Spontaneous Spawning of Cobia, *Rachycentron canadum*, Induced by Human Chorionic Gonadotropin (HCG), with Comments on Fertilization, Hatching and Larval Development

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ABSTRACT

Two mature female cobia, *Rachycentron canadum*, injected with a single dose of human chorionic gonadotrophin (HCG) at 275 IU/ kg of body weight, and one non-injected ripe male spawned spontaneously in captivity. Oocytes aspirated from each female prior to injection were maturing vitellogenic oocytes nearing the final oocyte maturation (FOM) stage and averaged 625 μ in diameter. Both females spontaneously spawned ~ 42 hours post-injection; spawned oocytes ranged 1.1 - 1.3 μ in diameter. Fertilized eggs hatched ~26 hours later. Estimates for number of eggs spawned (both females combined) and hatched were 3.2 million and 320,000, respectively. Aspects of embryogenesis and larval growth/development were observed. Critical survival period for larvae was day 3 at which time termination of yolk sac absorption occurred and first feeding commenced. Enriched rotifers, wild zooplankton, and artificial food were offered larvae during larval rearing treatments. Larvae contained in a black tank and fed a high density diet of enriched rotifers exhibited highest survival and were reared through day 13, post-hatch. The study documents the spontaneous spawning of wild-caught male and female *R. canadum* from the Gulf of Mexico, and provides comments on fertilization, hatching and larval development. Results of the study strongly suggest that *R. canadum* exhibits potential as an aquaculture species.

KEY WORDS: *Rachycentron canadum*, HCG injection, spontaneous spawning

INTRODUCTION

Cobia, *Rachycentron canadum*, are large, migratory, coastal pelagic fish of the monotypic family Rachycentridae and are distributed worldwide in tropical and subtropical seas, except for the eastern Pacific (Shaffer and Nakamura 1989). In the western Atlantic Ocean, cobia occur from Massachusetts and Bermuda to Argentina, including the Caribbean Sea, but are most common along the U. S. south Atlantic coast and in the northern Gulf of Mexico (Shaffer and Nakamura

1989) where the species supports valuable fisheries and is highly sought for its excellent flesh.

Life history information on cobia from the Gulf of Mexico (Gulf) and U. S. Atlantic coast includes: occurrence, distribution and description of early life stages (Dawson, 1971; Ditty and Shaw, 1992); age and growth (Joseph et al. 1964; Richards 1967 1977, Thompson et al. 1991, Smith 1995, Franks et al. 1999); reproductive biology (Thompson et al. 1991, Biesiot et al. 1994; Lotz et al. 1996, Brown-Peterson et al. in review); feeding habits (Knapp 1951, Franks et al., 1996; Meyer and Franks, 1996); seasonal migratory patterns (Franks et al. 1991, Biesiot et al. 1994); and population genetics (Hrincevich 1993). Life history information on cobia from the Caribbean Sea is scant.

Lotz et al. (1996) and Brown-Peterson et al. (in review) observed that females caught in spring and summer in the northern Gulf had ovaries in the final oocyte maturation (FOM) stage, as well as oocytes in less developed stages, indicating that cobia are batch (indeterminate) spawners. Cobia spawning season in the northern Gulf extends from April through September, and highest gonosomatic index (GSI) values for males and females occur during April and May (Lotz et al. 1996, Brown-Peterson et al. in review).

Studies on aquaculture-related aspects of cobia are few. Ryder (1889) collected fertilized cobia eggs from U.S. east coast waters and described the development of eggs and larvae. Hassler and Rainville (1975) collected fertilized cobia eggs from the Gulf Stream off North Carolina, successfully hatched most of them and reared larvae through juvenile stage. Biesiot et al. (1994) successfully stripped wild-caught female Gulf cobia which had been injected with human chorionic gonadotropin (HCG) and also induced spontaneous spawning in a female cobia using HCG injections but were unsuccessful in attempts to fertilize eggs with cryopreserved sperm. Liao et al. (1995) reported that *R. canadum* is a promising aquaculture species in Taiwan.

