INDUCED SEX INVERSION, MATURATION, SPAWNING AND EMBRYOGENY OF THE PROTOGYNOUS GROUPER, *Mycteroperca microlepis*

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INDUCED SEX INVERSION, MATURATION, SPAWNING
AND EMBRYOGENY OF THE PROTOGYNOUS GROPER,

Mycteroperca microlepis

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ABSTRACT

Female gag (Mycteroperca microlepis) were collected from the Eastern Gulf of Mexico, maintained in closed systems and spawned. Sex inversion of this protogynous grouper was induced with oral application of methyltestosterone. Ovotestes of transitional males were described. Photoperiod, temperature, and hormone application were utilized to induce maturation and ovulation of females. Approximately 26,000 eggs were produced in 2 spawns. Fertilization rate was 50%. Larvae hatched after 45 hours at 21.0°C. Embryonic and early larval stages were described.

INTRODUCTION

The gag (Mycteroperca microlepis) is a valuable food fish sought by commercial and sport fishermen along the Gulf of Mexico and Southeast Atlantic Coasts. Gags range in distribution from Massachusetts to Rio de Janeiro (Briggs 1958; Smith 1971). Centers of abundance are reef communities along the Gulf Coast of Florida (McErlean 1963) and along the coast of the southeastern United States (Manooch and Haimovici 1978). Gag are protogynous hermaphrodites (McErlean 1963). Protogynous Serranidae ovotestes are not segregated into ovarian and testicular segments but transit "in toto" (Smith 1965). Characteristically, sexes in protogynous hermaphrodites are functionally (temporally) distinct; small individuals are females and recently transformed males have degenerating advanced and/or immature oocytes in the gonads (McErlean and Smith 1964). Age and growth data for transitional gag in the Gulf of Mexico suggest that transition occurs around 5 years (or 750 mm SL) (Schlieder, unpublished MS). The significance of this pattern of reproduction for a study concerned with artificial breeding of captured specimens is that occurrence of male fish in collectable samples is less frequent than that of females.

In fact, males of this species are scarce and, when found, are in such deep water that decompression trauma renders them moribund before they are useful. Females, conversely, are abundant in shallower waters (12-15 m) and can be collected without significant mortality. Since
this species has inherent capacity for sex reversal, it was presumably feasible to utilize hormone-induced sexual transition to provide males for a breeding study. Furthermore, female Epinephelus tauvina, Malaysian grouper, were artificially sex reversed with methyltestosterone and spawned with females induced to ovulate by application of Human Chorionic Gonadotropin (HCG) and salmon-snapper pituitary extract (Chen et al. 1977; Chen 1979).

The purpose of this paper is to report preliminary results of experiments to maintain, artificially sex reverse, and spawn captive brood stock of gag using photoperiod, temperature, and hormone application as stimuli, and to rear eggs and larvae.

METHODS AND MATERIALS

Females were collected with hook-and-line, offshore Egmont Key, Florida, in 12-15 m water depth and transported to the laboratory in two 1,000 liter recirculating live-well tanks. Experimental regimes consisted of controlled photoperiods and temperatures in 16,000 liter circular tanks. Holding facilities, water quality management, filter designs, handling and sampling techniques follow Roberts et al. (1978a). Ovotestis biopsies were made periodically using catheterization and surgical procedures. Fish were anesthetized with Quinaldine (20 mg/l seawater), biopsied, examined, measured in a tagging cradle, and returned to respective tanks.

Eight fish (3.1 kg mean weight; 537.5 mm mean SL) were randomly assigned to one test tank and one control tank in Experiment I. Testosterone (17a methyltestosterone) was administered to four fish orally at a dosage of approximately 1.0 mg per kg body weight (b.w.) per day for 150 days. The androgen was injected into daily food rations of squid along with a vitamin supplement. Subsequent experiments to condition and spawn gag brood stock over a 10 month period involved changes in photoperiod and temperature regimes. The final regime combined short photoperiod (10 hours light) with warm initial temperatures (28-30°C) followed by decreased temperature (21-23°C).

