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INDUCED SEX INVERSION, MATURATION, SPAWNING
AND EMBRYOGENY OF THE PROTOGYNOUS GROUPER,
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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 methyl-testosterone. Ovotestes of transitional males were described. Photoperiod, temperature, and hormome 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for transitional gag in the Gulf of Mexico suggest that transition occurs around 5 years [or 750 mm SL] (Schlieder, unpublished MS).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 cacadylate 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-Trichlors-Androrethyl I Phosphanate) 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 nucleo-
lar dispersal and appearance of nuage in the cytoplasm. All stages of
spermatogenesis could be found in the ovotestis, primary spermatocytes
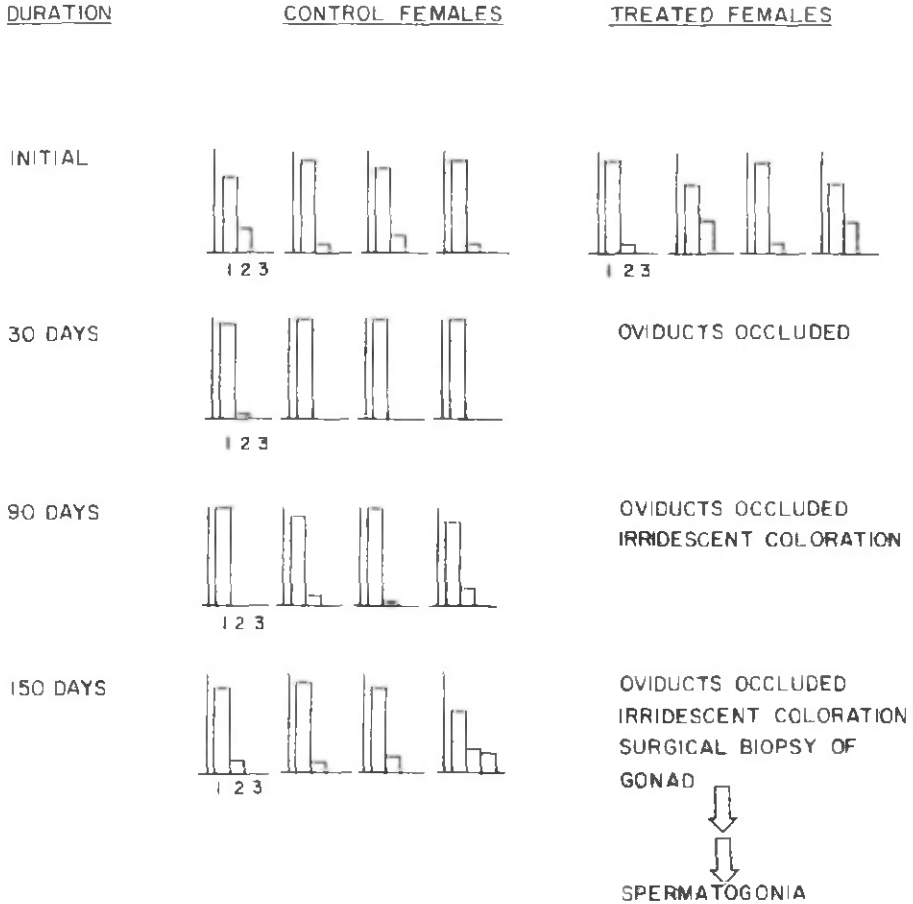being most abundant (Fig. 3).

