	2 weeks post spawning.	0	3
<u>EGGS GIVEN TO Sh MALES</u>			
7	Before courtship.	0	7
4	During courtship - before spawning.	0	4
3	Immediately after spawning.	3	0
1	1 week post spawning	1	0
<u>EGGS GIVEN TO G MALES</u>			
5	Immediately after spawning.	5	0
<u>EGGS GIVEN TO G-H MALES</u>			

TABLE II. SUMMARY OF RESULTS OF EGG
TRANSFERS

From this table it may be seen that Sh males would not accept eggs before the addition of the females i.e., before courtship, nor would they accept eggs during courtship. There was one case where a male which was dark and nest building and apparently close to spawning did at least initially, accept a number of eggs that were added. But usually a male in this condition would eat eggs immediately. All G males tested showed identical behavior. However, once spawning has occurred, i.e. clasping and oviposition, both Sh and G males appear to accept the addition of fertilized eggs. Sh eggs were transferred back and forth when several spawnings occurred on the same day and each accepted the other's eggs. A rough idea of how long following spawning this parental acceptance phase lasts was obtained by giving males eggs at one week post spawning and at two weeks post spawning. The Sh males accepted eggs after one week in all cases and rejected them after two weeks. This indicates an egg acceptance period from immediately after or during spawning until somewhere between one and two

weeks later. It was interesting to note that in both the one and the two week tests, some tanks contained living fry and others did not (eggs having been removed in total before hatching). Therefore, the presence or absence of fry did not affect egg acceptance at one week nor did it effect egg rejection at two weeks.

The G males which spawned (GII) accepted fertilized eggs in place of their own infertile eggs in all cases. One such male accepted eggs after one week's duration. An opportunity for testing at two weeks duration did not present itself but it may be assumed they would reject as did the Sh males.

The G-H treated males were given fertilized eggs immediately following spawning and accepted in all cases. No other tests were made on these fish.

Some interesting observations were made on the parental behavior displayed by the GII males and the G-H males regarding their own eggs. In all these cases, the eggs produced were checked and found to be infertile. However, 30-40 percent of the eggs were always left with the fish. Here it was noted

that initially (during and immediately following spawning) the males collected and concentrated the eggs in the nest in the usual manner. However, as time passed, the eggs began to disappear and by the next morning in all cases there was hardly an egg to be found. It was assumed that when the eggs began to disintegrate as a result of bacterial and fungal attack, they were weeded out and eaten by the male so that by morning, all had disappeared. This assumption is based on the fact that the eggs previously removed were still intact the next morning but were white with fungus.

Discussion

The experiments described above were carried out as part of an examination of the gonadal role in all aspects of reproductive behavior i.e., is parental behavior under separate control from sexual behavior?

An examination of the results would appear to indicate that something to do with the act of spawning itself, or the sight of eggs in the nest, triggers the mechanism of parental behavior. Virtually no eggs were accepted by any male who had not performed

the spawning cycle at least once. The usual or natural reaction of any male which is not in the parental phase is to eat the eggs immediately on discovery. Therefore some mechanism set off by the spawning act inhibits this usual tendency.

Apparently this inhibition only lasts for approximately ten days after which time eggs are eaten again. The interesting point here is that fry are not eaten after ten days. Indeed, males have been left with their fry for up to a month with no apparent decrease in the number of fry. Yet, if fry are placed in a tank with an unspawned male, they are quickly eaten as are the eggs. One male which had resided with several dozen fry for about two weeks was removed and castrated then given two days in a recovery tank after which it was returned to the tank containing the fry.

Apparently no fry were eaten on its return.

It may be postulated then, that the spawning act triggers a neural or endocrine mechanism which causes the inhibition of egg eating and probably stimulates the characteristic parental behavior. As time

progresses, the effect of this mechanism slowly decreases, but long before it has ceased to be effective, the eggs have hatched into fry which the male also is inhibited from eating. Possibly by a process of learning the male avoids eating the fry or in other words becomes used to their presence. This learning process lasts long after cessation of the initial inhibitory mechanism. This seems even more probable when one remembers that fertile eggs only last about twenty four hours; thus the fish has very little time in which to "learn" to avoid them whereas the fry may be present for many weeks. Thus it is possible that a certain time after spawning, fresh fertile eggs may be offered to a male and be immediately eaten, while at the same time hundreds of fry are milling about the feeding male.

