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CASTRATION AND REPRODUCTIVE BEHAVIOR IN THE PARADISE FISH, *MACROPODUS OPERCULARIS*

by
Trudy Ann Villars

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Psychology)
in The University of Michigan
1977

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To Murray whose support and encouragement

have been very important to me.

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INTRODUCTION

The bony fishes comprise a diverse class of vertebrates which have been evolving for 400 million years independently from tetrapods. Still, in many ways the endocrine system of fishes is similar to that of other vertebrates (Pickford and Atz, 1957; Dodd, 1960; Hoar, 1962a, 1969). Because of the diversity of species and their varied habitats and habits teleosts provide a rich source of material for the comparative study of hormonal controls of vertebrate behavior. From an applied viewpoint fish represent an important source of protein to a growing world population. Understanding the factors which regulate reproduction in fishes may be important both in aquaculture and in the prevention or remedy of ecological disruptions to the reproduction of important commercial fishes.

Compared to mammals, little is known about hormonal control of teleost reproductive behavior (reviews: Beach, 1948, 1970; Aronson, 1958; Liley, 1969; Fiedler, 1974; Hoar, 1962a, 1965; van Tienhoven, 1968). Only 20-25 species have been studied out of approximately 20,000, representing only 3 of the 8 major teleost supraorders. Few of the species have been studied extensively. Species represented have been selected for study because of their value as a food source (e.g. Tilapia), because of the background information available (e.g. goldfish or stickleback) or because of their conspicuous sociosexual behavior (e.g. cichlids or

anabantids).

Androgen control of morphological characteristics in teleosts is extensively documented in a number of species (Yamamoto, 1969), including Gasterosteus (Bock, 1928; Ikeda, 1933), Xiphophorus (Noble and Borne, 1940; Tozawa, 1923), Tilapia (Aronson, 1951), Lebistes (Blacher, 1926), Oryzias (Okada and Yamashita, 1944; Niwa, 1965 a,b), Carassius (Tozawa, 1929), and Trichogaster (Johns and Liley, 1970). The development of these morphological sex characteristics prior to breeding is dependent on gonadal hormones (Hoar, 1969).

The importance of androgens in the development or maintenance of behavior is less clear. Research has centered on administration of androgens to intact males or females, and on removal of androgen by castration. After androgen treatment, female platyfish and swordtails showed male courtship and copulation movements (Cohen, 1946; Tavolga, 1949; Hildemann, 1954; Laskowski, 1954, Noble and Borne, 1940), but male patterns were not completely obtained (Tavolga, 1949; Laskowski, 1954). Female medakas treated with testosterone were masculinized morphologically and behaviorally (Okada and Yamashita, 1944). Administration of methyl testosterone to intact male and female bluegill sunfish induced male-like behavior in both sexes (Avila, 1976). On the other hand female guppys which were morphologically masculinized did not show male sexual behavior (Clemens et al., 1966). Liley (1969) has attributed

this last finding to inadequacy of environmental rather than hormonal factors.

The effects of gonadectomy vary with the species. Castration reduced or blocked sexual behavior in Gasterosteus (Hoar, 1962b; Baggerman, 1966; Ikeda, 1933), Salmo (Jones and King, 1952), Xiphophorus (Chizinsky and Aronson, in Aronson, 1958) and Cymatogaster (Wiebe, 1967). Replacement therapy with methyl testosterone reversed castration effects on Gasterosteus (Wai and Hoar, 1963). On the other hand, castration had no effects on the cichlid Hemichromis and the anabantid Betta (Noble and Kumpf, 1936), and sexual activity of Bathygobius was increased (Tavolga, 1955). Trichogaster trichopterus showed marked individual differences in response to castration (Johns and Liley, 1970). These investigators attributed continuation of spawning behavior in some castrate T. trichopterus to undetected testicular tissue or extra-gonadal sources of androgen.

Alternatively, where castration did not alter socio-sexual behavior, the behavior in question may be directly regulated by gonadotrophins. Gonadotrophin regulation of behavior has been suggested with agonistic behavior of Starlings (Mathewson, 1961; Davis, 1964). Starlings administered luteinizing hormone (LH) show an increase in their dominance position (Mathewson, 1961) while testosterone injections are followed by a decrease in dominance position (Davis, 1957). Davis (1964) discusses how gonadotrophin might account for hitherto unexplained failures

of the testosterone theory in birds.

Some fish researchers (Hoar, 1962 a,b; Tavolga, 1955; Baggerman, 1966) have turned to a gonadotrophin theory to account for some of their results. Hoar observed that the effectiveness of castration in reducing agonistic behavior was dependent on the light cycle. Studying the three-spined stickleback Gasterosteus aculatus (forma lieurus)he found that castration was less effective in reducing aggression when fish were maintained on a long (16 hour) light cycle than when they were on a short day cycle (8 hour). He hypothesized that longer daylight resulted in greater stimulation of the pituitary and that gonadotrophin maintained the aggressive behavior of castrates on the long day cycle.

Baggerman (1966) reported some data which provided further indirect evidence for a gonadotrophin theory. In Gasterosteus (of the trachurus form) she noted that castration reduced agonistic behavior only after nestbuilding began. Castration had no effect on agonistic behavior in the pre-nesting period. Referring back to Hoar's work, she interpreted her data to mean that early, prenesting agonistic behavior was under the control of gonadotrophins, which were rising in response to lengthening daylight. Once androgen levels had risen and nestbuilding began, maintenance of agonistic behavior shifted to androgens.

In the one study on sexual behavior, Tavolga (1955) found that Bathygobius increased sexual behavior following

castration, while hypophysectomy blocked sexual behavior. Though inconclusive, these studies, taken together, suggest that direct gonadotrophin control of behavior is a possibility worth considering.

The comparison of a gonadotrophin theory with an androgen theory requires manipulation of gonadotrophins. Such manipulations are complicated by the difficulty in successfully hypophysectomizing fishes. Until recently the only chemical inhibitors of gonadotrophins were steroidal agents which produced many or all of the effects of gonadal hormones. They were of little use in dissecting out pituitary--gonad function. A dithiocarbamoylhydrazine (I.C.I. 33,828, methallibure) has been developed which is a non-steroidal inhibitor of gonadotrophins. It has been shown to cause gonadal atrophy in both mammals and fish (for brief review see: Hyder, 1972; Davis et al., 1975). In goldfish it has been demonstrated to reduce plasma levels of gonadotrophin (Bretan, Jalabert and Billard, 1973). In that same study pituitary levels were not reduced suggesting an interference with releasing factors. Studies in rodents have suggested methallibure acts at the level of the hypothalamus not the pituitary (Malvin, 1971). It has recently been suggested that it exerts its effect via an interaction with catechol amines (Davis et al., 1975) which may be involved in regulation of releasing factors (Brown and Fawke, 1972; Kalra and McCann, 1973; deWied and deJong, 1974).

