Successful seed production of cobia *Rachycentron canadum* and its prospects for farming in India

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Introduction

Availability of adequate quantity of high value marine finfish seed is the major prerequisite for initiation and expansion of finfish mariculture. Breeding and seed production of marine finfishes of high value have been expanding in recent years internationally. Large quantities of hatchery produced seeds meet the need for sea cage farming in many countries (Hong and Zhang, 2003). It is well understood that the first step towards seed production technology is the development of broodstock. Prior to 1980s, broodstock of finfishes were grown mainly in indoor concrete tanks. Since early 1980s, wild-caught broodstock have been raised either in outdoor earthen ponds or in sea cages. It has been proved that broodstock development in sea cages was highly effective in improving gonadal development for most finfish groups like groupers, pompano, red seabream, cobia, Japanese flounder and yellow croaker. The development of hatchery technology for commercial level seed production of marine finfishes is still in its infancy in India, except for the Asian seabass, *Lates calcarifer.* Hence, research and development need to be focused in evolving technologies for the seed production and farming of highly priced marine food fishes.

In recent years, seed production and farming of cobia (*Rachycentron canadum*) is rapidly gaining momentum in many Asian countries (Liao and Leano, 2007). Cobia is distributed worldwide in warm marine waters. They are found throughout the water column and are caught in both coastal and continental shelf waters, although they are typically considered to be an offshore species. Wild-caught cobia does not support a major commercial fishery and generally is considered as incidental catch. Sexual maturity is reported in males at 1-2 years and in females 2-3 years, with females growing larger and faster with maximum size upto 60 kg (Shaffer and Nakamura, 1989).

Fast growth rate, adaptability for captive breeding, low cost of production, good meat quality and high market demand especially for *sashimi* industry are some of the attributes that makes cobia an excellent species for aquaculture. Under culture conditions, cobia can reach 3-4 kg in body weight in one year and 8-10 kg in two years. The species has a protracted spawning season and it can spawn in captivity. The fecundity is very high. Aquaculture research on cobia was first reported in 1975 with the collection of wild caught cobia eggs off the coast of North Carolina. Larval development was described and after the termination of 131 day rearing trial, it was concluded that cobia has a good aquaculture potential because of its rapid growth and good flesh quality. Further research on cobia was conducted in the late 1980s and early 1990s in the USA and Taiwan Province of China. Research continued and by 1997 the technology to raise large quantities of cobia fry was developed and Taiwan Province of China was producing cobia juveniles for grow-out mostly in nearshore cage systems. Cobia production is also reported in the United States, Puerto Rico, Bahamas, Martinique, Belize, Brazil and Panama (Bennetti *et al.,* 2008). Envisaging the prospects of cobia farming in India, broodstock development was initiated at the Mandapam Regional Centre of the Central Marine Fisheries Research Institute in sea cages in 2008 and the first successful induced breeding and seed production was achieved during March - April 2010.

Cobia broodstock development - general aspects

The capacity to produce large, dependable quantity of quality seeds is the key for establishing

reliable and sustainable cobia aquaculture. The major bottlenecks in the development of commercial aquaculture are the control of reproductive processes of fish in captivity and production of biosecure and quality-certified fry. Broodstock management usually includes collection, selection and domestication of brooders as well as control of maturation, spawning and egg collection. Wherever available, cobia broodstock can be procured from the wild during the spawning season, then transfer them to culture systems for rather short time, and either spontaneous natural spawning or hormone induced spawning can be obtained.

Cobia being a very active fish which grows to large size, broodstock development is mostly practised in sea cages in order to ensure good water exchange and healthy environment. Brood fishes can be stocked at a density of about 2 kg/m^3. Trash fishes (sardines, scads *etc.*) are fed once in a day at the rate of 5% of biomass or till satiation. The trash fish has to be supplemented with vitamins and HUFA (fish oil, squid liver oil). Broodstock nutrition is very important and there is positive correlation between HUFA in the broodstock diets and in the eggs and larvae.

Cobia attains maturity when the fish is about two years old. It has a protracted spawning season in the Indian seas. It spawns under captivity naturally as well as on inducement. It has high fecundity, ranging from 0.05 to 0.25 million eggs per kg. Bigger fishes of 10-15 kg weight having normal shape without any deformity and with healthy behaviour are selected as broodstock. The other important criteria for broodstock include bright colour and with anus easily recognisable. Broodstock nutrition plays a key role in the quality and viability of the larvae. Best temperature for maturation is around 27 ºC and the best salinity range is 30-34 ppt. Separation of males and females from the broodstock cage is required for conditioning the fish for breeding. It is required for controlling the breeding and planning the seed production. The best time for separation is one month before the breeding inducement. Conditioning the brooders ensures best maturation as well as egg and larval quality. Cannulation can be done to assess the maturity condition of the female. The maturation characteristics of female include egg with size above 0.7 mm, non-adhesive, white colour and round shape. In the case of mature males, by gently pressing the belly, the milt oozes out. Brooders are characterised by big belly, chasing behavior and red, swollen anus. The selected brooders can be brought from the cages and transferred to cement tanks. Usually two males and one female are introduced to the spawning tank. Natural spawnings also can be obtained if brooders are selected properly. Induction of spawning can be done by injecting LHRHa 20 μg kg^{-1} for females and 10 μg kg^{-1} for males. Spawning occurs within 12-24 h after the injection. Egg collection can be done manually from the tank by employing 500 μm net.