The trigger for egg acceptance is likely associated with the actual physical or tactile sensations produced by the spawning act and not by any chemical nature of the fertilized eggs themselves. This seems likely since G males and G-H males on spawning initially cared for their infertile eggs in the same manner as the Sh

males. The infertile eggs were only eaten after they began to decompose. The eating in this case was likely a hygienic response which any male, regardless of gonadal state would perform, since likely, not every single egg in a fertile spawning is viable.

The final point which comes from these results is simply that the egg acceptance mechanism triggered during spawning is not mediated by androgen from the testes. This is demonstrated by the fact that GII males spawned and accepted eggs in the usual way. When given fertile eggs, these were cared for as well. As was mentioned earlier (Chapter 4) on examining the Cat II G males at the conclusion of all experiments, no trace of testicular tissue could be found in two of the three. Therefore, the presence or absence of testes apparently has no effect on egg acceptance once spawning is achieved. However, this evidence does not rule out the possibility that testosterone may still be important, i.e., if it was being made available from some other source.

CHAPTER 8

AN ANALYSIS OF THE AGONISTIC BEHAVIOR BETWEEN GONADECTOMIZED AND SHAM OPERATED MALES

Introduction

Agonistic behavior displayed between males was tested in a series male-male encounters. Different combinations of males were used. Sh males were paired with each other and with G males. Also G males were paired with each other. Any differences noted could be attributed to the effects of gonadectomy.

Procedure

The testing procedure was identical to that used for male-female interactions. In this case the two males to be tested were placed in a tank and separated by a black partition. On the morning of Day 4 the partition was removed and one behavioral recording was made which was terminated when one male established itself as dominant. Three classes of male interactions

were tested with four trials in each class. Table III indicates the classes and their trials. All of these records were made prior to the commencement of hormone treatment.

TABLE III

FISH COMBINATIONS USED TO TEST AGONISTIC
BEHAVIOR

<u>Class</u>	<u>Trial</u>	<u>Combination</u>
1. Sh male vs Sh male	1	Sh ₆ - Sh ₄
	2	Sh ₆ - Sh ₅
	3	Sh ₇ - Sh ₃
	4	Sh ₇ - Sh ₄
2. Sh male vs G male	1	Sh ₅ - G ₂₃
	2	Sh ₆ - G ₁₅
	3	Sh ₅ - G ₁₂
	4	Sh ₄ - G ₂₅
3. G male vs G male	1	G ₁₆ - G ₂₁
	2	G ₁₂ - G ₂₃
	3	G ₂₆ - G ₂₅
	4	G ₁₂ - G ₂₆

Results

During the recording of the three groups tested it became apparent to this observer that there was very little difference between them. Sh males paired with each other or G males, or G males with each other all behaved in a similar fashion to that described in Chapter 3. One observed difference was that the G males did not become as dramatically black as the Sh males although they were very dark. The bout lengths varied considerably within each class. It appeared that the lengths of the disputes varied with size differences between the two participating fish rather than with their gonadal state. The closer the two were to each other in size, the longer the bout.

The frequencies of four behavior patterns (Approach, Display, Butting and Fin Tugging) were taken for each fish in each bout. Since all bout lengths varied, the raw frequencies were computed as rates i.e., number per 10 minutes. In each bout the winner and loser were computed separately. A mean rate for each behavior pattern recorded for the winner and the loser was compiled from the four trials in each class. Fig. 21 shows the resulting mean

rates presented as a series of winner and loser bars for each class and each behavior pattern used.

Fig. 22 indicates the mean bout length for the three classes. From this graph it can be seen that the overall average bout length was about fifteen min. The longest dispute observed was twenty seven minutes, while the shortest was three minutes.

From the bar graph the following observations were made:

1. There is little difference in the mean rates for Approach, Display or Fin Tugging between the three classes or between the individual winners and losers.

2. A considerable amount of Butting occurred for the three classes.

3. There is a tendency for the winner to Butt less than the loser in all three classes. This Tendency is apparent only for Butting.

4. The frequency of Fin Tugging is very low. This may be misleading since each Fin Tugging episode may last several seconds whereas a single Butt is more or less instantaneous.

