Advances in Cobia Seed Production and Hatchery Management in India

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Cobia *Rachycentron canadum*, also known as black kingfish, is the only species in the family Rachycentridae. It is commonly found throughout the world, except the central and eastern Pacific (Shaffer and Nakamura 1989). Cobia is a pelagic species found in the water column of the open ocean from the surface to a depth of 1200 m (Shaffer and Nakamura 1989). In recent years, cobia has become popular as a potential candidate species for aquaculture, related to its excellent growth performance and high market demand.

Several successful attempts have been made to breed cobia. In India, the first successful breeding of cobia was achieved in 2010 at the Mandapam Regional Centre of Central Marine Fisheries Research Institute, Tamil Nadu (Gopakumar *et al*. 2010, 2012), followed by the Rajiv Gandhi Centre for Aquaculture at Pozhiyoor, Kerala (Samraj *et al*. 2011). Presently, seed production of cobia is being carried out on a commercial scale by both organizations.

FIGURE 1. *Cobia broodstock in a confined system. Photo: Fish Identification blogspot.*

FIGURE 2. *A cobia broodfish being held for cannulation. Photo: NOAA photo library.*

Broodstock Source and Management

Broodstock transportation and hatchery acclimation. Broodfish were caught in the open ocean by angling and transported in 500- to 1000-L tanks at 50 kg/m^3 to the hatchery. From these wild-caught fish, appropriate age and size fishes were selected as broodstock (Fig. 1). During handling, dissolved oxygen concentration in the broodstock tank was maintained at 8-12 mg/L with compressed oxygen cylinders. Water temperature was maintained at 22-26C with ice packs. Clove oil (10-40 ppm) was used as an anaesthetic, facilitating ease of handling during transportation (Benetti *et al*. 2007, 2008).

After reaching the hatchery, broodfish were treated with fresh seawater for two minutes and given a one-minute dip treatment with 100 ppm formalin to remove external parasites and treat lesions, then set aside in quarantine tanks for about 2-3 weeks for acclimation to hatchery conditions. Wild-sourced fish were shifted to the broodstock section when they were completely acclimated to hatchery conditions.

Mortality occurred when fish were reared in the flow-through system because of a protozoan infection (Samraj *et al*. 2011). Broodfish were then reared in recirculating aquaculture system and subjected to strict quarantine conditions to evaluate ecto- and endo-parasites and other suspected diseases. Fish were anesthetized with tricaine methanesulfonate (MS-222) to facilitate tagging and shifting to RAS from the quarantine tanks. Gonadal biopsy with a 1-mm diameter catheter was used to differentiate sexes.

Broodstock diet. Quality seed production depends on good broodfish diet quality. An ideal broodstock diet is a pre-requisite to obtain greater fecundity, gamete quality and hatchling survival rate (Watanabe 1985, Bromage 1995). Good nutrition is essential to ensure egg yolk quality and endogenous nutrition for growing larvae (Rainuzzo *et al*. 1997). Protein and omega-3 fatty acid deficiencies in feed results in poor gamete viability and larval survival. An inappropriate dietary ratio of polyunsaturated fatty acids (PUFA) alters the circulating level of androgens, resulting in asynchrony of maturation between the sexes (Cerda *et al*. 1997). Vitamin C was added to the diet improved sperm concentration, motility, and fertility (Mangor-Jensen and Holm 1994) while PUFA boosts egg and larval development (Ma *et al*. 2005). Because there was no information available about standard cobia broodfish diets, fish were fed sardines and squid on an alternative basis in two rations, corresponding to 3-5 percent of fish body weight daily.

Induced Spawning

Captive breeding. Male cobia become sexually mature at 53 cm total length whereas female cobia mature at 68 cm total length. Cobia has a protracted reproductive season in Indian seas and

generally breeds from March to September, but can be induced to spawn year round via hormonal stimulants and photothermal manipulation. During off-season spawning, there is a greater possibility of obtaining poor-quality eggs. Meeting broodstock nutritional requirements and providing appropriate environmental manipulation ensures that obtained spawn is of highest quality.