Optimisation of captive broodstock management protocols still remains a challenge to establish reliable bio-secure hatcheries with genetic diversity and consistent production of high quality eggs and larvae.

Broodstock development and captive breeding at Mandapam

The broodstock at Mandapam was developed in sea cages (Fig. 1 & 2) of 6 m diameter and 3.5 m depth (Gopakumar, 2008). The wild collected cobia brood fishes in the size ranging from 2-10 kg weight were stocked during December 2008 to February 2009. The fishes were stocked without separating sexes. All the fishes were collected from the hooks and line commercial catches. After transporting to hatchery, the fishes were treated with 100 ppm formalin for 2-5 min and conditioned for two to three days in 10 t FRP tanks before transferring to cages. These fishes were fed twice daily at 0900 and 1530 hrs with sardines (*Sardinella* sp.) and other fish species like *Pellona* and *Ilisha* and occasionally with squids and portunid crabs @5% of their body weight. Vitamin and mineral supplements were also given

Fig. 1. Broodstock cages for cobia at Mandapam

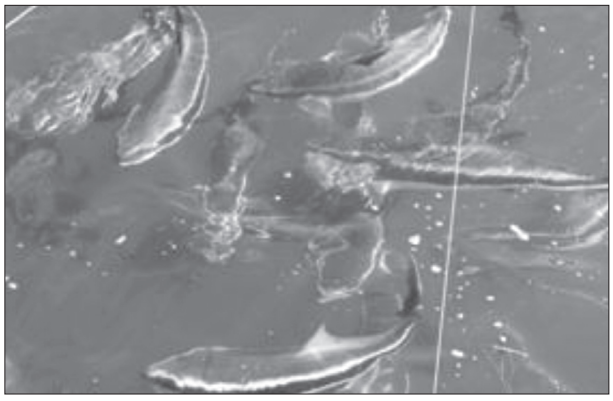

Fig. 2. Cobia broodstock in cage

Fig. 3. Cannulation of cobia to assess the maturity

twice a week along with the feed in order to complement any possible nutritional deficiencies in their diet. A total of 40 fishes were stocked in four cages. The length range and corresponding weight range of the brood fishes recorded during April 2009 ranged between 80 and 127 cm, and 4 and 20 kg respectively. The sexes were separated by cannulation using a flexible catheter (2 mm inner diameter) in June 2009 and stocked in separate cages. Thereafter, the females were cannulated (Fig. 3) every fortnight to assess the diameter of the intra-ovarian eggs.

On 11.03.2010, one of the females with intra-ovarian eggs (Fig. 4) of around 0.7mm size was selected for induced breeding. The size of the female was 120 cm in total length and 23 kg in weight. Two males were also selected from the male cage. The sizes of the males were 100 cm and 103 cm in total length and weighed 11 kg and 13.5 kg, respectively. On the same day, the selected brooders were introduced in a 100 t roofed cement tank holding about 60 t of seawater. At around 1300 hours, the brooders were induced for spawning with HCG at doses of 500 IU per kg body weight for female and 250 IU per kg body weight for males (Fig. 5). Spawning (Fig. 6) was noticed at 0430 hours on 13.03.2010. The total eggs spawned were estimated as 2.1 million. About 90% fertilisation was recorded. Fertilised eggs (Fig. 7) amounted to 1.9 million. The eggs were collected by a 500 µm mesh and stocked in incubation tanks at varying densities.

The eggs were hatched after 22 h of incubation at a temperature range of 28-30 ºC (Fig. 8 & 9). The percentage of hatching was 80% and the total number of newly hatched larvae was estimated as 1.5 million. The newly hatched larvae measured 2.2-2.7 mm in total length. The mouth opening was formed on 16.03.2010 (on 3rd day post-hatch) at a length of around 200 µm.