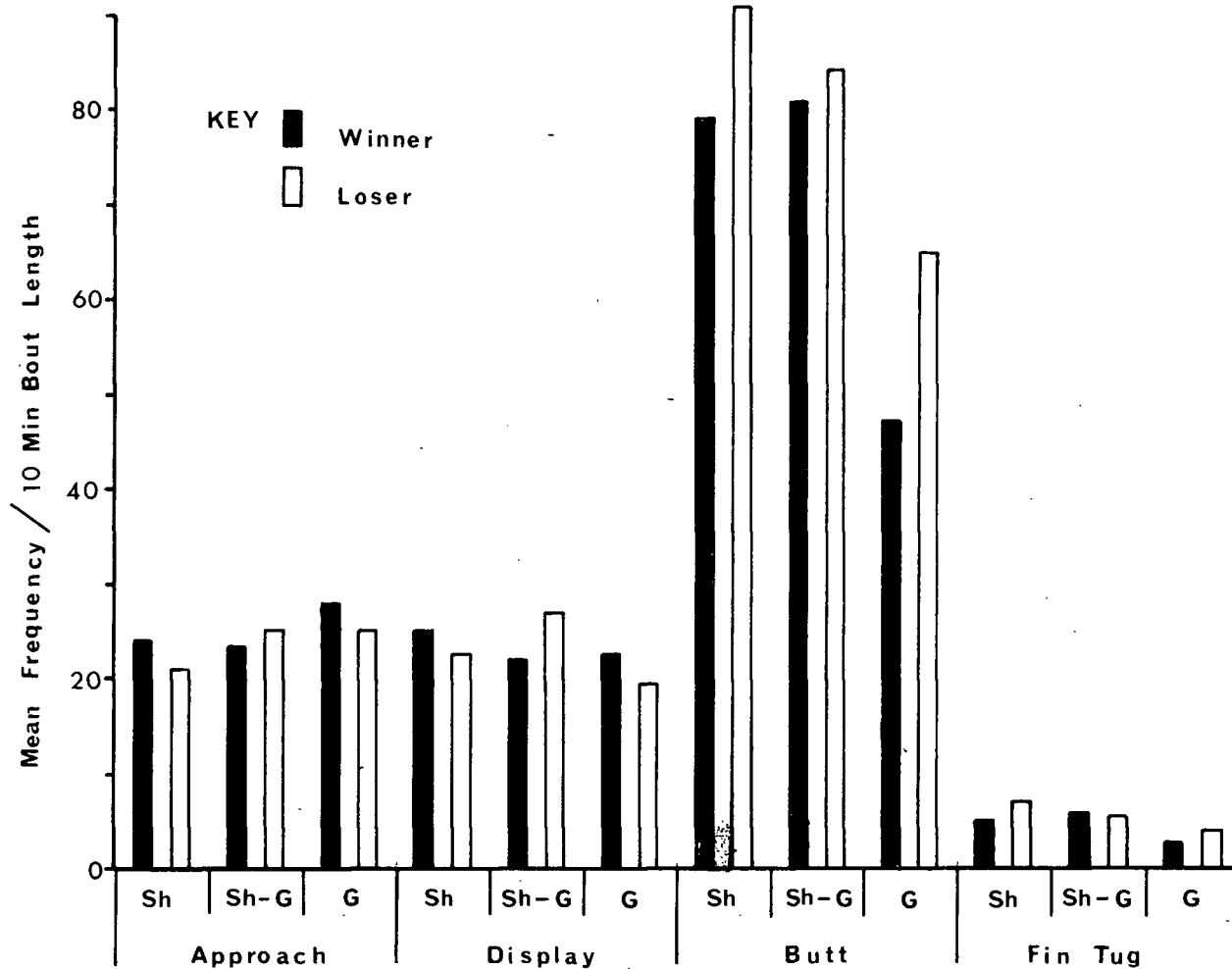


FIGURE 21. Mean frequencies of behavior patterns recorded for Sh-Sh, Sh-G, and G-G male encounters.