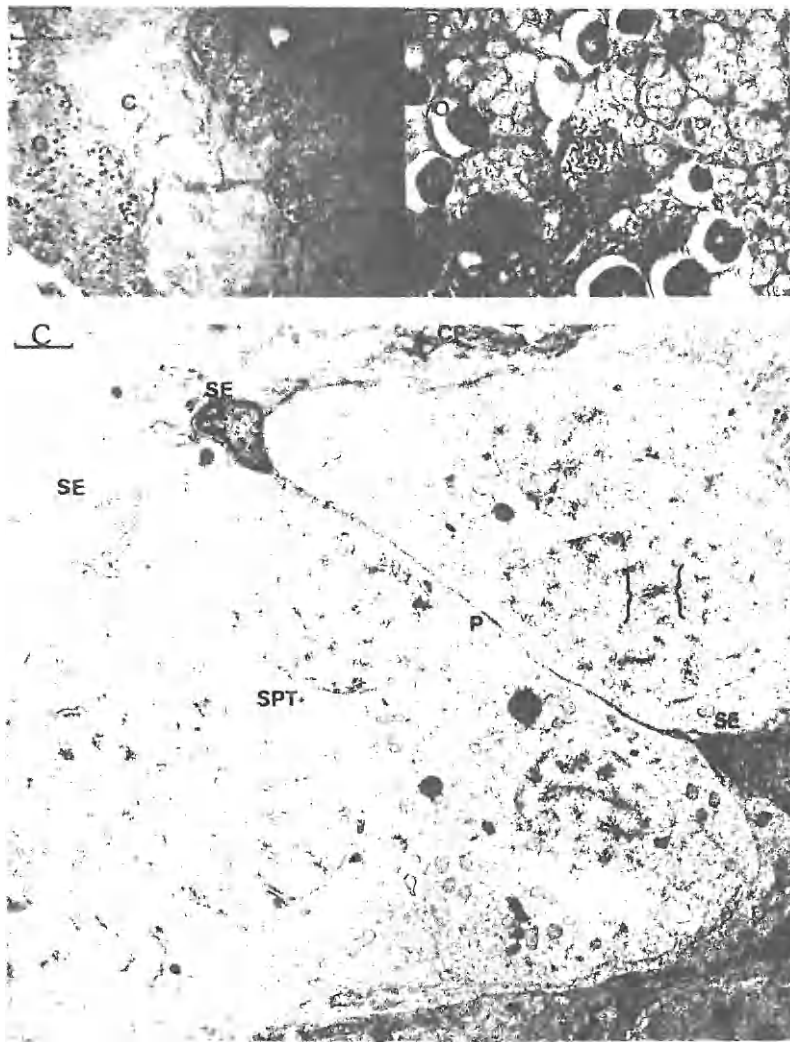Figure 1. Effects of oral application of 17a methyltestosterone on
ovaries of female gag.

Figure 2. Biopsied ovotestis from methyltestosterone treated gag.
A) ovotestis transection (35X). B) adjacent seminiferous
tubules and degenerating oocytes (400X). C) electronmicro-
graph of two adjacent seminiferous tubules (640X). Germ tis-
sue (G), connective tissue (C), oocyte (O), seminiferous tu-
bules (ST), Sertoli cell (SE), Sertoli cell process (P), zygo-
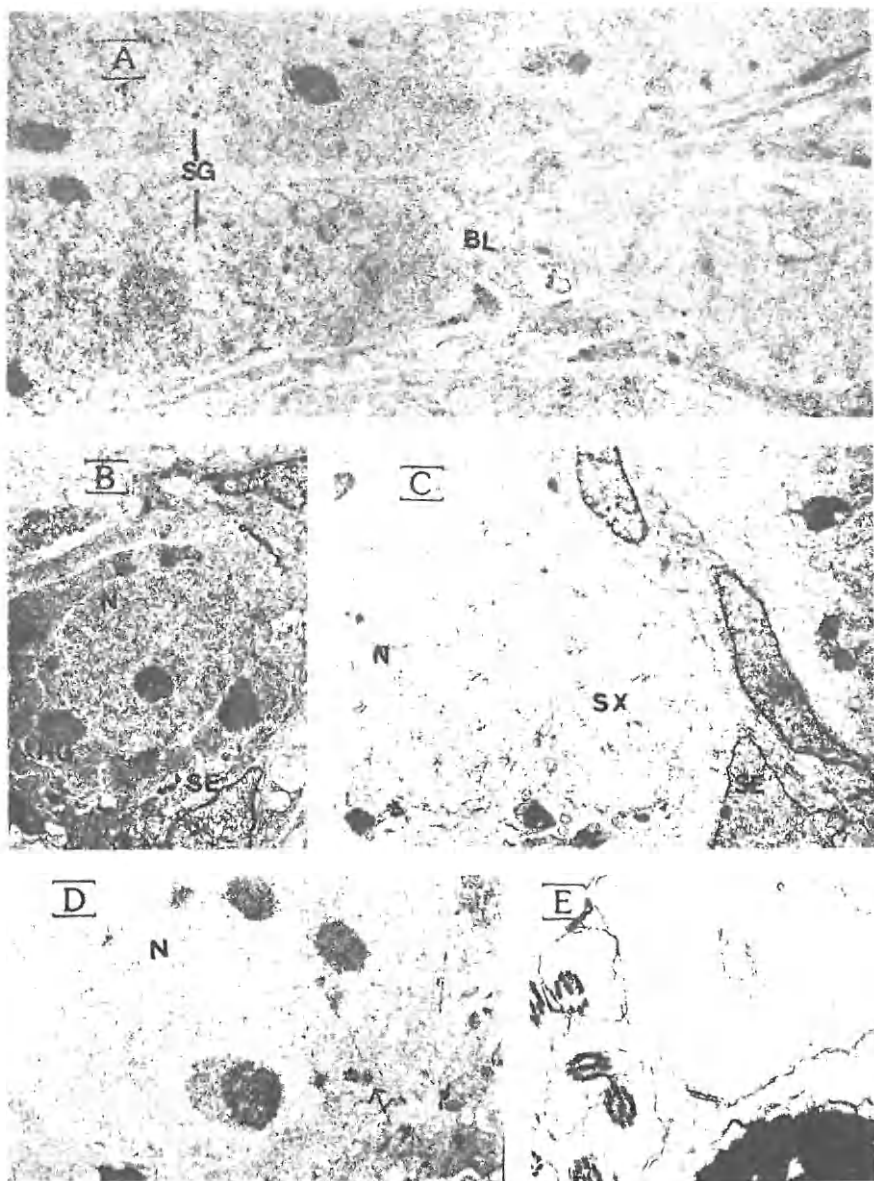tene spermatocytes (SPT), capillary (CP), boundary cell (BC).