In addition to methallibure's effects on gonadotrophins, some impairment of thyroxin production may occur (Tulloch, Crooks and Brown, 1963; Mackay, 1973). Apparently no effect on ACTH or adrenal function is produced (Bielanski, 1973). Its effect on other pituitary factors is unclear, although it may inhibit growth hormone (Cargill-Thompson, 1963) and stimulate prolactin release (Dies and Vermouth, 1973).

The present study examined the effects of testicular hormone on sexual behavior of the anabantid Macropodus opercularis, the paradise fish, using castration and methallibure administration. Macropodus is closely related to B. splendens and T. trichopterus which have been subjects in studies of androgen control of reproductive behavior (Noble and Kumpf, 1936; Johns and Liley, 1970; Machemer, 1971).

Preliminary studies (Davis, Mitchell and Dolson, 1976; Villars and Davis, 1976) suggested that Macropodus was not dependent on gonadal androgens for the maintenance of sexual behavior. Procedural issues however confounded the interpretation of their results. Davis, Mitchell and Dolson (1976) demonstrated testicular atrophy following five week treatment with the antigonadotrophin methallibure, while sexual behavior of treated males appeared normal. While those results suggested that sexual behavior is independent of gonadal control, the males were not treated with methallibure during the five day spawning trial and recovery of testicular function could have occurred during that time. Males castrated two weeks prior to a spawning trial and reoperated prior to successive trials showed normal sexual behavior and only

slightly reduced fertility (Villars and Davis, 1976). Examination four weeks after the last operation revealed significant amounts of testicular tissue, suggesting that regeneration had occurred.

The present investigation consisted of three parts. First, the effects of castration on sexual behavior one week and two weeks following the operation were determined. Second, the effects on sexual behavior of castration plus methallibure-induced gonadotrophin suppression were examined. Finally, the behavioral effects of testosterone replacement were investigated. If sexual behavior is influenced by testicular hormone, castration should produce behavioral changes and replacement of testosterone should reverse those behavioral effects. Independent groups were tested one and two weeks postoperatively following an indication in pilot studies that some testicular regeneration occurred within two weeks. Methallibure treated groups were included to test for effects of gonadotrophin, directly on behavior, or indirectly by facilitation of testicular regeneration.

METHODS

Adult paradise fish, Macropodus opercularis (Belontiidae: Teleostei (Liem, 1963) were obtained from Gossington Tropical Fishes, Del Ray Beach, Florida, or from a local dealer of Florida stock. Subjects were kept in 190 liter community tanks for at least seven days before an experiment, and cared for as previously described (Davis, Harris and Shelby, 1974). They were fed Tetramin Staple Food or frozen brine shrimp one or two times daily. The 24° to 26° C. water was filtered continuously and replaced weekly. Daylight was supplemented by flourescent lights to produce 16 hours of light and 8 hours of dark. Males and females were distinguished on the basis of secondary sex characteristics; adult males have longer medial fins, relative to body length and wider mouths than females.

SURGERY.

Surgery was performed using ophthalmic surgical instruments and a stereoscopic dissecting microscope. Fish were anesthetized with Finquel (Tricaine Methanesulfonate, Ayerst) and placed on a cool, damp platform. An incision was made into the caudal end of the body cavity just dorsal of the urogenital papilla (Figure 1). The incision was extended dorsally to visualize the distal end of the ipsilateral testes which was then freed from the supporting mesentary and lifted out. The contralateral lobe was similarly removed through the same opening. The testes were weighed and a

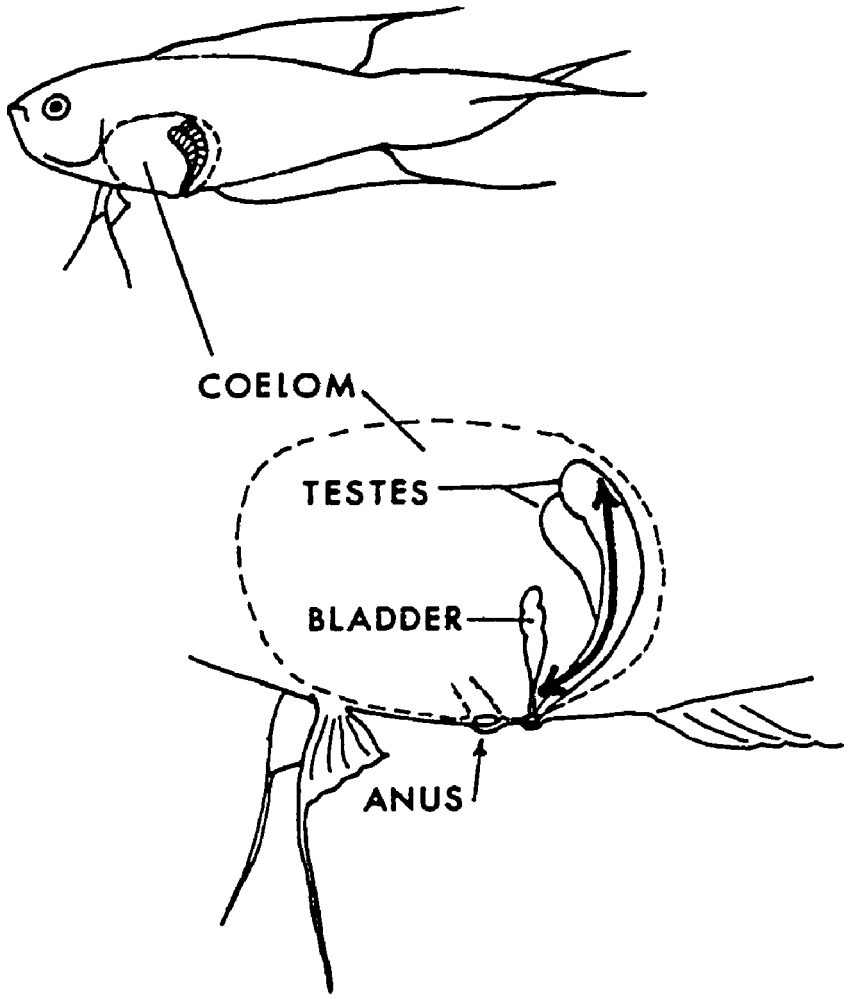

FIGURE 1: SURGERY. The testes are loosely attached to the back wall of the coelom. The incision, indicated by the double-pointed arrow, was approximately one cm in length.

pretrial gonadosomatic index (G.S.I) determined as (testes wt/body wt) X 100. The wound was closed using two sutures of silk cardiovascular thread (Deknatel). Sutures were removed two or three days later. In the sham-operated controls an incision was made, the testes manipulated and the incision sutured. Between surgery and testing subjects were held in individual 9 liter isolation tanks.

DRUGS.