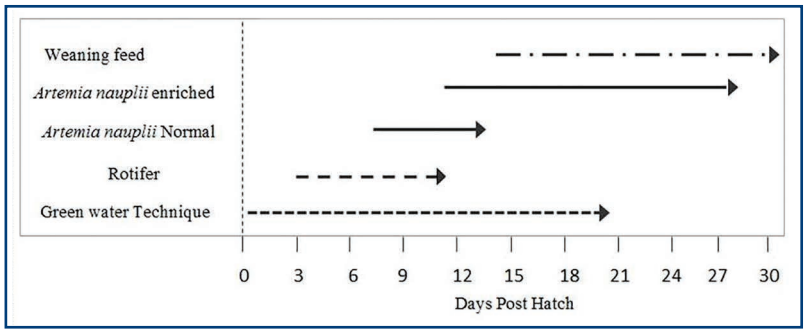

FIGURE 3. *Feeding protocol for cobia larvae.*

Fish were inspected periodically to check for maturation stage of oocytes (Fig. 2). When oocytes are more than 600 μm diameter (> 700 μm is ideal) and males are actively producing milt, broodfish were ready for induced breeding. Selected broodfish were injected with luteinizing hormone-releasing hormone (LHRH) at 20 µg/kg for female and 10 µg/kg for male fish (Nhu *et al*. 2011, Samraj *et al*. 2011). Broodfish can be induced to spawn using intramuscular injection of human chorionic gonadotropin hormone (HCG) at 500 IU/kg for females and 250 IU/kg for males (Gopakumar *et al*. 2011). Fish were anesthetized during handling and horomone injection to avoid stress. Typically, a sex ratio of two males to one female at a stocking density of 1-1.9 kg/m^3 provides consistent success (Benetti *et al*. 2008). Spawning occurs 39 h after hormone injection.

Eggs and collection. Eggs were positively buoyant, regulated by oil globules in the egg. Eggs were cream-colored, transparent, and spherical, between 1.0-1.1 mm in diameter (Gopakumar *et al*. 2011). A 35-kg broodfish released about 3.5 million eggs (Samraj *et al*. 2011) and a 23 kg fish released about 2.1 million eggs, with a fertilization rate of up to 90 percent (Gopakumar *et al*. 2010, 2011). Eggs can be collected with the use of an egg collector using an air lift or with a hand net with a mesh size of 500 μm (Gopakumar *et al*. 2010, Samraj *et al*. 2011). Collected eggs were disinfected with a dip of 5 percent iodine for one minute to avoid fungal infection (Samraj *et al*. 2011). Volumetric methods were used to calculate the total volume of eggs spawned.

Water parameters of spawning tank. Sterilized broodstock tanks, filled with de-chlorinated seawater, were maintained at an ideal water temperature of 26-29 C with adequate dissolved oxygen of 7 mg/L. Chillers were used for temperature maintenance, while air was supplied from an oil-free compressor and supplemental oxygen was supplied from a compressed oxygen cylinder. Salinity was maintained between 25 and 35 ppt. Other important water quality parameters such as pH, hardness and alkalinity were kept within desirable limits (Gopakumar *et al*. 2011).

Egg Hatch-out and Larval Feeding Practices

Tank preparation and egg hatch-out. The tank was filled with dechlorinated seawater to about 60 percent of the total water holding capacity of the tank and probiotics (INVE at 5 ppm) were added to improve environmental conditions. Hatching tanks were stocked with 10-15 eggs/L. The perivitelline space was thin and the embryo was pigmented. The incubation period depended on water temperature and egg size but required about 22 h at 28-30 C. Newly hatched fry were 2.2-2.7 mm (Gopakumar *et al*. 2010).