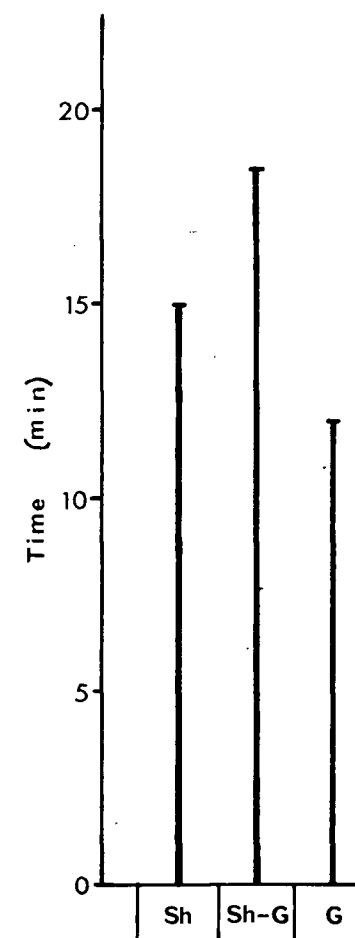


FIGURE 22. Average bout length for each class.

5. There appears to be a correlation between the mean rates of Display and Approach in all three Classes.

Discussion

From the results presented it would appear that gonadectomy has little effect on the initial agonistic behavior. Both the Sh and G males follow the same sequences of Approach, Display, Butting and Fin Tugging in alternate fashion with accompanying darkening. G males however, were not observed to be as dark as Sh males. It is suggested that colour darkening may be under the influence of androgens since all the G males used were of the GIII group which had not spawned (Chapter 4) nor had they exhibited colour darkening when paired with females. All GII and GIII males under hormone treatment showed intense colour change when spawning.

Certain GIII males when paired with each other or Sh males were initially treated as females since they were highly female-like in appearance (Chapter 6). The male-female behavior was of short duration, since the female-like males did not turn and Flee but remained and

returned the Butting. The rates for Approach and Display are very similar since just about every Approach resulted in Display.

The lower rate for Butting in G-G pairing is probably a result of the fact that two of the G-G trials were of very short duration (three-four min.) Since the first 30 seconds of any encounter is taken up with Approach, Pelvic Contact and Display, the high rate of Butting does not become apparent with such a short bout.

The winning fish in every case was slightly larger than its opponent. Of the four G-Sh trials, three were won by Sh fish and one by a G fish. Here again the winning G male was the largest. A fairly definite trend appears in Butting where the losing fish tends to Butt more than the winner.

This again probably results from size difference. It would seem that from a strictly physical basis, a greater number of Butts from a smaller individual would be necessary to achieve the same result as a smaller number of Butts from a larger individual. The moment of decision was quite dramatic. The losing fish for no apparent reason, suddenly blanched, ceased all attacks and swam away.

Several of the Sh fish had bubble nests at the time of release. There was no apparent attempt to defend this nest on the part of its owner. The dispute ranged throughout the tank equally. However, the test was terminated following completion of the dispute. Other observations, suggest that nest or territorial defence would develop sometime following the initial encounter.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

I General Discussion

a) Normal Reproductive Behavior

It seems worth mentioning at the beginning of this discussion that all of the behavior observed, described and recorded in this thesis occurred in a laboratory, i.e. aquarium situation. This was also true of Miller's (1964) work. All results therefore must be interpreted with this in mind. This observer feels that all of the behavior patterns described would be seen in the field, but not necessarily at the same frequencies or durations since the natural environment would be less confining.

Interesting also is the point that a very reliable method for predicting spawning evolved from this study. In 70 percent of the trials involving inexperienced fish of both sexes, spawning occurred within two or three days after the single opaque partition was removed. Miller describes a nonreproductive or social phase occurring between reproductive periods. This

aspect of behavior was never clearly observed in the present study. The animals would enter into a reproductive phase whenever the environment was manipulated in the manner described. Those failing to spawn in this situation always showed a certain amount of sexual behavior.