Methallibure (Imperial Chemical Industries, Ltd., 33,828) was administered perorally, mixed with the standard diet in a concentration of 10 mg methallibure per gm dry food (Davis, Mitchell and Dolson, 1976). Methallibure was weighed and dissolved in acetone. Standard Tetramin diet was powdered with a mortar and pestle, and a premeasured amount of powdered food was soaked with dissolved methallibure. The acetone was evaporated, leaving dry, drug-treated food. The treated food was mixed with tapwater to form a paste which was then placed in a 10 cc plastic syringe. The paste was extruded from the syringe to form a pellet containing approximately 0.3 mg of methallibure. Fish were fed once daily in the morning. During the spawning trial females were removed from the tank allowing subjects and females to be fed separately. Subjects were fed drugged or control food one hr before the morning observation.

Testosterone enanthate (Schering AG, Berlin) was injected intraperitoneally in sesame oil with a 100 microliter syringe and a 26 guage needle. Fish received 0.5 mg testosterone enanthate in 10 microliters of oil five days

and one day before the spawning trial.

SPAWNING TRIAL.

Spawning was tested in 38 liter glass aquaria, 62 X 20 cm filled to 25 cm with dechlorinated water. The walls adjacent to other tanks were covered with opaque white plastic to prevent subjects in different tanks from seeing each other. Tanks were drained and refilled with water before each spawning trial. Water temperatures were kept between 23 and 27° C. The daily light period consisted of 16 hours of flourescent illumination directly above the aquaria. The water was undisturbed by filtration to facilitate foamnest building. Two plastic plants and a brick provided some cover.

Previously spawned, ripe females were selected on the basis of appearance. Females were spawned no more often than every two weeks, an interval adequate to restore spawning ability (Hall, 1966). The day before the spawning trial, the female was placed in the tank and fed. The following morning the males were fed and placed in the tank an hour later. Pairs remained together for five days or until spawning occurred. Spawning was considered to have occurred when eggs were seen in the nest. The proportion of males which spawned in each experimental group was recorded.

Eggs were removed shortly after spawning appeared complete, or immediately after eggs were seen in the nest. The eggs were allowed to develop for 24 to 36 hours prior to counting the number of live and dead eggs. The percent of fertile (live) eggs was then calculated. At the end of the

trial subjects were sacrificed, and examined for testicular remnants. Any remnants were weighed and a post-trial GSI was calculated.

BEHAVIORAL OBSERVATIONS.

Observations. The behavior of the males was recorded during two daily 10-min observation periods. The first period began immediately after the male was placed in the spawning tank. The male was otherwise observed in the morning and the late afternoon until spawning took place or, if spawning did not occur, for five days. In addition, pairs were checked every two to three hours to determine if curving or clasping sequences had begun.

Behavior. The following units of behavior were recorded using a keyboard connected to electromechanical counters and a running time-meter (Grason-Stadler): lateral display, frontal display, approach, pursue, attack, nestbuild, curve and clasp. These behaviors have been described previously (Hall, 1966; Schwagmeyer et al, in prep.).

Lateral display (Figure 2, A.) was recorded when the male fully spread its anal, caudal and dorsal fins. Frontal display (Figure 2, B.) was recorded when the opercula were extended. Approach was recorded whenever the fish moved to within one body length of the female. Pursue was recorded when the male swam behind the withdrawing female for a distance of at least four inches. Attack was recorded when the male lurched toward the female and appeared to make contact. A curve (Figure 2, C.) was recorded when the male

FIGURE 2: SOCIOSEXUAL DISPLAYS OF M. OPERCULARIS. Four of the eight recorded units of behavior are illustrated. A. Lateral Display: fully spread anal, caudal and dorsal fins, B. Frontal Display: extension of opercula, generally while approaching or facing a conspecific. C. Curve: the male curves his body around the female, who positions her body vertically. D. Clasp: inversion of the female following a curve.

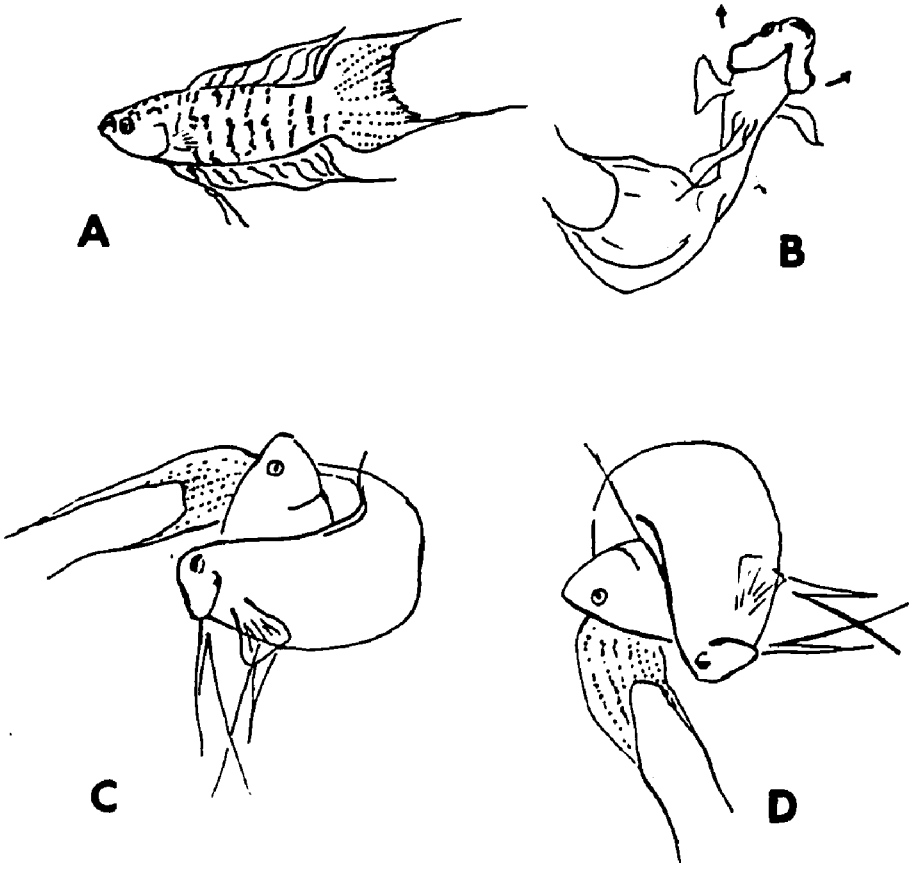

FIGURE 2: SOCIOSEXUAL DISPLAYS OF M. OPERCULARIS

curved its body around the female's abdomen bringing the urogenital openings into juxtaposition. A clasp (Figure 2, D.)was recorded whenever the male successfully inverted the female.

The total time spent nestbuilding and the latency to first begin nestbuilding was recorded. The nestbuilding male gulps air, descends several centimeters below the surface and releases a stream of bubbles. The sequence may be repeated several to many times in a bout. A bout was denoted as terminated when the male made a non-nestbuilding response or paused for more than 5 seconds. Nestbuilding was considered to have begun during the first observation in which any nestbuilding responses were seen.