Human chorionic gonadotrophin has been used successfully to accelerate oocyte maturation and induce ovulation and spawning in many marine fish species, as reviewed by Lam (1982) and Donaldson and Hunter (1983). To the best of our knowledge, the present study represents the first published account of the spontaneous spawning (not stripped) of wild-caught, captive male and female *R. canadum* from the Gulf of Mexico using HCG to induce ovulation and release of eggs, with observations on subsequent fertilization, hatching and larval development.

MATERIALS AND METHODS

Two female cobia (9.0 and 16.0 kg TW) were opportunistically caught by hook and line gear in the northern Gulf of Mexico from Mississippi coastal

waters on 3 June 1996 and transported to the University of Southern Mississippi, Institute of Marine Sciences' Aquaculture Facility located at the Gulf Coast Research Laboratory, Ocean Springs, Mississippi, USA. Since cobia do not exhibit sexual dimorphism, laboratory examination was required to determine their sex and gonadal condition. Intra-gonadal samples gently aspirated from each fish using a small polyethylene cannula (3 mm diameter) revealed large, maturing vitellogenic oocytes nearing the FOM stage. Fish were held in a large recirculating seawater tank, with continuous aeration.

The following morning (4 June 1996) at 1030 h, approx. 20 hours after capture, each female was injected intramuscularly with HCG dissolved in distilled water at a dosage of 275 IU/kg body weight (BW). The amount of HCG used was based on the recommended dosage for induction of ovulation in striped bass (Harrell et al. 1990) and cobia (Biesiot et al. 1994). Injections were administered posterior to the base of the left ventral fin using a disposable syringe and an18G hypodermic needle. Both fish were minimally resistant to handling during the injection procedure. Fish were transferred to a circular fiberglass tank (3.7 m diameter x 1.2 m height, 11,350 L capacity) which was a component of an indoor, recirculating seawater system described by Ogle (1992). A male cobia (9.0 kg TW) previously captured on 2 May 1996 was available for the spawning attempt and was placed in the tank with the females. Prior examination of milt from the male revealed active sperm. The male cobia was not injected.

Water in the spawning tank was continuously aerated and filtered, and temperature and salinity were maintained at 23.0°C and 28.0 ‰., respectively. Fish were held under a 12 hour light-dark photo period and were not fed.

Spontaneous spawning occurred two days later (6 June 1996). Floating eggs were collected by an egg collector (180 μm mesh) described by Ogle (1992). Total numbers of spawned eggs were estimated volumetrically. Fertilized eggs were placed in 120 L hatching tanks and maintained at 23.0°C and 28.0‰. The number of larvae in hatching tanks was estimated volumetrically. The ratio of the number of larvae to total number of eggs incubated multiplied by 100 was taken as an estimate of hatching rate.

Surviving yolk sac larvae were stocked into circular, fiberglass tanks: two black tanks (0.6 m diameter x 0.4 m height, 120 L) and three light blue tanks (1.13 m diameter x 0.4 m height, 410 L) for larval rearing experimentation. The water was gently aerated and periodically cleaned of dead larvae and debris. Water temperature and salinity were maintained at 23.0°C and 28.0‰, respectively, in all tanks. No significant differences in general water quality were observed between the experimental groups. At day three, larvae were offered several types of food at varying densities. Experimental diets included rotifers (*Brachionus plicatilis*, 130 - 340 μm) enriched with protein Selco (Inve,

Animal Health, Gent, Belgium), wild zooplankton, and a commercially available "artificial plankton" (AP-R, Ocean Star International). The rotifers were cultured on-site using the diatom *Chaetocerous gracilis*, and wild zooplankton was collected from nearby coastal waters by plankton net (335 μm mesh). Larvae were fed two times a day (morning and afternoon). Aeration kept the larvae and food in suspension during larval rearing treatments. The generalized details of the larval rearing treatments (five), including specific diet and pertinent observations, are presented in the results section of this paper.

Ten larvae from one of the tanks (Treatment 1) were arbitrarily chosen every day over the duration of the study and preserved in 95% ethanol. Total length (TL, mm) measurements of these larvae were made using a dissecting microscope with an ocular micrometer. Unless otherwise noted, reference to "day" throughout this paper refers to the number of days post-hatch (including hatch date).

All research activities conducted during this study were conducted in clarified, natural estuarine water (16.0‰) adjusted to a salinity of 28.0‰ with artificial sea salt (Marine Environment).

