

# Castration and Reproductive Behavior in the Paradise Fish, *Macropodus opercularis* (L.) (Osteichthyes: Belontiidae)

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VILLARS, T. A. AND R. E. DAVIS. *Castration and reproductive behavior in the paradise fish, Macropodus opercularis (L.) Osteichthyes: Belontiidae*. *PHYSIOL. BEHAV.* 19(3) 371-375, 1977. - The effects of castration on male spawning behavior, nestbuilding and fertility were measured in a five day spawning trial with an intact female. Spawning and fertility decreased one week following castration while nestbuilding was unaffected. Sham operated males showed normal spawning and fertility. Two weeks postoperatively, fertility and spawning were increased paralleling partial regeneration of the testes. The antigonadotropin methallibure blocked testes regeneration and recovery of spawning in castrated males but it had no effect on reproductive functions of intact males. Spawning did not decrease in one-week castrated males which were administered testosterone enanthate. The results suggest that gonadal androgen facilitates sexual behavior in *Macropodus*.

Castration    Testosterone    Methallibure    Fish sexual behavior

HORMONAL control of fish reproductive behavior has not been extensively investigated. Gonadal hormones participate in the differentiation and maintenance of secondary sex characteristics [29] but the function of gonadal hormones in the activation and maintenance of sex behavior is controversial. Castration decreased sexual behavior in male *Gasterosteus*, *Salmo*, *Xiphophorus* and *Cymatogaster* [19]. Replacement therapy with methyl testosterone reversed the effects of castration in male *Gasterosteus*. In contrast, castration had no effects on reproductive behavior of the cichlid *Hemichromis* and the anabantid *Betta*, and the sexual activity of male *Bathygobius* was increased. *Trichogaster* showed marked individual differences in response to castration [19,14].

The present study examined the effects of castration and testosterone administration on sexual behavior of the anabantid *Macropodus opercularis*, the paradise fish. Previous studies suggested that *Macropodus* is not dependent on gonadal androgen for the maintenance of sexual behavior. Davis, Mitchell and Dolson [6] demonstrated testicular atrophy in males which received the antigonadotropic agent methallibure for five weeks, but the sexual behavior of the males appeared normal. The males did not receive methallibure during the spawning trial, and testicular functions could have recovered. Other males castrated two weeks prior to a spawning trial and reoperated prior to successive trials continued to show normal sexual behavior and only slightly reduced fertility [27]. Examination four

weeks after the last operation revealed that substantial testicular 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 administration were examined. Finally, the behavioral effects of testosterone replacement were investigated.

## METHOD

Adult paradise fish, *Macropodus opercularis* (Osteichthyes: Belontiidae), 4.2 g mean body weight, were obtained from Gossington Tropical Fishes, Del Ray Beach, FL, or from a local dealer of Florida stock. Fish were kept in 190 liter community tanks for at least seven days before an experiment, and cared for as previously described [4].

## Surgery

Fish were anesthetized with Fiquel (Ayerst), weighed 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 (Fig. 1). The incision was extended dorsally to visualize the distal end of the ipsilateral testes which was then freed from the supporting mesentery and lifted out. The contralateral lobe was similarly removed through the same opening. The testes were weighed and a

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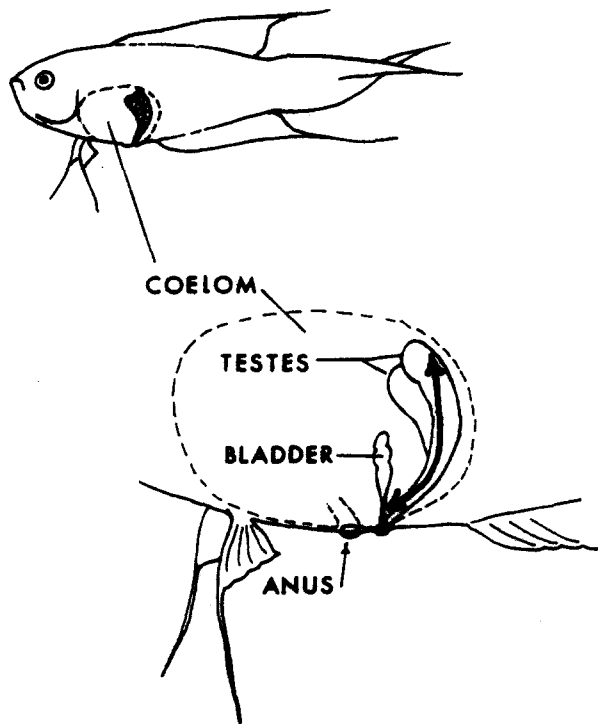