The mean frequency per 10 min observation of lateral display, frontal display, approach, pursue and attack was calculated for each subject using all observations prior to spawning. Observations in which curve or clasp occurred were not included. Data for all subjects in a group were pooled and an overall mean for the group was obtained. Likewise, the mean nestbuilding duration per 10 min observation was calculated for each subject and data for subjects pooled in each group. Nestbuilding duration was averaged only over observations after nestbuilding had begun, therefore nestbuilding duration is independent of nestbuilding latency. The median latency to begin nestbuilding and the proportion of males nestbuilding by the third observation was determined for each group.

EXPERIMENTAL GROUPS.

Eight groups of males were administered the five day spawning trial. The males of group I (n=12) were placed in individual isolation tanks for 2 days prior to testing. Group II (n=8) was sham-operated 8 days prior to the spawning trial. Groups III and IV were castrated 8 days (n=11) and 14 days (n=6) prior to the spawning trial. Group V (n=8) consisted of intact males which were administered 0.3 mg methallibure daily for 8 days prior to the spawning trial and during the trial. Groups VI and VII were castrates which were tested 8 days (n=13) or 14 days (n=7) postoperatively and administered methallibure as was group V. Group VIII (n=14) was treated the same as the methallibure-treated one-week castrates except that they also received testosterone.

RESULTS

Spawning. Spawning data are summarized in Table 1 and Figure 3. A chi-square test for k independent samples (Siegel, 1956) indicated significant differences among the groups ($p < .05$). Each group was compared to the controls using a test for differences between two proportions (Bruning and Kintz, 1968). The results for groups III and IV showed that the proportion of castrates spawning decreased one week but not two weeks following surgery ($p < .05$). The sham operation had no effect on spawning probability. Spawning latency of castrates which did spawn was the same as control latency.

Methallibure combined with castration resulted in decreased spawning at two weeks as well as one week following testicular removal. There was no significant difference in the probability of spawning in one-week castrates and methallibure-treated one-week castrates. Testosterone replacement resulted in an increased spawning probability in the castrated, methallibure-treated group, which was not significantly different from that shown by the controls.

Fertility. The fertility data are summarized in Table 1 and Figure 4. A one-way analysis of variance revealed that the percent of fertile eggs varied significantly between groups ($F = 16.8$, $df = 7,30$ $p < .0001$). Individual groups were compared with each other using the Sheffé method for the analysis of simultaneous contrasts between groups (Sheffé,

TABLE 1: PERCENT OF MALES SPAWNING, FERTILITY AND SPAWNING LATENCY. Columns 1 and 2 give the group and the number of subjects included in each group respectively. Roman numerals in the group column refer to the group number as indicated in the methods. Column 3 presents the percent of the males in each group which spawned. The percent of live eggs was calculated for each brood, and the mean percent fertility of all broods in each group is presented in Column 4. Column 5 presents the median latency to spawn in days, for those subjects which did spawn. If a male spawned during or prior to the morning observation it was counted as spawning on the previous day. The bottom row presents the significance level obtained in the analyses of variance.

TABLE 1: SPAWNING SUCCESS, FERTILITY AND LATENCY

	GROUP	N	PERCENT SPAWNED	FERTILITY (± s.e.)	MEDIAN LATENCY (range)
I	CONTROL	12	75	90 (4)	3 (1 - 4)
II	SHAM	8	75	91 (2)	3 (1 - 5)
III	GDX-1	11	45 *	34 (22)*	4 (1 - 5)
IV	GDX-2	6	67	62 (11)	4 (2 - 5)
V	MTB	8	88	88 (5)	3 (1 - 5)
VI	GDX-1 + MTB	13	38 *	.3 (.2)*	2 (2 - 3)
VII	GDX-2 + MTB	7	14 *	0 *	1
VIII	GDX-1 + MTB + TESTOSTERONE	14	79	11 (8) *	3 (1 - 5)
$p <$			.05	.0001	NS

* significantly different from controls ($p < .05$)

FIGURE 3: PERCENT OF MALES SPAWNING. Starred (✡) groups are significantly different from controls ($p < .05$). The number (N) of subjects included in the calculations is shown under each column. (CONTROL= Group I, untreated controls; SHAM= Group II, sham- operated controls; MTB= Group V, intact, methallibure-treated subjects; GDX-1= Group III, castrates tested one week postoperatively; GDX-2= Group IV, castrates tested two weeks postoperatively; GDX-1 + MTB= Group VI, one week castrates treated with methallibure; GDX-2 + MTB=Group VII, two week castrates treated with methallibure; GDX-1 + MTB + Testosterone= Group VIII, one week castrates, treated with methallibure and with testosterone enanthate.)