After working with the blue gourami for over a year and observing many spawnings, this observer has not been able to define clearly any particular behavior patterns as "courtship". From the analysis of Sh prespawning behavior (Chapter 4) a number of trends were described. Initially, it appears that Approach, Pelvic fin ray contacting and alternate Displaying are important. These give way to male Butting and Chasing and female Fleeing and Hiding. The Sh male asserts his dominance over the female very soon after release. Male aggressive behavior towards the female increases steadily with the result that she is intimidated into Hiding more frequently and for longer periods. The increased aggression is accompanied by gradual darkening and an increase in the frequency and duration of Nest Building. Until actual spawning begins there is no ritualized courtship behavior

such as is found in other species, e.g. stickleback, bitterling and guppy. Furthermore, the start of the spawning cycle, which is the only part of the behavior that could be described as at all ritualized, appears to be completely dependent on the female. Here is the most interesting question of this study. What causes a female which has been Butted and Chased, often having the whole distal portion of her anal fin torn away, to suddenly swim forth, apparently of her own volition, and initiate spawning?

A possible explanation is that during the time from release until spawning and as a result of seeing the male in the act of nest building, a final maturation of the female gonad is triggered. This might involve a readying of a certain number of eggs for release plus an increase in gonadal secretions. When these secretions, presumably steroids, reach a certain level as a result of constant visual stimulation, sexual behavior is initiated. This could occur through direct action on the brain by the steroids or by referral, through the pituitary. Lehrman (1965), describes mechanism of this type in the female ring dove. He claims it is the sight of the male in the

act of nest building which brings the female into reproductive condition. This is accomplished through a neural-endocrine link i.e. from the eye to the brain to the pituitary to the gonad.

Due to the lack of complete understanding of the contribution of the female in terms of prespawning behavior, it has had to be assumed during this study that all females would react in the same manner to a presented male. Thus any large differences in the time from the release until spawning, or the lack of spawning altogether, were attributed to some change or failure in male behavior as a result of gonadectomy.

In summary, a mature male when in the presence of a mature female will usually commence nest building. Nest building is accompanied by colour change and territorial behavior. This leads to the following hypothesis: there is no ritualized prespawning behavior in this species. It is the sight of the Black, Nest building male which brings the female into reproductive condition.

The discussion to follow attempts to show that nest building, colour change and the morphological secondary sexual characteristics in the male are the important factors

in bringing the female into condition and that all three are mediated by androgen.

b) Effects of Gonadectomy

The performance of the Sham operated males following one month's recovery from operation indicates that the differences observed on the part of gonadectomized males on retesting could not be attributed to any physical effects of the operation itself. The behavioral evidence presented in Chapter 4 showed that eleven out of sixteen castrated males (GIII) did not succeed in eliciting a spawning response in the females.

Paralleling the change in behavior was a dramatic reduction in the size and shape of their dorsal fins (Chapter 6). As described in Chapter 3, the size and shape of the dorsal fin is the only gross morphological difference between the sexes.

In the case of the five G males which did spawn (GII), their behavioral records indicated that a lower level of aggression was reached than by the Sh males. But still this was above the aggression level of the GIII males and included nest building. Correspondingly there was a slight reduction in the dorsal fin size for these

five over the Sh males but this was well above that of the majority or G III males.

It has been mentioned earlier that the GIII males were extremely female-like in appearance and furthermore initially were displaying typical agonistic behavior with the females. This observer suggests that the females could not distinguish the GIII males from females and treated them accordingly. Picciolo (1964) conducted a number of tests involving visual, chemical, auditory, lateral line and pelvic fin ray stimuli. He concluded that sexual discrimination in the blue gourami is strictly visual. He placed males with dorsal fins clipped to look like females in glass containers floating in aquaria. Unclipped males were placed in other glass containers. When females were placed in the aquaria he noted they approached the containers holding unclipped males far more often than those containing clipped males. This evidence backs the present findings regarding the failure of the females to discriminate and their resulting agonistic behavior.

The author feels that apart from the morphological

changes resulting from gonadectomy, the most important behavioral change was the failure on the part of the males to build nests. As mentioned earlier it seems likely that the sight of a fully mature male in the act of nest building with typical dark coloration brings the female into reproductive condition. The high level of aggression accompanying nest building is undoubtedly a function of it, i.e. strictly territorial in nature, with little or no direct connection with courtship. An observation made prior to the experiments may help to illustrate this. Two unoperated males and females were left together in a 100 liter tank. The larger of the two males built a nest at one end and defended it vigorously against both females and the other male. These females were not driven into hiding but merely to the other end of the tank which was much larger than the regular tanks used. Then after a day or so both females were observed spawning with the large male alternately. In fact one female was observed rapidly Approaching and Butting the male who was already engaged in Claspings the other female. The Clasp was broken and the male commenced to Rub the intruder, Here then, both

females had come into reproductive condition at the same time as a result of the sight of the dark, nest building male.