RESULTS

Ovarian samples aspirated from females prior to injection with HCG contained large, maturing vitellogenic oocytes that were spherical in shape. Twenty of the large oocytes from each fish averaged 625 μ in diameter. On the morning following the day that all fish were placed in the spawning tank, the male displayed what was interpreted as courtship behavior, included constant circling the females and swimming with its snout at their vent. We observed slight redness around the vent of the females, as previously reported by Biesiot et al. (1996) for females in a near-spawning state.

Ovulation and Spawning

A single administration of HCG 275 IU/kg BW was effective in producing hydration and the spontaneous shedding (not stripped) of eggs. Spawning commenced approximately 42 hours (0430 h, 6 June 1996) after injection with HCG. We presume that both females released eggs simultaneously. Based on the numbers of eggs collected, we estimated 3.2 million eggs were spawned (eggs from females combined). We were unable to determine the number of eggs spawned per female and the percentage of eggs that were viable, hydrated oocytes. The mean diameter of spawned ooctyes was 1.2 mm. The male spontaneously released sperm, simultaneous with the release of eggs by the females.

Incubation and Hatching

Fertilization rate was not determined but was presumed to be moderately high. At 12 hours post-spawn, eggs ranged 1.3 - 1.5 in diameter (mean = 1.4 mm), the embryo was developed on a three-quarter circumference, and the embryo, as well as a single yellow oil droplet, were bright yellow. Eggs basically appeared as described by Hassler and Rainville (1975) and Ditty and Shaw (1992). Live, fertilized eggs were buoyant and were also distinguishable from unfertilized, "dead" eggs by the lumpy, opaque appearance of the yolk in dead eggs. Dead eggs were periodically removed from hatching tanks.

Eggs were incubated in hatching tanks at 23.0°C. Hatching commenced ~24 h post-spawn, and most eggs hatched within four hours. An estimated 320,000 eggs hatched (presumed hatching success of 10%). At hatching, mean length of larvae was 2.5 mm, the yolk sac was large, and larvae appeared similar to that illustrated by Ditty and Shaw (1992).

Larval Rearing Treatments

An estimated 140,000 day one, yolk sac larvae were used in our small-scale larval rearing treatments. All treatments were conducted simultaneously and were unreplicated; the results of one treatment (Treatment 1) were superior to all others. Larval survival at day three appeared high. By day three, yolk sac absorption was nearing completion for most larvae, and food was offered all larvae. Mean size of larvae on day three was 4.3 mm TL. Summary results of treatments are presented as follows:

Treatment 1: 120 L black fiberglass tanks; larvae offered high density diet of enriched rotifers — The tank was stocked with ~10,000 larvae (84,000/m^3). Larvae were fed a high density of "standard-size" enriched rotifers 2 X per day in an attempt to maintain a feeding density of 200/ml which represents a density of prey considerably greater (~5 - 10X) than often recommended for the culture of some fish larvae (Ludwig 1994, Puvanendran and Brown 1999). First feeding was observed to commence on day three for some larvae, concurrent with the near-final absorption of remaining yolk. Feeding response was positive, and by day 5 many larvae were observed swimming actively and "striking" at rotifers in an S-shaped strike maneuver described by Hassler and Rainville (1975).

Survivability of the larvae appeared relatively high until day 13 when an aeration system malfunction resulted in the death of all remaining larvae. Although some larval mortalities (numbers unknown) occurred during the treatment period prior to day 13, possibly linked to the lack of successful first feeding, this treatment represents the most successful larval rearing experiment conducted during the study.

Treatment 2: 120L black fiberglass tank; larvae offered standard density diet of enriched rotifers — The tank was stocked with ~10,000 larvae (84,000/m^3). Larvae were fed "standard size"enriched rotifers 2 times per day in an attempt to maintain a feeding density of 20/ml. A few larvae were observed feeding on day 3, however, the overall feeding response was not positive. The food density probably resulted in insufficient prey surrounding the larvae. Most larvae never initiated first feeding, while others, although possibly successful at initiating first feeding, probably encountered prey too infrequently to survive. Total mortalities occurred by day seven.