Vitellogenic stages of oocytes follow Roberts et al. (1978a) and Schlieder (unpublished MS). Ovotestis biopsies were made approximately every 30 days for 150 days in Experiment I and periodically in subsequent experiments. Immediately after biopsy, samples were fixed in Davidson's solution, dehydrated, infiltrated with paraffin, sectioned at 4-7 μm and stained with either Harris' hematoxylin and eosin or Gomoris' trichrome. For transmission electron microscopy, biopsies were fixed for 2 days in 3% glutaraldehyde and 3% paraformaldehyde fixative made with a 0.2 M sodium cacodylate stock buffer adjusted to pH 7.6 and a final osmolality of 353 milliosmoles. After four 75% strength buffer rinses for 10 minutes each, tissue was postfixed for one hour in buffered 1% OsO4 at 232 milliosmoles. Tissue was rinsed in 50, 25 and 12.5% stock buffer and distilled water 10 minutes each, dehydrated in a series of 30, 50, 70, 95 and 100% ethanol, and embedded in epoxy resin (Spurr 1969). Sections were prepared with a Sorvall MT-2B ultramicrotome and a diamond knife. They were collected on coated slot grids, stained with uranyl acetate and lead citrate, and micrographed with an Hitachi HS-9 electron microscope.

Ovulation of females was induced with HCG after biopsies indicated
females had oocytes in the tertiary yolk stage. Fish were strip-spawned (Roberts et al. 1976). Fertilized eggs were incubated and larvae reared (Roberts et al. 1976, 1978b).

RESULTS AND DISCUSSION

SEX INVERSION/MATURATION

In all sex transition/maturation experiments, gag remained generally fit. Occasional monogenetic trematode infestations of Benedina sp. occurred on eyes, gills, and integument of test fish and controls. These were successfully treated with Dylox (Dimethyl 1 222-Trichloro-Androethyl I Phosphonate) at 1.0 mg per liter. Water quality parameters (pH, NO$_3$-N, NO$_2$-N, NH$_4$-N, O$_2$) remained within tolerable limits throughout the experiment. Fish were docile in confined conditions, easily handled, and never suffered prolonged handling stress.

Effects of 17a methyltestosterone on ovotestes of female gag in Experiment I are presented in Figure 1. Histograms represent oocyte stage frequency distributions for 4 females in the control group and 4 females in the treated group for the initial and 3 subsequent sampling periods. All 4 females in both groups demonstrated oocytes in stages 1 and 2 of development at the initial sampling period. Control females continued to demonstrate early oocytes for 90 days and at the end of 150 days one female in the control group had oocytes in the tertiary yolk stage, indicating advanced vitellogenesis. No fish in the treatment group demonstrated true vitellogenesis. The testosterone treated females, however, had occluded oviducts after 30 days. This condition existed for the duration for all 4 treated females.

After 90 days, these fish began to display iridescent coloration. A blue-black sheen appeared along the dorsal epaxial musculature. Similar color patterns developed around the anal, pectoral and pelvic fins. After 150 days, a section of the anterior left lobe of the ovotestis of one experimental fish was surgically biopsied and found to be late transitional/early male, based on structure of the gonad and meiotic stages of spermatogenesis.

Morphology and cytology of the transitional ovotestis was presented (Roberts 1980, abstract; Grier et al. 1982, abstract; Grier et al., in preparation; Roberts et al., in preparation). The male phase of the ovotestis is generally characterized as "Epinephelus type" (Smith 1965), "unrestricted spermatogonial testis type" (Grier et al. 1981), or "lobule" type (Billard et al. 1982). However, there are distinct differences currently being detailed. In gross appearance, the ovotestis appeared masculinized, angular, and atrophied. Histologically, connective tissue of the tunica albuginea was continuous through the germinal epithelium, partitioning the lobe (Fig. 2). Atretic and perinucleolus oocytes were surrounded by crypts of spermatogenically active tissue. Lamellar organization was still evident, but appeared regressive. The whole organ, especially connective tissue, was heavily vascularized. Wildstock males near the same stage of transition demonstrated large areas of connective tissue in the medullary portion of the ovotestis, but not to the extent depicted here. That the ovotestis was masculinized and not juvenilized was substantiated by testicular organization of spermatogonia (gonial cells found in cysts formed by Sertoli cells and their cytoplasmic processes), size of spermatocytes during identifiable
stages of meiotic prophase, size of spermatocytes at the time of nucleolar dispersal and appearance of nuage in the cytoplasm. All stages of spermatogenesis could be found in the ovotestis, primary spermatocytes being most abundant (Fig. 3).