If the hypothesis is correct, then the failure of spawning among the GIII fish can be explained in two ways. First, as a result of gonadectomy and presumably the lack of gonadal hormones, these males had lost their secondary distinguishing features and failed to nest build with accompanying colour change and territorial aggression. Secondly, the females initially failed to distinguish them as males, and behaved towards them as they would to normal females. On receiving no further stimuli from the males, the females failed to come into reproductive condition.

From the evidence presented in Chapter 8 it can be seen that gonadectomy had virtually no effect on male agonistic behavior. Here, all G males tested were of Category III. The only observed difference was the fact that these G males did not undergo as dramatic a colour change as the Sh males. Therefore it is suggested that colour change is partly under gonadal control since these fish showed very little darkening when paired with

females. However, gonadectomized males are quite capable of agonistic behavior and even of establishing dominance over an Sh rival. Further, the GIII males behaved towards normal females in the same manner as they did toward the Sh males i.e. a high rate of Display, Butting and Fin Tugging. Females also behave initially towards each other in the same manner but for a shorter duration and without colour darkening. Thus both males and females are capable of agonistic behavior but the female form lacks the intensity and accompanying colour change. It is assumed the females would not normally be secreting androgens.

With regards to parental behavior (Chapter 7), it was shown that no males either operated or unoperated would accept eggs or fry until they had gone through the physical process of spawning at least once. GIII males never achieved spawning so it was impossible to test whether they were capable of parental behavior. GII males did not spawn however and entered into a parental phase in the same manner as the controls. These males initially cared for the inviable eggs and only ate them when they began to decompose. When given fertile eggs they were looked after in the usual way.

These observations suggest that some neural or endocrine mechanism triggers parental behavior and associated egg eating inhibition as a result of spawning and that this mechanism is not necessarily associated with the testes. Further this inhibitory mechanism seems to last only a short while (approximately ten days) after which the males will no longer accept eggs but they will continue to avoid fry. Perhaps during the period of inhibition the males become used to the fry through constant association i.e. by a learning process.

When all G males were reopened following completion of the hormone experiment it was noted that two of the four GIII males and one of the three GII males showed some testes regeneration. The four GIII controls on retest (Chapter 5) showed no more inclination to Nest build than they did previously. Their dorsal fin size was on the average slightly smaller than before and yet regeneration was discovered in two. On the basis of the results of the hormone treatments, it was felt the only plausible explanation could be that this regeneration was either non existent or too small at the time of testing to have much effect, i.e. to produce a hormone level sufficient to promote Nest building.

In support of the above suggestion, it may be pointed out that reoperation took place a month after termination of the hormone experiment. At this time, two instances of slight regeneration were found. The dorsal fins of these two were noticeably longer at the time of reoperation than when previously measured. It was felt therefore that this regeneration must have occurred to a large extent following the second testing.

The GII controls present a more interesting picture still. These fish when tested first showed only slight dorsal fin atrophy and their behavior was of a sufficient intensity to cause spawning but they failed to fertilize eggs. At the time of their second testing their dorsal fins were practically identical to the Sh males and further the time from release to spawning was also similar to Sh males. Their aggression level was closer to the Sh males than was that of the G-H males. All of these observations suggested regeneration of testes; yet on reoperating, none could be found in two of the three. Two possible explanations suggest themselves. Either their behavior was being mediated by some other endocrine or neural factor, or by androgen from some other source. If either of

these mechanisms were functioning, then the most interesting question of all arises i.e. how did such a substitute system become established in some fish, obviously from very soon after gonadectomy, and not in others? Further, was the behavior of the single GII regenerate being mediated by its testes all along, or was it too under some other control and the regeneration only a recent occurrence? The latter was thought to be the case since the amount of regeneration was very similar to the amount found in the two GIII males. A further check (not possible here) will be to carry out a histological examination of the pituitaries to see if they show the hypertrophy characteristic of castrated animals.

c) The Effects of Methyl Testosterone Replacement

Methyl testosterone administered to GIII males brought back nest building, territorial aggression, colour darkening, elongation and pointing of their dorsal fins and in four out of five cases, spawning resulted. This evidence indicates almost beyond doubt that androgen is in fact very much concerned with mediating sexual

behavior and in regulating the secondary sexual characteristics in the males.