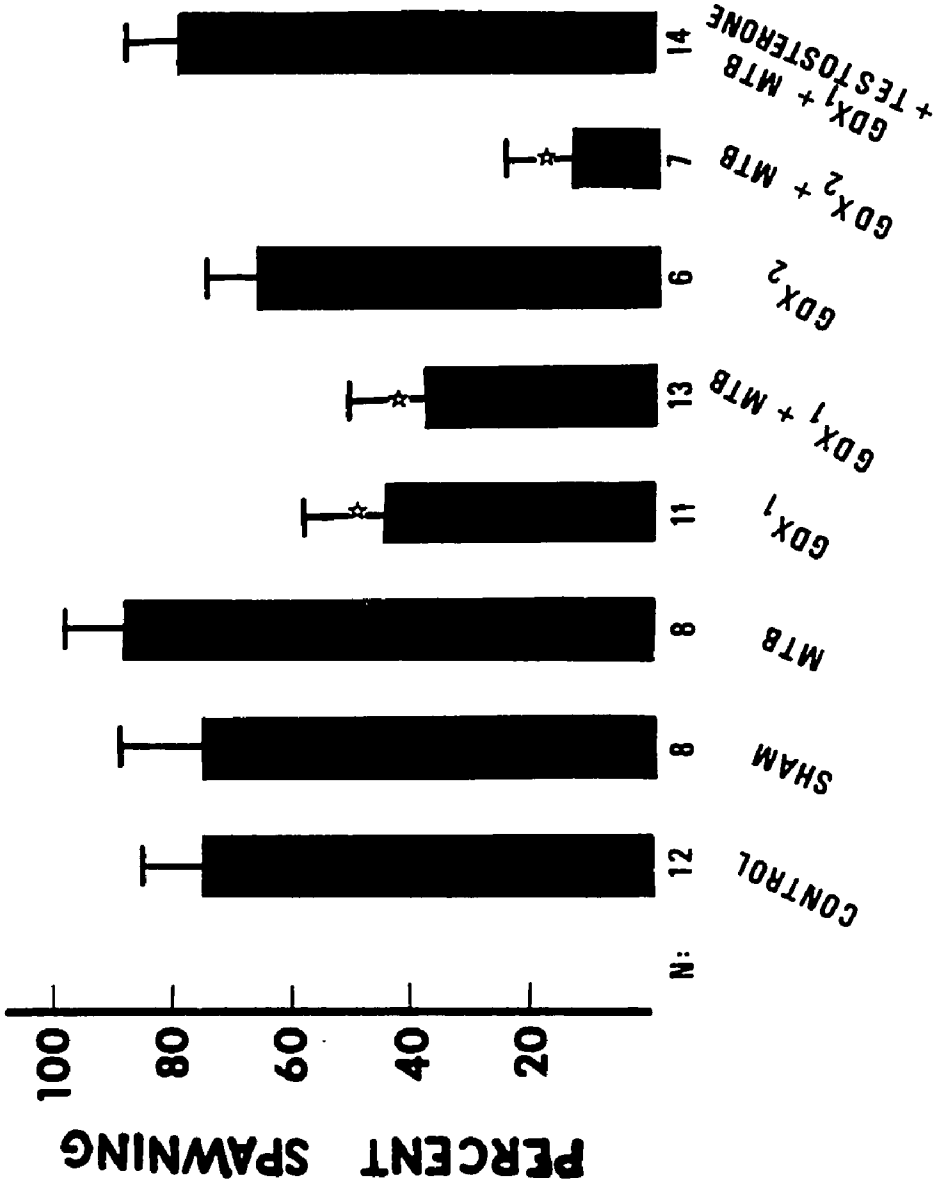

FIGURE 3: PERCENT OF MALES SPAWNING.

FIGURE 4: FERTILITY AMONG SPAWNERS. Starred (*) groups are significantly different from control, sham and intact methallibure groups ($p < .001$) but not from each other. (CONTROL= Group I, untreated controls; SHAM= Group II, sham-operated controls; MTB= Group V, intact, methallibure-treated subjects; GDX-1= Group III, castrates tested one week postoperatively; GDX-2= Group IV, castrates tested two weeks postoperatively; GDX-1 + MTB= Group VI, one week castrates treated with methallibure; GDX-2 + MTB=Group VII, two week castrates treated with methallibure; GDX-1 + MTB + Testosterone= Group VIII, one week castrates, treated with methallibure and with testosterone enanthate.)

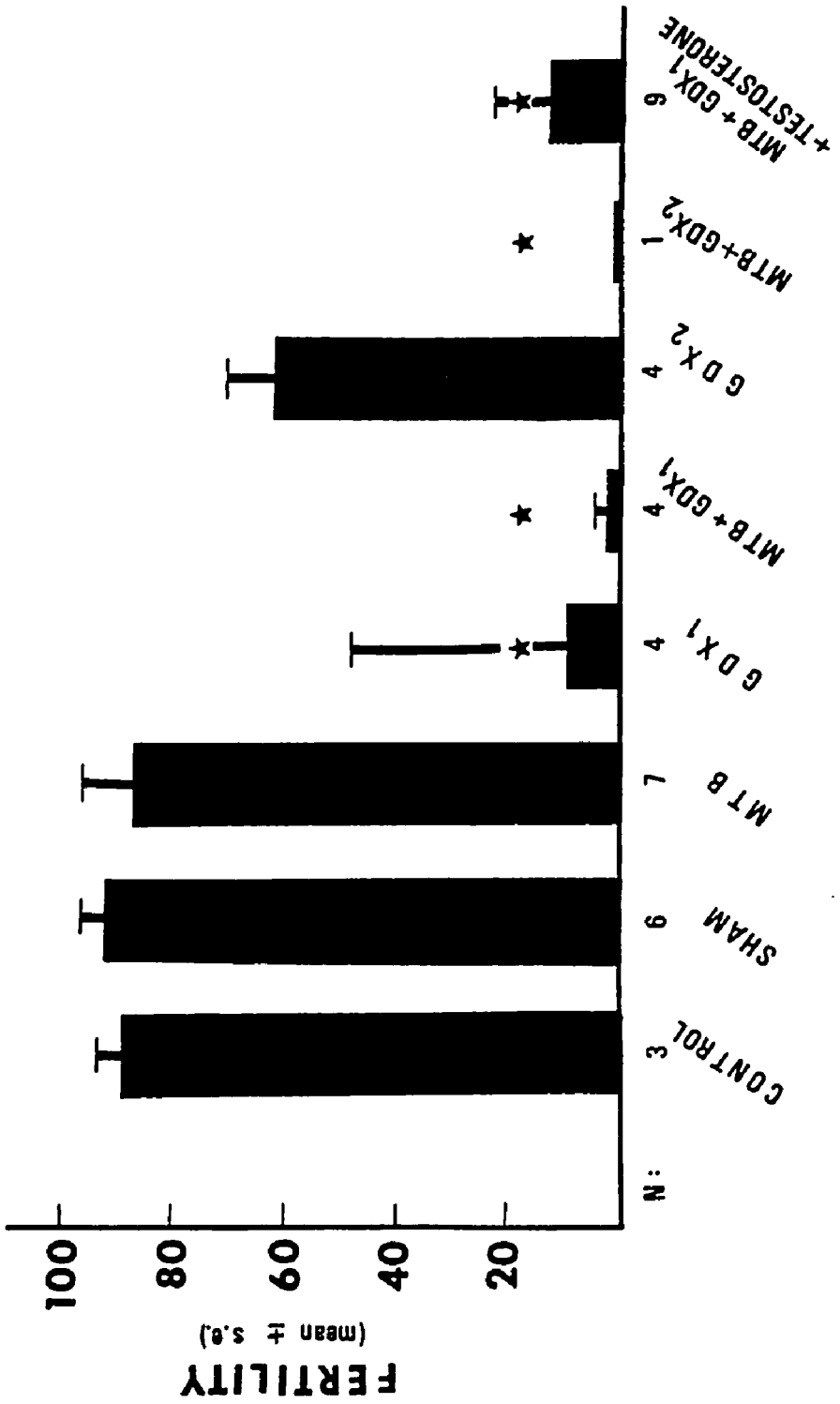

FIGURE 4: FERTILITY AMONG SPAWNERS

1959). The control, sham and methallibure-only groups showed high fertility and were not significantly different from each other ($p < .75$). They were significantly higher than one-week castrates, methallibure treated one-week and two-week castrates, and testosterone treated subjects ($p < .001$) which were not different from each other. Fertility of the two-week castrates was higher than that of methallibure-treated one-week and two-week castrates and testosterone treated subjects ($p < .0002$) .0004 and .01 respectively) but only marginally higher than the one week castrates ($p < .06$). The two-week castrate fertility was lower than in control, sham and methallibure-only groups, but those differences were marginally significant or insignificant ($p < .09$, .04 and .06 respectively).

Gonadosomatic Index. The mean GSI was compared among the castrated groups (Table 2). Pretrial GSI values did not vary significantly ($F = 2.10$, $df = 4,35$ $p < .10$) but post-trial GSI values did ($F = 4.7$, $df = 4,46$ $p < .003$, Figure 5). Males castrated three weeks prior to autopsy (group IV) had a mean GSI value which was two to three times that of the other four castrated groups, a highly significant effect ($p < .0001$). The differences between the other groups were not significant, when examined using Scheffé analysis.