Treatment 3: 410 L light blue plastic tank; larvae offered standard density diet of enriched rotifers — The tank was stocked with ~40,000 larvae (96,000/m^3). Larvae were fed "standard size"enriched rotifers 2 times per day in an attempt to maintain a feeding density of 20/ml. A few larvae were observed to commence feeding on day three, however most did not feed, and total mortalities occurred by day five. A high larvae:low prey density ratio presumably was the primary cause of the mortalities.

Treatment 4: 410 L light blue fiberglass tank; larvae were offered wild zooplankton — The tank was stocked with ~40,000 larvae (96,000/m^3). Composition of the zooplankton was not analyzed, but cursory observation revealed prodigious numbers of adult copepods. Zooplankton densities were not determined but were considered very low. Close scrutiny revealed that none of the larvae commenced first feeding, and total mortalities occurred by day five.

Treatment 5: 410 L light blue plastic tanks; larvae offered commercial food — The tank was stocked with ~40,000 larvae (96,000/m^3). Large quantities of a viable feed ("artificial plankton", 100 μm) were offered larvae 2 times per day (specific amount undetermined). First feeding was never initiated, and total mortalities occurred by day five.

General observations on growth and survival — Although not calculated, daily mortalities of yolk sac larvae, prior to yolk sac absorption on day three, appeared low in all tanks. On day five, observations indicated that a large percentage of the larvae in Treatment 1 had food in their gut, close to the time of 100% mortality in most other treatments.

The following information was based on examinations and observations of larvae from Treatment 1 only. Reported lengths include specimens with and without food in their gut, and lengths were recorded for specimens preserved in 95% ethanol for a period of four months. The percentage shrinkage of larvae in the preservative was unknown. Twenty-four hour-old yolk sac larvae exhibited a

large yolk sac, averaged 3.5 mm TL, and were colorless. The yolk sac was absorbed in most larvae by the end of day three, at which time we observed relatively well developed mouth parts and a near functional digestive tract; mean size, 4.3 mm TL. First feeding was actually initiated by some three day-old individuals. By day five, larvae averaged 4.5 mm TL, and the development of the body, particularly the head and mouth, permitted continuous swimming, accompanied by rapid, darting motions, and an active feeding behavior. At day 10 (mean TL, 6.3 mm) the head was large and the eyes were well developed, and larvae were a yellowish-golden color. Mean length of 13 day-old larvae was 8.0 mm

Daily survival rates in Treatment 1 were not calculated, and assumptions about high survivorship in that treatment were based on the existence of a large number of well developed larvae on day 13.

DISCUSSION

Because northern Gulf cobia have a relatively well-defined spawning season, induced spawning of mature females caught during peak reproductive potential appears to represent a viable approach for obtaining large numbers of eggs in late maturation stages. Males reportedly are not as abundant as females (Franks et al. 1999), but are available. Our research was conducted opportunistically which necessarily limited the sample size of adults.

Our small-scale study demonstrated the effectiveness of gonadotropin (HCG) to induce ovulation and spawning in mature, wild-caught female *R. canadum* with well-developed oocytes prior to administration of HCG. The study also demonstrated that captive, ripe male cobia do not require administration of gonadotropin to released viable sperm in the presence of spawning females.

Administration of a single dose of gonadotropin to successfully induce ovulation and spawning in fishes is not unique, and the single dosage of HCG (275 IU/kg BW) applied in our experiment is similar to that often used with other marine fish (Harrell et al. 1990). The success of induced ovulation and spawning in fishes is dependent, in great part, on females exhibiting advanced stage, vitellogenic oocytes, which effectuates a greater response to the injection of hormones (Lam 1982). Furthermore, studies by De Montalembert et al. (1978) and Roland (1985) showed if degeneration of mature oocytes has begun in mature ovaries, success of hormone-induced ovulation and spawning is greatly reduced. In our study, there was no apparent significant variation in the microscopic appearance of advanced-stage oocytes aspirated from either female cobia prior to injection with HCG. Interestingly, Rowland (1988) observed that the lowest dosage of HCG required to induce ovulation or spawning generally resulted in the highest hatch rates.