Live Feed and Feeding Protocol. Production of high-quality fry represents the greatest constraint to the development of cobia aquaculture around the world. Existing rearing technologies, feeding protocols and survival remain poor. There is a major larval die-off during metamorphosis, when fish shift their feeding strategy, highlighting our inadequate knowledge of cobia larval nutrition. Studies have been conducted on larval stocking density, feeding quantity and feeding frequency. Water quality management and feeding protocols have been an underlying theme to attain greater survival of cobia fry (Fig. 3).

Microalgae, rotifers and *Artemia* nauplii were used as live feeds in the cobia hatchery (Radhakrishnan *et al*. 2017). Typically, *Nannochloropsis salina* or *Isochrysis galbana* (at 1 × 10^5 cells/mL) were used to maintain the greenwater technique from 0 to 20 days post-hatch (dph). Newly hatched larvae nourished themselves with the yolk sac for the first three days. The rotifer *Brachionus plicatilis* was then introduced and maintained at 10-15/mL up to 11 dph. Rotifers were enriched with *N. salina* or *I. galbana* or other commercial products, including Algamac 3050. Newly-hatched *Artemia nauplii* (7-13 dph) and enriched *Artemia nauplii* (11-28 dph) were fed to larvae at 1-3/mL. Cobia larvae weaning started with 200-300 μm size feed at 14 dph and then gradually increased to 300-500 μm, 500-800 μm, and 1200 μm up to 30 dph (Samraj *et al*. 2011).

Cobia larvae were fed ad libitum using a pulse feeding technique (five times per day) and size grading is needed to reduce cannibalism and enhance survival at this stage. The first siphoning takes place during 23-30 dph, depending on the degree of accumulation of debris and dead larvae. Under a natural environment, a cycle of 13-14 h of light and 10-11 h of dark is needed throughout the nursery phase.

Water Quality Maintenance. During the larval rearing phase, utmost care must be taken to preserve excellent water quality. Dissolved oxygen concentration was maintained between 7-12 mg/L. Strong aeration was avoided has it can cause stress to the larvae. Hence, mild diffused aeration from the tank bottom and pure oxygen should also be provided if possible. The concentration of ammonia should be < 0.1 mg/L and pH of 8 is desirable. Water samples for water quality assessment should be collected prior to water exchange of larval rearing tanks. To maintain water quality, probiotics were applied at 10^3-10^5 CFU/mL (5 percent) The predetermined quantity of probiotics was mixed with low-salinity or fresh water, aerated for one hour for multiplication of bacteria and slowly added to the tank. Water exchange started between 15-20 dph, depending on water quality, especially ammonia concentration.

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Juvenile and Grow-out Phase

Juvenile. Cobia fry were normally reared in cement cisterns, composite tanks or earthen ponds. A suitable volume for a fry rearing tank was 3-10 m^3 with an average depth of 1.0-1.5 m. Cobia growth was influenced by stocking density, feeding rate and culture environment. The desirable stocking density ranges from 5-15 kg/m^3; growth rate is inversely related to stocking density.

Fry were fed with a 35 percent protein diet at 6-8 percent body weight, three times per day. Cobia fry typically reach 30-43 g at 90 d. Size grading is necessary to reduce cannibalism (Xan 2005). During fry and fingerling rearing, 100-200 percent of water was exchanged daily, but this increases electricity cost. Thus, water flow rate can be adjusted according to the water quality and routine siphoning can reduce the water exchange rate.

Grow-out. The grow-out of cobia in India has been successfully accomplished in cages in the open sea (Philipose *et al*. 2013). The suggested stocking size in marine cages was about 50-100 g. Fish less than 50 g are not suitable for offshore culture because of their weak resistance to water currents that can result in mortality (Xan 2005). Better survival is obtained at a greater stocking size. Cobia reach about 50 g (40-80 g) in 110 d and 100 g (65-120 g) in 150 d. The growth rate of cobia varies broadly depending on the culture environment but can reach about 2-6 kg in one year. At 13 $fish/m^3$, fish will reach an average weight of 6 kg, and at 23 $fish/m^3$, fish will reach 3.5 kg.

Notes

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