Prespawning Behavior. The effects of all treatments on prespawning behavior is presented in Table 3. A one-way analysis of variance using the Kruskal-Wallis H test (Siegel, 1956) showed significant differences between groups in mean frequency of lateral display ($H = 16.5$, $df = 7$ $p < .02$), frontal display ($H = 21$, $df = 7$ $p < .004$) and approach ($H = 27$, $df = 7$ $p < .0003$) but not pursue or attack ($p < .05$).

Nestbuilding. Nestbuilding behavior was assessed in

TABLE 2: PRE- AND POST-TRIAL GONADOSOMATIC INDEX (GSI). Columns 1 and 2 indicate the groups and the number of subjects included in calculations for that group. Roman numerals refer to the group number as indicated in the methods. Gonadosomatic Index was calculated by dividing testes weight by body weight and multiplying by 100. Pretrial GSI reflects the amount of testes removed at castration. For shams none was removed. Post-trial GSI values indicate the amount of testes found upon inspection at the termination of the spawning trial. The post-trial value for shams is an estimate of normal GSI values. The sham group was not included in the ANOVA on post-trial GSI differences.

TABLE 2: PRE- AND POST-TRIAL GONADOSOMATIC INDEX

GROUP		N	PRETRIAL (+ s.e.)	POST-TRIAL (+ s.e.)
II	SHAM	8	-	.157 (.013)
III	GDX-1	11	.122 (.006)	.010 (.003)
IV	GDX-2	6	.157 (.022)	.038 (.006)*
VI	GDX-1 + MTB	7	.143 (.021)	.015 (.006)
VII	GDX-2 + MTB	7	.177 (.020)	.008 (.007)
VIII	GDX-1 + MTB + TESTOSTERONE	9	.133 (.005)	.012 (.003)
$p <$			NS	.003

* significantly different from groups III, VI, VII and VIII ($p < .0001$)

FIGURE 5: POST-TRIAL GONADOSOMATIC INDEX OF CASTRATED GROUPS. Testicular remnants found in castrated subjects at the end of the spawning trial were weighed and a gonadosomatic index (GSI) calculated; the weight of any remaining testicular tissue was divided by the subject's body weight and multiplied by 100. The mean GSI of all subjects in each of the five castrated groups was then calculated.

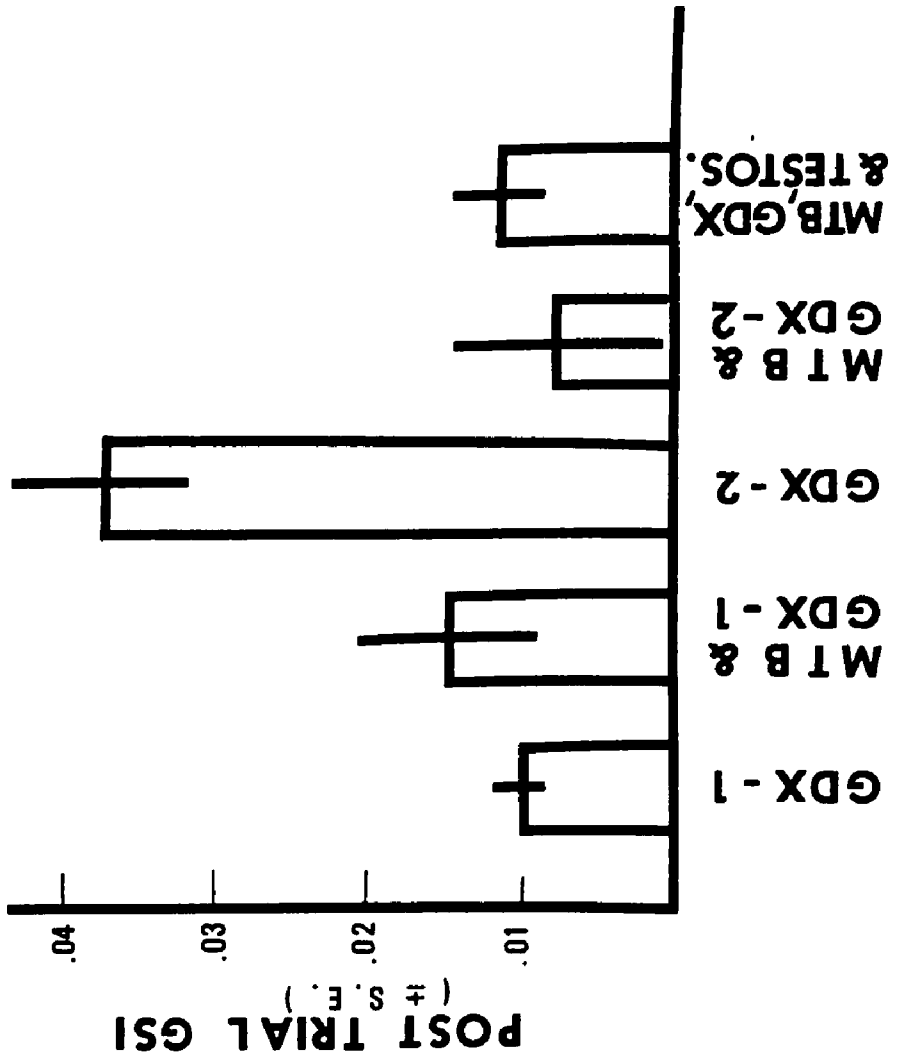

FIGURE 5: POST-TRIAL GONADOSOMATIC INDEX OF CASTRATED GROUPS

TABLE 3: FREQUENCY OF SOCIOSEXUAL DISPLAYS. The mean frequency of displays per 10 min observation was calculated for each subject over all observations prior to the beginning of spawning. This table presents the mean of each group of individual values.

TABLE 3: FREQUENCY OF SOCIAL BEHAVIOR

GROUP		LATERAL DISPLAY	FRONTAL DISPLAY	APPROACH	PURSUE	ATTACK
I	CONTROL	4.9	2.4	3.0	0.4	0.8
II	SHAM	3.0	1.9	4.1	1.4	1.2
III	GDX-1	4.4	1.8	3.9	0.3	1.8
IV	GDX-2	5.6	3.4	4.5	0.6	2.9
V	MTB	5.2	2.0	2.0	0.6	0.7
VI	GDX-1 + MTB	4.4	1.6	2.1	0.8	1.5
VII	GDX-2 + MTB	2.0	0.4	1.4	0.9	1.5
VIII	GDX-1 + MTB + TESTOST.	4.2	1.9	3.0	0.6	1.5
$p <$		.02	.004	.0003	NS	NS

terms of latency to begin nestbuilding and maximum nestbuilding occurring after it had begun (Table 4). Nestbuilding latency varied between groups ($H=14$, $df=7$ $p<.05$). Nestbuilding began by the third observation, which occurs on the morning of day 2, in all controls, shams, and two-week castrates. Median latency to nestbuild was increased in the methallibure-only group, even though all subjects but one were nestbuilding by the third observation. In methallibure-treated one-week castrates three fish began nestbuilding by the fourth observation, one by the eighth observation and one never began nestbuilding. In methallibure-treated two-week castrates three fish began by the third observation, three others waited until the fourth, fifth and seventh observation and one never initiated nestbuilding. In the testosterone-treated group the median latency to nestbuild was the same as controls, but 5 males delayed nestbuilding past the third observation.