FIG. 1. Illustration of the location of the testes in the coelom and the incision used in the castration procedure.

pretrial gonadosomatic index, G.S.I., was determined as the  $(\text{testes wt/body wt}) \times 100$ . The wound was closed with silk cardiovascular thread (Deknatel). Sutures were removed two or three days later. In the sham operation the tests were manipulated then the wound closed. Between surgery and the trial fish were kept in individual, 9 liter isolation tanks.

#### Drugs

Methallibure (I.C.I., 33,828) was administered perorally, mixed with the standard diet in a concentration of 10 mg methallibure per g dry food [6]. 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 pellets containing approximately 0.3 mg of methallibure. Each fish received one pellet daily in the morning. During the spawning trial the males were fed drugged or control food one hr before the morning observation. The females were fed frozen brine shrimp.

Testosterone enanthate (Schering AG, Berlin), a long-lasting androgen, was injected intraperitoneally in sesame oil with a 100  $\mu\text{l}$  syringe and a 26 ga needle. Fish received 0.5 mg testosterone enanthate in 10  $\mu\text{l}$  of oil.

#### Spawning Trial

Spawning was tested in 38 liter glass aquaria, 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 14 hr of fluorescent illumination directly above the aquaria. The

water was undisturbed by filtration to facilitate foamnest building. Two plastic plants and a brick provided cover.

The day before the spawning trial a ripe female was placed in the tank and fed. The following morning the male was fed and placed in the tank. The pair 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.

The fertility of the male was measured as the proportion of live eggs in a large sample of the brood 24 to 36 hr following spawning. Eggs were removed after spawning appeared to be complete, incubated for 24 to 26 hr and the number of live and dead eggs were counted. At the end of the trial the male was sacrificed and its body weight was measured. The testes or the remaining parts of testes were weighed to determine a posttrial GSI.

#### Behavioral Observations

The behavior of the males was observed during two daily 10-min observation periods. The male was observed in the morning and the late afternoon until spawning took place or, if spawning did not occur, for five days. The pair was additionally checked every two to three hr to determine if nestbuilding, sexual curving or clasping sequences had begun. A curve was noted when the male curved its body around the female's abdomen. A clasp was recorded when the male successfully inverted the female [16,21]. In nestbuilding, the fish gulps air, descends below the surface and releases a stream of cohesive bubbles which accumulate in a foamy mass. The proportion of the males in each group that were seen to begin nestbuilding in the first 24 hr of the trial was recorded.

#### Procedure

Eight groups of males were administered a five day spawning trial (Table 1). The males of Group 1 were placed in individual isolation tanks for 2 days prior to the trial. Group 2 was sham-operated 8 days prior to the spawning trial. Group 3 and 4 were castrated 8 days and 14 days prior to the spawning trial. The males of Groups 5 to 8 were administered 0.3 mg of methallibure daily for eight days prior to and during the spawning trial. In addition to receiving methallibure, Group 5 was unoperated, Groups 6 and 8 were castrated eight days prior to the spawning trial, and Group 7 was castrated 14 days previously. Group 8 also received testosterone five days and one day prior to the spawning trial.

#### RESULTS

The results are presented in Table 1. There were overall significant differences between groups in spawning ( $\chi^2 = 16.3$ ,  $df = 7$ ,  $p < 0.05$ ), fertility,  $F(7,30) = 16.8$ ,  $p < 0.0001$  and nestbuilding ( $\chi^2 = 58$ ,  $df = 7$ ,  $p < 0.001$ ). Posttrial GSI values differed between groups  $F(4,46) = 4.7$ ,  $p < 0.003$  but pretrial GSI values did not  $F(4,35) = 2.10$ ,  $p < 0.10$ . The groups showed no change in body weight during the experiment.