Our spawn estimate of 3.2 million eggs (both females combined) is 2.5X greater than the estimated mean batch fecundity (53 eggs/g ovary-free body weight) reported for cobia by Brown-Peterson et al. (in review). Post-mortem examination of ovaries conducted shortly after the spawning event (females succumbed to poor water quality conditions in holding tanks a few days after spawning) suggested recent spawning. It is presumed that not all spawned oocytes were viable, hydrated ooctyes, i.e., the affects of HCG also may have caused the release of less developed oocytes.

We considered our estimated hatch rate of 10% moderately successful, however we have no data on the actual number of viable eggs spawned. Ayson (1989) commented that the choice of hormone for induced spawning should be based not only on its capacity to stimulate egg release but also its effect on egg quality and hatch potential. Our observations suggest that the dosage of HCG administered in our study had no apparent adverse effect on the general quality of cobia eggs, as suggested by a presumed high rate of fertilization, a successful hatch, and the number of yolk sac larvae produced. Most early larvae examined in our study appeared "normal" with morphological features and pigmentation basically as that described by Hassler and Rainville (1975).

A critical time in the life of larval fish is the onset of exogenous feeding, and failure to initiate feeding before the yolk is totally exhausted generally results in mortality (Blaxter 1986). The combination of prey concentrations and larval density significantly influence growth and survival of larvae. The larvae stocking density of 84,000/m^3 (83/L) in Treatments 1 and 2 was comparable to that reported for seabream (Fukusho 1990) but high when compared with that for rockfish (Ko and Lee, 1991) and some other species. Larval stocking density of 97/L in Treatments 3, 4, and 5 were high. We believe growth and survivability of larvae in Treatment 1 were directly related to the offering of suitable live food at the proper time and at high density, probably augmented by some visual advantage in locating prey against a black background.

Interestingly, Olsen et al. (1999) reported that the size distribution of prey organisms may be more important than the total number of prey per unit volume for the growth and survival of some marine fish species. Naas et al. (1996) illuminated "first feeding tanks" of various colors and found that black was best suited to reproduce natural illumination conditions, suggesting that black background may be important to creating an optimum first-feeding environment for larval cobia. The mortalities associated with the unsuccessful treatments suggest larvae were unable to initiate or maintain sufficient feeding. Although small particles of prey were observed in stomachs of some larvae from Treatment 2 (also a black tank), results strongly suggest that encounters with prey were not sufficient to prevent mass starvation mortality.

Low densities of wild zooplankton were presumed to be the cause of

mortalities in Treatment 4. Field sampling efforts produced inadequate amounts of zooplankton, and larvae did not initiate first feeding. Adequate densities of zooplankton of appropriate composition and size may have potential as food for cobia larvae. Wild zooplankton, particularly copepods, may produce the best growth and survival rates for some larval fishes (Holmefjord et al. 1993), however, a zooplankton diet can be a limiting factor due to the uncertainty of supply, the occasional dominance of unfavorable species in the plankton fauna, and the variability in its nutrient content (Holmefjord et al. 1993). Although large quantities of a proven commercial food were offered larvae in Treatment 5, none of the larvae initiated feeding, suggesting that cobia require live food at first feeding.

Experimental rearing of pelagic fishes from fertilized eggs yields important knowledge on the biology of the species. Our successes with induced ovulation and spontaneous spawning of cobia, coupled with moderate success at larval rearing, are encouraging and enlarge the catalogue of possible areas for cobia cultivation. In fact, we consider cobia a promising aquaculture candidate. Adult brood stock, juveniles and sub-adults adjust well to captivity, lack aggressive behavior, and tolerate crowding.

Artificial inducement of final gonad maturation and spawning in cobia can provide extended access to viable gametes and may prove useful in future mass culture of cobia for commercial markets or in future efforts to rebuild stocks. Under exemplary conditions, captive adult cobia might be conditioned to spawn voluntarily.

ACKNOWLEDGMENTS

We thank Kathy Wilson, Dyan Wilson, Melanie Griggs, Nikola Garber, Amber Garber, Nate Jordan, and Diane Scott for their valued assistance during the study. The study was supported in part by the University of Southern Mississippi/Institute of Marine Sciences, the Mississippi Department of Marine Resources, and the Sport Fish Restoration Program of the U. S. Fish and Wildlife Service, Atlanta Georgia.