Significant differences between groups occurred in the duration of nestbuilding ($F=4.8$, $df=7,71$ $p<.0002$). One methallibure treated group, methallibure-treated two-week castrates, showed the least nestbuilding, however the methallibure-only group showed the highest nestbuilding scores while one-week castrates, methallibure-treated one-week castrates, testosterone-treated castrates and two-week castrates showed intermediate nestbuilding rates.

Egg-eating. In three groups, one-week castrates, methallibure-treated one-week castrates and testosterone

TABLE 4: NESTBUILDING LATENCY AND DURATION. Nestbuilding latency indicates the median observation by which males in each group were first seen to nestbuild. The adjacent column presents the percent of males which were first seen to nestbuild during observation three, or in preceding observations. The last column gives the mean duration of time spent nestbuilding in minutes per 10 min observation. Means were calculated for each individual over all observations following and including the observation in which nestbuilding had begun, excluding any observations in which curving occurred. Individual means were pooled to obtain a group mean.

TABLE 4: LATENCY AND DURATION OF NESTBUILDING

GROUP		PROPORTION OF MALES NESTBUILDING BY OBS. 3	NESTBUILDING LATENCY (range)	NESTBUILDING DURATION (± s.e.)
I	CONTROL	100%	2.0 (2 - 3)	3.88 (.42)
II	SHAM	100	2.0 (1 - 2)	.28 (.31)
III	GDX-1	91	2.0 (2 - 4)	2.45 (.59)
IV	GDX-2	100	2.0 (1 - 3)	1.7 (.51)
V	MTB	87	2.5 (2 - 4)	4.44 (.91)
VI	GDX-1 + MTB	62	3.0 (2 -11)	2.10 (.53)
VII	GDX-2 + MTB	43	4.0 (1 -11)	.65 (.39)
VIII	GDX-1 + MTB + TESTOSTERONE	64	2.0 (1 - 5)	1.68 (.37)
$p <$			.05	.0002

treated castrates, there was evidence of egg-eating by a few males. For one male each among the one-week castrates and the methallibure treated one-week castrates, and for four testosterone treated castrates, eggs were seen to be eaten or missing from the nest and presumed to have been eaten.

Incomplete Spawning Sequences. In four subjects, one each in one-week castrate and methallibure-treated one-week castrate groups and two in methallibure-treated two-week castrate group, curving and clasping was observed but no egg-release. One one-week castrate was observed to invert the female in a clasp one half hour before the lights went out at the end of the day. Only three clasps were observed before the lights went out and no egg release was seen. The other three subjects were actively spawning two to three hours before the end of the day, showing 14 to 18 curves per 10 minutes and successfully clasping the female. All were observed until the lights went out and no egg release was observed. In none was spawning observed to continue the next day and none were counted as spawners.

Continuous observations were made on three spawnings by sham-operates. In all three, spawning was complete with over 500 eggs in the nest from two to three hours after the first clasp was observed. The maximum number of curves seen in any 10 minute period was 9 to 13, and 17 to 19 clasps were seen over an entire spawning.

DISCUSSION

The foregoing results suggest that gonadal androgen stimulates sexual behavior in male Macropodus. Incomplete recovery from traumatic effects of castration might have contributed to the decreased spawning one week following the surgery. However, sexual behavior and fertility of sham-operated controls was unimpaired. In addition, administration of testosterone enanthate, a long-lasting androgen (cf. Dericks-Tan and Tauber, 1975; Mauss, Börsch, Bormacher, Richter, Leyendecker and Noche, 1975) restored spawning in one-week castrates. The recovery of spawning and fertility between one and two weeks following castration paralleled an increase in testicular tissue, suggesting that regeneration had occurred. The testes remnants in two-week castrates were heavier than those recovered in one-week castrates even though the amount of tissue removed at castration in the two groups was the same. Methallibure blocked the recovery of spawning and fertility in castrates as well as testes regeneration. On the basis of these results, it can tentatively be concluded that castration results in a temporary reduction in gonadal androgen which produces a corresponding decrease in the probability of spawning.

The effects of castration and methallibure on prespawning social behavior were minimal. Differences between groups in lateral display, frontal display and approach, though statistically significant were small. It is unlikely that effects of castration on spawning probability

were mediated via changes in social displays.

Persistence of Sexual Behavior. Some males spawned despite extensive removal of testes tissue. Persistence of sexual behavior following castration has been observed in other teleost species (Noble and Kumpf, 1936; Wiebe, 1967) and marked individual variability within species in response to testes removal has been reported in fishes as well as other vertebrates (Beach, 1948, 1958, 1970; Johns and Liley, 1970; van Tienhoven, 1968; Young, 1961; Hart, 1974). Two possible explanations are that testis remnants might produce sufficient androgens to maintain behavior (Johns and Liley, 1970; Liley, 1969) or that compensatory increases in pituitary gonadotropin following castration (van der Hurk and Testerink, 1975; Nagahama, 1973) might directly stimulate sexual behavior (Hoar, 1962b; Baggerman, 1966; Tavolga, 1955). Gonadotropin might act indirectly by augmenting the function of any testicular remnants. The fact that administration of methallibure did not reduce the proportion of spawnings in the one-week castrated males suggests that gonadotropin levels were neither directly nor indirectly involved.

The antigonadotropic effects of methallibure in fishes is well established. Methallibure produced a reduction in gonadal weight, atrophy of steroidogenic cells and blockade of gametogenesis in Carassius and Gasterosteus (Hoar et al., 1967), Cymatogaster (Hoar et al., 1967; Wiebe, 1968), Tilapia (Hyder, 1972; Hyder et al., 1974; Dadzie, 1972, 1973) and

Jenynsia (Calvo and Morriconi, 1972). In Macropodus, peroral administration of methallibure for five weeks reduced testes weight (Davis, Mitchell and Dolson, 1976). Replacement with teleost pituitary extract or mammalian gonadotropin reversed the physiological effects of methallibure in Tilapia and Jenynsia (Calvo and Morriconi, 1972; Dadzie, 1973). In the present experiment, methallibure administration blocked testicular regeneration.

Interrenal tissue in the head kidney might secrete sufficient androgenic hormone to maintain sexual behavior (Liley, 1969; Johns and Liley, 1970; Warren and Aronson, 1956; Hart, 1974). Whether androgenic steroids are secreted by teleost interrenals has apparently not been demonstrated (Colombo, Pesavento and Johnson, 1972; Butler, 1973). In mammals, however, adrenal androgens do not account for the persistence of sexual behavior in castrates (Beach, 1970; Bloch and Davidson, 1968; Warren and Aronson, 1956; Cooper and Aronson, 1958; Schwartz and Beach, 1954).