#### Effects of Castration

The effects of castration on spawning one and two weeks postoperatively were evaluated by comparing the experimental groups to the control group using a test for

TABLE 1  
SPAWNING, NESTBUILDING, FERTILITY AND GONADOSOMATIC INDEX DATA

Group	N	Spawned (%)	Nestbuilding within 24 hr (%)	Fertility ( $\bar{x}$ %)	G.S.I. ( $\bar{x}$ )
(1) intact males	12	75	100	90	0.160
(2) sham castrate 1 week	8	75	100	91	—
(3) castrated 1 week	11	45	91	34	0.010
(4) castrated 2 weeks	6	67	100	62	0.038
(5) intact males methallibure 1 week	8	88	87	88	—
(6) castrated 1 week and methallibure	13	38	62	0.3	0.015
(7) castrated 2 weeks and methallibure	7	14	43	0	0.008
(8) castrated 1 week methallibure and testosterone	14	79	64	11	0.012

differences between two proportions [1]. Spawning was decreased at one week (Group 3,  $p < 0.05$ ) but not two weeks following surgery. The sham operation had no effect on reproduction.

Castration resulted in decreased fertility one and two weeks postoperatively ( $p < 0.001$ , Scheffe *t*-test). The post-trial GSI of two-week castrates (Group 4) was four times greater than that of the one-week castrates in Group 3 ( $p < 0.0004$ ). Analysis of differences between the proportions of males nestbuilding within 24 hr showed no effect of castration.

#### Effects of Methallibure and Testosterone

Following administration of methallibure alone (Group 5), the proportion of males which began nestbuilding within 24 hr, the proportion which spawned, and fertility were unimpaired. In castrates, methallibure had time-dependent effects. In males spawned one week following castration (Group 6), methallibure resulted in a significant decrease in fertility ( $p < 0.05$ , Scheffe) and nestbuilding ( $z = 2.52$ ,  $p < 0.01$ ), while spawning probability and the GSI were not decreased compared to undrugged castrates in Group 3. In two week castrates (Group 7), methallibure resulted in decreased spawning probability, in addition to decreased nestbuilding, fertility and GSI compared to the undrugged castrates in Group 4.

Testosterone administered to methallibure-treated, one-week castrates (Group 8) resulted in an increased proportion of males spawning compared to Group 6 ( $p < 0.001$ ). Spawning probability was in fact equivalent to that shown by the control group. Testosterone did not alter fertility, nestbuilding or the GSI.

#### DISCUSSION

The foregoing results suggest that gonadal androgen maintains sexual behavior in male *Macropodus*. Surgical removal of testicular tissue, the most direct procedure for reducing the level of endogenous androgens, results in only a temporary decrease in reproductive behavior and fertility.

While the decreased spawning seen one week following castration might be interpreted as a result of surgical trauma, the males which received the sham castration surgery showed normal spawning and fertility. Two weeks after castration, there is an increase in spawning, fertility and testicular tissue which can be blocked by prior administration of the antigonadotropin methallibure. When castrates receive methallibure and exogenous testosterone, spawning one week postoperatively is increased but fertility and the GSI are not. Since administration of testosterone counteracted the inhibitory effects of methallibure on spawning in castrated males, we infer that androgenic steroids participate in the activation of male sexual behavior in *Macropodus*. Postoperative application of antigonadotropin may make it possible to obtain a more complete blockade of testicular secretions than can be achieved by surgery alone.

The frequency of occurrence of nonsexual social behaviors was recorded during the daily observation periods. Male and female *Macropodus* both perform lateral and frontal displays, approach, bite-butt, and chase responses in bisexual and in unisexual encounters [5]. The mean frequencies of these responses did not vary significantly among the groups of males in this investigation and the data were omitted. Males show a higher frequency of lateral display than females do in a brief trial following a period of social isolation [4]. This sex difference suggests that in certain social situations lateral display may be facilitated by androgenic hormones.