LITERATURE CITED

Ayson, F.G. 1989. The effect of stress on spawning of brood fish and survival of larvae of the rabbitfish, *Siganus guttatus* (Bloch). *Aquaculture* **80**: 241-246.

Biesiot, P.M., R.M. Caylor, and J.S. Franks. 1994. Biochemical and histological changes during ovarian development of cobia, *Rachycentron canadum*, from the northern Gulf of Mexico. *Fish. Bull., U. S.* **92**:686-696.

Blaxter, J.H.S. 1986. Development of sense organs and behaviour of teleost

larvae with special reference to feeding and predator avoidance. *Trans. Am. Fish. Soc.* **115**:98-114.

Brown-Peterson, N J., R.M. Overstreet, J.M. Lotz, J.S. Franks, and K.M. Burns. (In review). Reproductive biology of cobia, *Rachycentron canadum*, from coastal waters of the southern United States.

Dawson, C.E. 1971. Occurrence and description of prejuvenile and early juvenile Gulf of Mexico cobia, *Rachycentron canadum*. *Copeia* **1960(3)**:171-180.

De Montalembert, G., G. Jalabert, and C. Bry. 1978. Precocious induction of maturation and ovulation in northern pike (*Esox lucius*). *Ann. Biol. Anim., Biochim., Biophys.* **18(4)**:969-975.

Ditty, J.G. and R.F. Shaw. 1992. Larval development, distribution, and ecology of cobia, *Rachycentron canadum* (Family:Rachycentridae), in the northern Gulf of Mexico. *Fish. Bull., U. S.* **90**:668-677.

Donaldson, E.M. and G.A. Hunter. 1983. Induced final maturation, ovulation and spermiation in cultured fish. Pages 351-403 in: W. S. Hoar, D. J. Randall, and E. M.. Donaldson (eds.), Fish Physiology, IXB. Acad. Press, London.

Franks, J.S., M.H. Zuber, and T.D. McIlwain. 1991. Trends in seasonal movements of cobia, *Rachycentron canadum*, tagged and released in the northern Gulf of Mexico. *J. Miss. Acad. Sci.* **36**(1):55.

Franks, J. S., N. M. Garber, and J. R. Warren. 1996. Stomach contents of juvenile cobia, *Rachycentron canadum*, from the northern Gulf of Mexico. *Fish. Bull., U. S.* **94**:374-380.

Franks, J.S., J.R. Warren, and M.V. Buchanan. 1999. Age and growth of cobia, *Rachycentron canadum*, from the northeastern Gulf of Mexico. *Fish. Bull.*, **97**(3):451-479.

Fukusho, K. 1990. Outline of aquaculture:red seabream culture. Kanagawa International Fisheries Training Center, Japan Int. Coop. Agency. 8 pp.

Harrell, R.M., J.H. Kerby, and R.V. Minton (eds.), 1990. Culture and propagation of striped bass and its hybrids. Striped Bass Committee, Southern Division, Am. Fish. Soc., Bethesda, MD. 323 pp.

Hassler, W. W., and R. P. Rainville. 1975. Techniques for hatching and rearing cobia, *Rachycentron canadum*, through larval and juvenile stages. Univ. N.C. Sea Grant Coll. Prog., UNC-SG-75-30, Raleigh, NC. 26 pp.

Holmefjord I., J. Guldbransen, I. Lein, T. Refstie, P. Léger, T. Harboe, I. Huse, P. Sorgeloos, S. Bolla, Y. Olsen, K.I. Reitan, O. Vadstein, G. Oie, and A. Danielsberg. 1993. An intensive approach to Atlantic halibut fry prduction. *J. World Aquacult. Soc.* **24**:275-284.

Hrincevich, A. W. 1993. Analysis of cobia *Rachycentron canadum* population

structure in the northern Gulf of Mexico using mitochondrial DNA. M.S. thesis, Univ. Southern Miss., Hattiesburg, MS, 91 pp.

Joseph, E.B., J.J. Norcross, and W.H. Massmann. 1964. Spawning of the cobia, *Rachycentron canadum*, in the Chesapeake Bay area, with observations of juvenile specimens. *Chesapeake Sci.* **5**:67-71.