Blockade of sex behavior might require prolonged deprivation of appropriate hormones, as reported in mammals. Mammals show gradual loss of sexual behavior over periods of weeks to months following castration (Hart, 1968, 1974; Phoenix, Slob and Goy, 1973; Rosenblatt and Aronson, 1958; Resko and Phoenix, 1972). The period of time that was allowed following castration in the present experiment may have been too short to observe a decline in sexual behavior. Testicular regeneration obviously complicates demonstration

of such an effect in the Macropodus.

Non-hormonal factors may interact with androgen stimulation as in mammals (Aronson, 1957; Beach, 1947; Hart, 1974). For example, prior sociosexual experience results in continued sexual behavior following castration in cats (Rosenblatt and Aronson, 1958; Rosenblatt, 1965) but not in rats or dogs (Bloch and Davidson, 1968; Beach, 1970). Such variables need further investigation in paradise fish.

Nestbuilding and Parental Behavior. The onset of nestbuilding was delayed slightly in all methallibure treated groups, however the most striking effect was the decrease in nestbuilding duration which was shown by the methallibur e treated one- and two-week castrates. Testosterone did not increase the proportion of males nestbuilding by the third observation, although it did decrease the median nestbuilding latency of the entire group. The duration of nestbuilding was suppressed in all castrated groups, with or without methallibure treatment. Again, testosterone treatment did not restore nestbuilding duration to control levels. The role of androgens in nestbuilding of other species has been examined. Castrated sticklebacks and sunfish show reduced nestbuilding (Smith, 1969; Smith and Hoar, 1967; Wai and Hoar, 1963; Hoar, 1962b; Baggerman, 1966). In sticklebacks replacement with methyl testosterone reverses the depression in nestbuilding (Wai and Hoar, 1963). In Macropodus Machemer (1971) has proposed that prolactin and androgen act synergistically to induce foamnest building.

The occurrence of egg-eating, as seen in the present

study, is very rare in intact male Macropodus. Egg-eating was seen in the groups which were tested one week following castration: the one-week castrates (group III), methallibure-treated one-week castrates (group VI) and testosterone-treated castrates (group VIII). Only six males were seen to eat their broods. While it is unlikely that undetected spawnings occurred at times other than the first few hours of the light cycle, other males could have spawned and consumed their eggs in the early morning before the first observation. Males who were seen to eat their broods did so immediately or within a couple of hours. It is doubtful that egg-eating was elicited by the increased proportion of infertile eggs in the brood, since eight completely infertile broods were not cannibalized. Perhaps it should not be surprising that parental behavior is disrupted along with sexual behavior. Of all the behaviors performed by Macropodus, parental behavior is one of the most specialized. Consequently it could be highly susceptible to disruption.

The importance of androgens in the control of parental behavior has been reported for Trichogaster and for Gasterosteus. Kramer (1972) obtained parental behavior in androgen-treated female Trichogaster, however castrated male Trichogaster continue parenting fertile eggs (Johns and Liley, 1970). Smith and Hoar (1967) reported that castration of Gasterosteus males following spawning reduced parental fanning, and the castrates were reported to remove (eat?) the eggs from the nest. In my experiment, exogenous

administration of testosterone which increased the probability of spawning in castrates did not prevent egg-eating. Thus both egg eating following spawning and decreased nestbuilding are effects of castration and methallibure treatment which cannot be readily attributed to decreased androgen levels. On the other hand, the dose of testosterone may have been inadequate to completely reverse the effects of castration on parental and nestbuilding behavior.

A number of closely related factors, including changes in sensory or motor functioning of the male, and changes in the attractiveness of the male to the female could be responsible for the reduced probability of spawning in castrates. Sensory changes in the male should have been reflected in the response of the male to the female, but differences in behavior of experimental males toward the female which might relate to reduced spawning were not apparent. Experimental males were seen to approach the female, lead them to the nest, and display at rates comparable to controls. Methallibure produced decreases in approach, even in the intact group which spawned normally. In the one-week castrates, which showed reduced spawning, the frequency of approaches were comparable to the frequency in controls. Lateral display and frontal display were reduced only in the methallibure treated two-week castrates even though spawning was reduced in two other groups as well.

Castration may impair the attractiveness of the male to the female. Incompleted spawning in some pairs suggests

that the male did not stimulate the female adequately. This agrees with the proposal of Johns and Liley (1970) that castrated Trichogaster males which did not spawn "failed to elicit a spawning response in females presented to them".

Some Speculations and Suggestions for Further Research. Based on available data it appears that inhibition of egg-eating in male Macropodus is not androgen dependent, however the present experiment was not designed to examine parental behavior. Detailed observations of parental behavior toward premeasured quantities of eggs (cf. Kramer, 1972) might provide a sensitive measure of both castration and methallibure effects. Variation in the androgen selected or the dose used might yet demonstrate a role for androgens in parental behavior. Machemer's (1971) suggestion of synergism between androgens and other hormones in the regulation of parental behavior needs further evaluation.

Detailed analysis of female behavior and of the sequencing of interactions between males and females could provide further evidence as to male attractiveness following castration and methallibure treatment. Androgen dependent pheromones may exist in Macropodus. Pheromones stimulate nestbuilding in Macropodus (Rossi, 1969; Mainardi and Rossi, 1968) and Davis and Pilotte (1975) demonstrated that both male and female Macropodus are attracted to conspecific stimuli.

Measurement of spawning in terms of the proportion of

males spawning is a gross criterion of castration effects. Hart (1974) has discussed various measures of sexual behavior in mammals and commented that measures such as ejaculatory rate or frequency is a better measure of hormone effects on male sexual behavior than the use of a proportion of males showing a sexual response (cf. Warren and Aronson, 1956). Observation periods were too brief and spaced too far apart to obtain such sensitive measures of sexual activity; continuous observation of a spawning sequence after it begins might be preferable. In the intact male, clasping and subsequent gamete release is preceded by repeated, incomplete spawning bouts in which the male curves around the female but does not invert her. These behavioral sequences culminating in either a curve or a clasp with inversion of the female are functionally equivalent to the mammalian sequences of mounting and intromission. It is possible that rate of clasping, the ratio of curves to clasps, the latency from first clasp to first egg release and the length of the entire spawning sequence from first curve to completion would be more detailed and perhaps sensitive measures of sexual response in the Macropodus.

The response of Macropodus to castration is grossly similar to that of a variety of mammals. Beach (1947) has suggested that in mammalian evolution the central nervous system has become increasingly important in mating behavior and the importance of gonadal hormones to sexual behavior has tended to decrease. In a variety of teleost

species sexual behavior is at least partially independent of hormonal control. Coupled with the observation of persistence of sexual behavior in some castrate males, it appears that partial independence of male sexual behavior from hormonal control is either phylogenetically quite old or has evolved independently in fishes. In fact independence of hormonal control may not follow a steady trend but rather may evolve repeatedly in response to environmental pressure , (cf. Hart, 1974). Likewise, the stimulatory functions of androgens on sexual behavior may depend on environmental pressures toward such a function. Further evaluation of androgen control of behavior of a variety of species and correlation of that data with the ecology of the species involved may provide more substantial evidence relevant to evolution of hormonal control of male sexual behavior.

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