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 ( ).

SPAWNING

Following the regime of short photoperiod and decreasing tempera-
tures, 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. fol-
lowed 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 un-
segmented yolk (telolecithal meroblastic cleavage) and were spherical
(diameter $\bar{x}$ = 920 μm). A single oil globule was isolated at the vegetal
pole with diameter approximately 24% the diameter of the egg. The peri-
vitelline 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 em-
bryonic 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 be-
fore 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 embry-
ogeny proceeded and multiple oil globules coalesced to a single oil glob-
ule by hatching. This coalescing of oil globules was not observed in
gag.

Newly hatched larvae were small ($\bar{x}$ = 2.05 mm TL). The yolk sac ex-
tended 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 primi-
tive 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 con-
tracted 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. Vi-
gorous 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 ex-
perimentation. Furthermore, successful rearing technology should allow
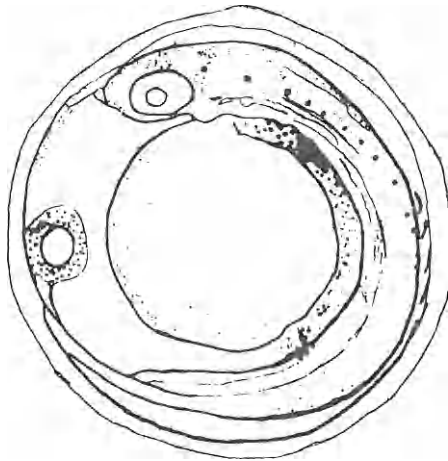a comprehensive larval description of this species.

Blastula 8 hrs.
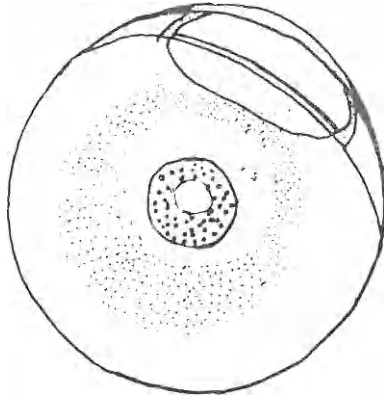
━━ 920 UM ━━━

Figure 4.   Pre-embryonic shield blastodisc (9 hours).

Pre-hatch 40 hrs.

━━ 900 um ━━━

Figure 5.   Pre-hatch embryo (40 hours).