Nestbuilding is sexually dimorphic in *Macropodus*. Males build nests in the spawning tank much more readily than females do. Neither castration nor testosterone replacement affected the latency to nestbuild. This suggests that decreased gonadal androgen secretion was not responsible for the decreased nestbuilding within 24 hr in the methallibure treated castrates. Kramer [17,18] found that both methallibure and the antiandrogen cyproterone acetate suppressed sexual behavior and nestbuilding in sunfish (*Lepomis gibbosus*). Machemer [21] reported that a dose of testosterone similar to that used in our experiment

stimulates nestbuilding in intact male *Macropodus*, and that nestbuilding also depends on increased prolactin secretion. Testosterone influences nestbuilding in other fishes, such as sticklebacks and sunfish [24,25].

Six experimental males ate some or all of their eggs. In our experience, egg eating is rare in intact males. One male each of Groups 3 and 6, and four of the 14 males which received testosterone were seen to eat eggs from the nest during spawning. Egg viability did not seem to be a factor. Egg eaters usually took eggs immediately after spawning, and males which did not eat eggs were seen to guard nests of dead eggs. The implications of this unusual behavior are unclear.

Some males spawned even though the postmortem examination revealed no testicular tissue. Persistence of sexual behavior following castration has previously been observed in fishes [19] and is common in mammals [11]. It should be pointed out that *Macropodus* testes fragment or tear easily during surgery, and that minute remnants could escape detection. Such remnants may produce sufficient androgens to maintain sexual behavior, as Johns and Liley [14] proposed in their experiment in *Trichogaster*. Increased pituitary gonadotropin is reported to occur in castrated fish [26] and it might directly stimulate sexual behavior (cf. [19]). In our experiment, administration of a dose of methallibure which blocked testicular regeneration, and thus presumably suppressed gonadotropin release, did not prevent some castrates of Group 6 from spawning one week postoperatively. This suggests that the persistence of spawning was not owed to gonadotropin stimulation.

The antigonadotropic action of methallibure in fishes is well established. Prolonged administration of methallibure variously produced a reduction in gonad weight, atrophy of steroidogenic cells, and blocked gametogenesis in *Carassius*, *Gasterosteus*, *Cymatogaster*, [12,28] *Tilapia* [13], *Jenynsia* [2], and *Macropodus* [6]. In unilaterally ovariectomized

*Tilapia*, postoperative administration of methallibure blocked the compensatory hypertrophy of the remaining ovary in two weeks [3]. Castrates may be more sensitive to methallibure than intact fish. Intact male and female *Macropodus* show no change in GSI or gametogenesis when methallibure is applied for less than two weeks [6]. In addition, the present experiment shows that a brief treatment with a dose of methallibure which does not noticeably effect reproductive functions in intact males, can result in decreased spawning, fertility, and GSI in castrated males.

The neural pathways by which sex steroids control reproductive behavior in fish have not been extensively investigated. Brain cells which concentrate estradiol and testosterone are restricted to areas of the ventral forebrain in *Macropodus* males [8]. The principal areas are the ventral telencephalon, the adjacent, rostral portion of the preoptic region, the posterior and inferior portions of the lateral tuberal nucleus, and the nucleus of the lateral recess proximal to ventricle III. Some of these brain areas are implicated in gonad function and sexual behavior by neural intervention experiments. Lesions selectively placed in the posterior portion of the lateral tuberal nucleus of *Carassius* result in gonad atrophy [22]. Electrical stimulation of the preoptic area of sunfish, *Lepomis*, evokes components of reproductive behavior including sperm release [9,10] and preoptic lesions in killifish, *Fundulus*, interfere with elicitation of the spawning reflex [20]. In *Macropodus*, bilateral ablation of the telencephalon obliterates sexual and parental behavior in most males [7, 15, 23]. The ablation, which includes removal of varying portions of the rostral preoptic region, has little, if any, effect on nonsexual social behavior, feeding, and activity [16]. The effects on reproductive behavior and gonad function of lesions restricted to individual sex steroid concentrating areas of the *Macropodus* brain are being investigated.

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