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Figure 1. Effects of oral application of 17a methyltestosterone on ovaries of female gag.
Figure 2. Biopsied ovotestis from methyltestosterone treated gag. A) ovotestis transection (35X). B) adjacent seminiferous tubules and degenerating oocytes (400X). C) electronmicrograph of two adjacent seminiferous tubules (640X). Germ tissue (G), connective tissue (C), oocyte (O), seminiferous tubules (ST), Sertoli cell (SE), Sertoli cell process (P), zygotene spermatocytes (SPT), capillary (CP), boundary cell (BC).
Figure 3. Meiotic stages of spermatogenesis. A) spermatogonia (9200X). B) leptotene (6400X). C) zygotene (6400X). D) pachytene (6400X). E) spermatozoids. Spermatogonia (SG), basal lamina (BL), nucleus (N), nuage (NG), Sertoli cell (SE), synaptone- mal complex (SX), centriole ( ).
Following the regime of short photoperiod and decreasing temperatures, males produced hydrated spermatozoa and females had oocytes in the tertiary yolk stage. Ovulation was induced in one female with intra-muscular injections of HCG. Dosage regime was 0.600 IU per g b.w. followed by 0.270 IU per g b.w. 24 hours later. Approximately 6,000 eggs were released naturally in the spawning tank 48 hours after the first injection. None were fertilized. An additional 12,000 eggs were strip-spawned from the same female and fertilized with milt stripped from two ripe sex-reversed males. Fertilization was approximately 50%. A second spawning attempt produced 8,000 eggs with 50% fertilization. Hatch rate was low and few of the resulting larvae survived past yolk absorption.

Embryonic development from the first rearing is presented in Figures 4, 5 and 6. The first blastomeres appeared 1.5 hours after fertilization. Hatching began about 44.5 hours later. Embryonic survival was 20% to neural streak and 4% to hatch. Viable spawned eggs were pelagic, had unsegmented yolk (telolecithal meroblastic cleavage) and were spherical (diameter $\bar{x} = 920 \mu m$). A single oil globule was isolated at the vegetal pole with diameter approximately 24% the diameter of the egg. The perivitelline space was narrow but increased as blastulation progressed. By 8 hours, a well defined blastodisc was apparent in most specimens. From 10 hours to 24 hours gastrulation progressed from formation of the embryonic shield to an embryo with 20 somites and sigmoid heart. Multiple oil globules were observed in the ventral yolk mass in some eggs. This condition may be the result of premature ovulation, i.e., ovulation before coalescence of lipid yolk. This is not necessarily a result of HCG induced ovulation, as this phenomenon has been observed in naturally spawned red drum (Sciaenops ocellatus) eggs; in red drum, normal embryogeny proceeded and multiple oil globules coalesced to a single oil globule by hatching. This coalescing of oil globules was not observed in gag.

Newly hatched larvae were small ($\bar{x} = 2.05 \text{ mm TL}$). The yolk sac extended well anterior to the tip of the snout in live specimens (Fig. 5), but was noticeably less extended in formalin fixed specimens. A single oil droplet was prominent in the ventral anterior yolk sac. The primitive gut was incomplete. Twenty-one myomeres (or partial myomeres) could be distinguished at hatching, 8 of them pre-anal and 13 post-anal. Contracted chromatophores were located posterior to the optic vesicle (1), anterior to the first myomere along the base of the dorsal portions of the continuous finfold and on myomeres 1, 3, 5, 6, 8, 9, 11, 14, 15, 18, 19, 20, and posterior to distinguishable myomeres. Ventral contracted chromatophores were observed on myomere 18, posterior margin of the yolk sac, and around the anterior portion of the oil droplet.

Larvae demonstrated typical pelagic behavior in rearing tanks. Vigorous swimming was followed by periods of drifting in the water column. Swimming periods increased in duration with age.

Preliminary rearing trials with gag larvae while unsuccessful do not indicate problems that cannot be solved after appropriate metabolic, physiological and trophic parameters have been profiled by ongoing experimentation. Furthermore, successful rearing technology should allow a comprehensive larval description of this species.
Figure 4. Pre-embryonic shield blastodisc (9 hours).

Figure 5. Pre-hatch embryo (40 hours).
Gamete maturation in gag is a response to photoperiod and temperature but to date the relationship of these parameters to the process is unclear. Constant cool water temperatures inhibit feeding which in turn has a negative effect on maturation.

Detailed cytology and morphology of the gag ovotestis is currently being summarized in separate manuscripts. Further experimentation to determine dosage and hormone effects on sex inversion in this and other serranid species is ongoing. Chen (1979) reported sex inversion in young androgen treated *F. taurina* with subsequent reversal after cessation of methyltestosterone treatment. In fact, these fish spawned as secondarily reversed females. Recent observations of gag indicate they may undergo secondary sex reversal after androgen treatment is terminated.

Gag have a rapid growth rate in nature and maintain a consistently high market value. The food quality of the fish is excellent. A quality product coupled with apparent potential hatchery suitability makes the gag an interesting mariculture species worthy of more extensive research.

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LITERATURE CITED