The effects of testosterone on the morphological secondary sexual characteristics of fish is well documented. Wai and Hoar (1962) found the effects of testosterone the same regardless of genetic sex. They measured the height of kidney tubule cells of the three-spine stickleback. They found testosterone caused an increase in the size of these cells in juveniles and adults of both sexes. Gorbman (1962) says "sex secondaries are almost universally regulated by sex steroids". He reports that in gobies, dorsal fin spines elongate with testosterone treatment, as does the sword of the swordtail (Xiphophorus) and the gonadapod of Platypoecilus. He further states that male secondaries will develop in females with androgen treatment. In the present work testosterone caused an increase in the dorsal fin length in GII and GIII males and in unoperated females.

Not only did the dorsal fins of the treated females increase but their behavior was modified as well.

Among the six treated females an increased amount of typically male agonistic behavior was observed. The important aspects here were the increase in intensity and duration of Displaying, Butting and Fin Tugging plus the fact that extreme colour darkening accompanied the behavior. The most interesting behavior of all was the fact that these females engaged in a limited amount of Nest building. On opening one of these females, a very much degenerate ovary was discovered but no indication of any other gross changes.

These results add further strength to the suggestion that androgen is in fact the missing ingredient following gonadectomy of the male and that it is responsible for nest building in the male. It seems likely it is also responsible for colour darkening which together with nest building form the visual stimuli for female reproductive behavior. Colour change was the only thing missing in the G male agonistic behavior. This fact raises a question. Why did testosterone bring on typical male agonistic behavior among the females whereas its lack, did not appear to reduce the intensity of the

behavior in the G males? A possible explanation here might be the role of experience. All of the males had had plenty of experience with male contacts before gonadectomy, whereas each had had only one spawning experience before the operation.

There has been much discussion recently as to the role of the pituitary in reproductive behavior. Wai and Hoar (1962) have suggested that gonadotropin is essential for the normal expression of behavior. Aronson (1957) said there is some evidence for the direct action of the pituitary on certain aspects of sexual behavior. Liley (1965) suggested the gonad appears to exert a regulatory influence superimposed on a more direct neural and/or pituitary control of receptivity in the female guppy. Clemens, et al (1966) treated guppies for 60 days after birth with testosterone. They obtained a sex ratio of nine males to one female. These males all showed male secondary coloration and produced copious amounts of sperm, yet only a few sired young. They suggest that the endocrine control of the pituitary was involved in the siring failure. They quote Hoar (1962) as finding endocrine controls from

the gonads and the pituitary are responsible for coordination and timing of reproductive activities.

In the present experiment in view of the above findings one might suspect a pituitary or neural mechanism was regulating the behavior of the GII male controls without testes. Yet the effects of testosterone on the GIII males in bringing back the morphological S.S.C. and sexual behavior, neither of which were lost in the GII males, suggest an extra-gonadal source of testosterone, perhaps the interrenals.

II Summary of Conclusions

1. Blue gouramis may be easily and predictably induced to spawn by manipulating the environment.

2. Male prespawning behavior in this species seems to be confined to the act of nest building, with accompanying territorial behavior and colour darkening. Spawning itself involves a stereotyped behavioral cycle.

3. It appears that the presence of the dark nest building male provides the stimulus situation necessary to bring the female into a sexually receptive condition. After a certain period of exposure to this stimulus she initiates spawning.

4. Sexual recognition or discrimination by the female appears to be a strictly visual process.

5. Castration eliminates nest building, territorial behavior and the spawning cycle, limits colour change, and causes atrophy of the morphological secondary sexual characteristics in the majority of males, however it does not reduce agonistic behavior in socially raised males. It was impossible to say from this experiment whether spawning was eliminated as a result of a lack of androgen or from the failure of prespawning behavior.

6. Parental behavior is apparently triggered by the act of spawning regardless of gonadal condition. The male is initially inhibited from eating the eggs by a chemical or neural mechanism of relatively short duration, during which time the fry hatch. Continued inhibition appears to be the result of a learning process.