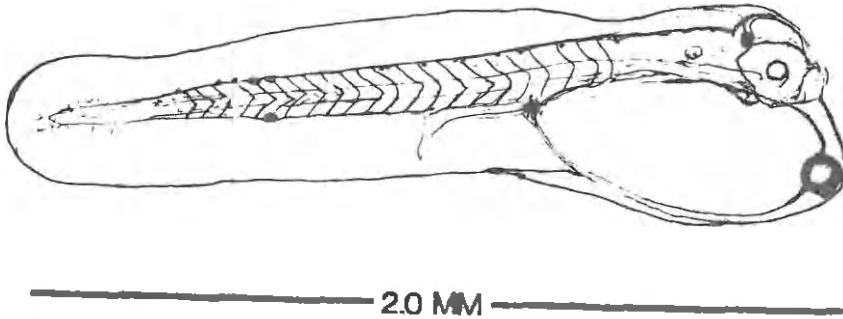## M. microlepis 4 hr. larva

◄────── 2.0 MM ──────

Figure 6. Newly hatched prolarva (46 hours).

Gamete maturation in gag is a response to photoperiod and tempera-
ture 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 *E. tauvina* with subsequent reversal after cessa-
tion 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 termi-
nated.

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.

morphology and for his sincere interest in this project.

LITERATURE CITED

Billard, R., A. Fostier, C. Weil, and B. Breton. 1982. Endocrine control of spermatogenesis in teleost fish. Canadian Journal of Fisheries and Aquatic Sciences 39:65-79.

Briggs, J. 1958. A list of Florida fishes and their distribution. Bulletin of Florida State Museum 2(8):223-318.

Chen, F. Y., M. Chow, T. M. Chao, and R. Lim. 1977. Artificial spawning and larval rearing of the grouper, *Epinephelus tauvina* (Foskal) in Singapore. Singapore Journal of Primary Industries 5(1):1-21.

Chen, F. Y. 1979. Progress and problems of netcage culture of grouper (*Epinephelus tauvina* F.) in Singapore. Proceedings World Mariculture Society 10:260-271.

Grier, H. J. 1981. Cellular organization of the testis and spermatogenesis in fishes. American Zoologist 21:345-357.

Huntsman, G. R., and R. L. Dixon. 1976. Recreational catches of four species of groupers in the Carolina headboat fishery. Proceedings Annual Conference Southeastern Association of Game and Fish Commissioners 29:185-194.

Manooch, C. S. III, and M. Haimovici. 1978. Age and growth of the gag, *Mycteroperca microlepis*, and size-age composition of the recreational catch of the southeastern United States. Transactions of the American Fisheries Society 107(2):234-240.

McErlean, A. J. 1963. A study of the age and growth of the gag, *Mycteroperca microlepis* Goode and Bean (Pisces:Serranidae) on the west coast of Florida. Florida State Board of Conservation Technical Series #41. 29 pp.

McErlean, A. J., and C. L. Smith. 1964. The age of sexual succession in the protogynous hermaphrodite *Mycteroperca microlepis*. Transactions of the American Fisheries Society 93(3):301, 302.

Roberts, D. E., Jr., B. V. Harpster, W. K. Havens, and K. R. Halscott. 1976. Facilities and methodology for culture of the southern sea bass, *Centropristis melana*. Proceedings World Mariculture Society 7:163-198.

Roberts, D. E., Jr., B. V. Harpster, and G. E. Henderson. 1978a. Conditioning and induced spawning of the red drum (*Sciaenops ocellata*) under varied conditions of photoperiod and temperature. Proceedings World Mariculture Society 9:311-332.

Roberts, D. E., Jr., L. A. Morey III, G. E. Henderson, and K. R. Halscott. 1978b. The effects of delayed feeding, stocking density, and food density on survival, growth, and production of larval red drum (*Sciaenops ocellata*). Proceedings World Mariculture Society 9:333-343.

Smith, C. L. 1965. The patterns of sexuality and the classification of Serranid fishes. American Museum Movitates No. 2207. 20 pp.

Smith, C. L. 1971. Secondary gonochorism in the serranid genus Liopropoma. Copeia (2):316-319.

Spurr, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructure Research 26:31-43.