Knapp, F.T. 1951. Food habits of the sergeantfish, *Rachycentron canadus*. *Copeia* **1951**:101-102.

Ko, T.S. and C.C. Lee. 1991. Mass production experiment of rockfish seed. *Tech. Rep. Natl. Fish. Res. Develop. Agency, Korea.* **90**:45-50.

Lam, T.J. 1982. Applications of endrocrinology to fish culture.Can. J. Fish. *Aquat. Sci.* **39**:111-137.

Liao, I.C., S. Mao-Sen, and C. Su-Lean. A review of the nursery and growout techniques of high-value marine finfishes in Taiwan, Pages 121-137 in: M. L. Kevan and C. Rosenfeld (eds.), *Proceedings: Culture of high-value marine fishes in Asia and the United States.* The Oceanic Institute, Honolulu, HI, ISBN 1-886608-01-6.

Lotz, J.M., R.M. Overstreet, and J.S. Franks. 1996. Gonadal maturation in the cobia, *Rachycentron canadum*, from the northcentral Gulf of Mexico. *Gulf Res. Rep.* **9**(3):147-159.

Ludwig, G.M. 1994. Tank culture of sunshine bass *Morone chrysops* X *M. saxatilis* fry with freshwater rotifers *Brachionus calyciflorus* and salmon starter meal as first food sources. *Aquaculture* **25**(2):337-341.

Meyer, G.H., and J.S. Franks. 1996. Food of cobia, *Rachycentron canadum*, from the northcentral Gulf of Mexico. *Gulf Res. Rep.* **9**(3):161-167.

Naas, K., I. Huse, and J. Iglesias. 1996. Illumination in first feeding tanks for marine fish larvae. *Aqua. Engin.* **15**(4):291-300

Olsen, A.I., Y. Attramadal, A. Jensen, and Y. Olsen. 1999. Influence of size and nutritional value of *Artemia franciscana* on growth and quality of halibut larvae (*Hippoglossus hippoglossus*) during the live feed period. *Aquaculture* **179**:475-487.

Ogle, J.T. 1992. Design and operation of the Gulf Coast Research Laboratory penaeid shrimp maturation facility I., *Penaeus vannamei.* Tech. Rpt. 4. Gulf Coast Research Laboratory, Ocean Springs, MS 39564, 41 pp.

Puvanendran, V and J.A. Brown. 1999. Foraging, growth and survival of Atlantic cod larvae reared in different prey concentrations. *Aquaculture* **175**:77-92.

Richards, C. E. 1967. Age, growth and fecundity of the cobia, *Rachycentron canadum*, from the Chesapeake Bay and adjacent Mid-Atlantic waters. *Trans. Am. Fish. Soc.* **96**:343-350.

Richards, C.E. 1977. Cobia (*Rachycentron canadum*) tagging within Chesapeake Bay and updating of growth equations. Chesapeake Sci.

18:310-311.

Rowland, S J. 1985.Aspects of the biology and artificial breeding of the Murray cod, *Maccullochella peeli* and the Eastern freshwater cod, *M. ikei* n sp. Ph.D. Thesis, Macquarie Univ., N.S.W., 253 pp.

Ryder, J.A. 1887. On the development of osseus fishes, including marine and fresh water forms. *Rept. U. S. Fish. Comm.* 1885, **13**:488-604.

Shaffer, R. V., and E.L. Nakamura. 1989. Synopsis of biological data on the cobia *Rachycentron canadum* (Pisces:Rachycentridae). FAO Fisheries Synop. 153 (NMFS/S 153). U. S. Dep. Comm., NOAA Tech. Rep. NMFS 82. 21 pp.

Smith, J.W. 1995. Life history of cobia, *Rachycentron canadum* (Osteichthyes: Rachycentridae), in North Carolina waters. *Brimleyana* **23**:1-23.

Thompson, B.A., C.A. Wilson, J.H. Render, and M. Beasley. 1991. Age, growth and reproductive biology of greater amberjack and cobia from Louisiana waters. Year 1. Rep. to U. S. Dep. Commer., NOAA, NMFS, Coop. Agreement NA90AA-H-MF089, Marine Fisheries Initiative (MARFIN) Prog., Coastal Fish. Inst., Louisiana St. Univ., Baton Rouge. 55 pp.