7. Testosterone replacement causes a regrowth of the morphological S.S.C., restores nest building, colour change and spawning in gonadectomized males.

8. Testosterone given to non-operated females caused a growth of male morphological S.S.C., and brought

on male-like agonistic behavior, colour change and some nest building.

9. A few males, following gonadectomy, retained their morphological S.S.C., continued to nest build, to show territorial behavior and colour change and to spawn. It is suggested that testosterone from an extragonadal source was being made available since no testes regeneration was found.

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APPENDIX

Introduction

This appendix contains the raw data concerning the fish measurements made in connection with Chapter 5. The following tables contain the snout to fin length, standard length and resulting ratio for Sh males, unoperated females, GII and GIII males following gonadectomy, G males following hormone treatment, GII and GIII males following control treatment, and unoperated hormone treated females.

Fish	Snout-fin (cm)	Snout length (cm)	Ratio
Sh ₃	5.3	5.2	1.02
Sh ₄	5.65	5.4	1.045
Sh ₅	5.7	5.6	1.02
Sh ₆	6.0	5.85	1.025
Sh ₇	5.3	5.0	1.06
Norm	5.2	5.0	1.04
Norm	5.0	4.9	1.02
Norm	4.85	4.6	1.055
Mean Ratio			1.035

TABLE IV Sh and Unoperated Male fin ratios

Fish	Snout-fin (cm)	Snout length (cm)	Ratio
1	5.2	5.9	0.882
2	4.75	5.4	0.880
3	4.95	5.45	0.908
4	4.55	5.1	0.893
5	4.5	5.0	0.900
6	4.5	5.1	0.882
Mean Ratio			0.891

TABLE V Unoperated female fin ratios

Fish	Snout-fin (cm)	Snout length (cm)	Ratio
G ₁₁	6.40	6.15	1.04
G ₁₄	6.15	6.15	1.00
G ₁₇	5.95	5.95	1.00
G ₁₈	5.80	5.70	1.02
G ₁₉	5.60	5.45	1.027
Mean ratio GII males			1.017
G ₁₂	5.75	5.65	1.022
G ₁₅	5.57	5.55	1.027
G ₁₆	5.37	5.70	0.924
G ₂₀	5.50	5.70	0.965
G ₂₁	5.10	5.50	0.927
G ₂₂	4.95	5.10	0.972
G ₂₃	5.00	5.44	0.927
G ₂₅	4.80	5.15	0.932
G ₂₆	5.20	5.70	0.912
Mean ratio G III males			0.957

TABLE VI Gonadectomized (GII and GIII) male
fin ratios

Fish	Snout-fin (cm)	Snout length (cm)	Ratio
G ₁₄	6.20	5.90	1.050
G ₂₀	5.70	5.65	1.008
G ₂₂	5.25	5.10	1.030
G ₂₅	5.40	5.20	1.039
G ₂₁	5.40	5.50	0.983
G ₁₂	6.10	5.85	1.040
Mean ratio GII 1 fish = 1.050			
Mean ratio GIII 5 fish = 1.020			

TABLE VII Gonadectomized, Hormone treated male
fin ratios

Fish	Snout-fin (cm)	Snout length (cm)	Ratio
Cat. II			
G ₁₁	6.35	6.25	1.015
G ₁₇	6.20	6.00	1.033
G ₁₉	5.90	5.70	1.035
Cat. III			
G ₂₆	5.40	5.90	0.915
G ₂₃	5.20	5.50	0.945
G ₁₅	5.80	5.75	1.008
G ₁₆	5.50	6.00	0.917
Mean ratio GII 3 fish = 1.028			
Mean ratio GIII 4 fish = 0.946			

TABLE VIII Gonadectomized control (GII & GIII)
male fin ratios

Fish	Snout-fin (cm)	Snout length (cm)	Ratio
1	4.4	4.6	0.958
2	4.5	4.8	0.938
3	4.55	4.65	0.980
4	4.60	4.55	1.010
5	4.55	4.75	0.958
6	5.00	5.00	1.000
Mean ratio =			0.975

TABLE IX Hormone treated unoperated female
fin ratios