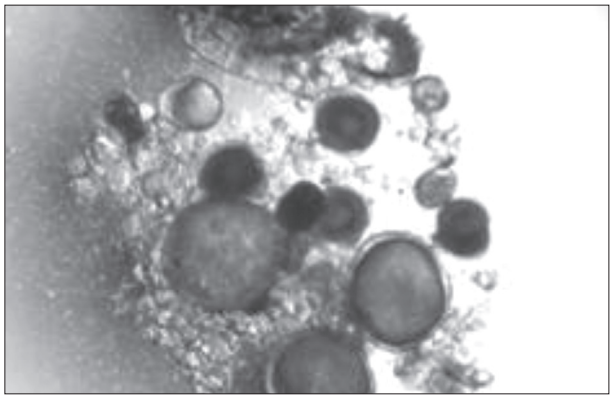

Fig. 4. Cannulated intra-ovarian eggs

Fig. 5. Administration of hormone for final oocyte maturation and spawning

Fig. 6. A view of the spawning behaviour of cobia inside the spawning tank

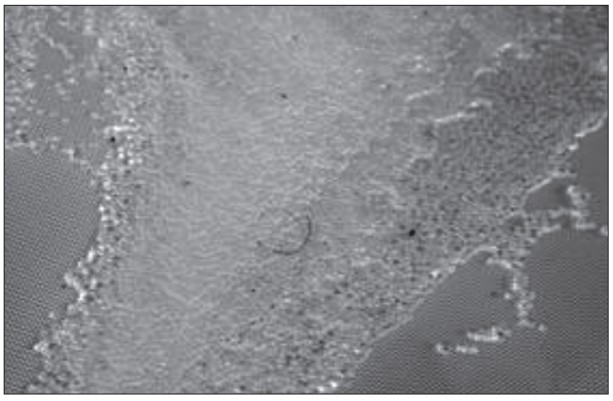

Fig. 7. Fertilized eggs collected on 500 µm mesh

Larviculture and seed production - general aspects

Cobia eggs are pelagic with single oil globule which is resorbed completely at 7 dph (day post-hatch). The egg diameter is 1.4 mm. Hatching occurs 26 h after spawning at 27 ºC. Newly hatched larvae measures 3.4 mm size (Holt *et al*., 2007). Though the larvae are vigorous, they are very sensitive to environmental conditions. However, they are more resistant to some stressors when compared to other tropical marine fish larvae (Liao *et al.,* 2004). Larval mouth opens at 2-3 dph (temperature dependent). Metamorphosis starts from 9-11 dph.

Newly hatched larvae (Fig. 10) generally start feeding at 3 dph and they can be fed with the enriched rotifer (*Brachionus rotundiformis*) @ 10-12 nos./ml, four times a day till 10 dph. From 8-10 dph, the larvae are fed with enriched *Artemia* nauplii @ 1-3 nos./ ml, 4-6 times per day. During the rotifer and *Artemia* feeding stage, green water technique is used in the larviculture system with the microalgae *Nanochloropsis oculata* at a cell density of $1x10^5$ cells/ml. Weaning to artificial larval diets is during 18 to 25 dph. While weaning, formulated feed has to be fed 30 min before feeding with live feeds. Continuous water exchange is required during weaning. Between 25-40 dph, the larvae are highly cannibalistic and hence size-grading is undertaken every four days to one week. During this stage the fry could be weaned totally to artificial diets. Larval rearing can be practised both intensively in tanks and extensively in ponds. The major factors affecting the growth and survival of larvae are nutrition, environmental conditions and handling procedures. Since there is high demand for essential fatty acids (EFAs), enrichment protocols are needed for live feeds. During the first 12 days of larviculture, water exchange has to be gradually increased from 10-100%. Surface skimmers are employed to remove oil particles from the water surface. After 18 dph, recirculation system is preferred. The environmental conditions required during the larviculture period are

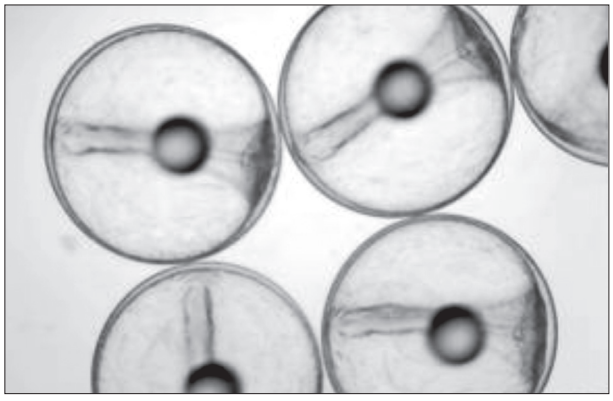

Fig. 8. Development of the embryo

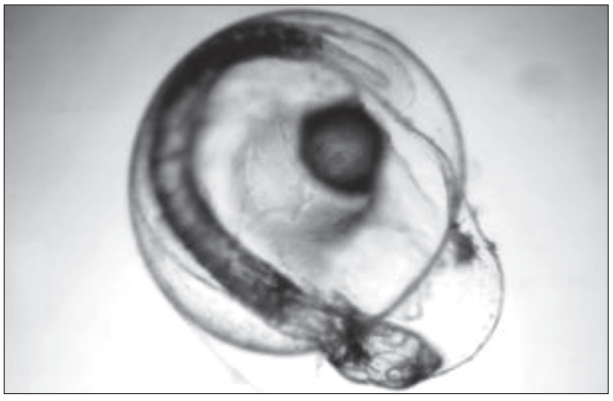

Fig. 9. Hatching of larva

Fig. 10. Newly hatched larvae

DO: >5 mg/l, NH_3: <0.1 mg/l, pH: 7.8–8.4, Salinity: 25-35 ppt, water temperature : 24-33 °C (Liao *et al.,* 2004).

Larviculture protocols developed at Mandapam

Larviculture protocols were developed by appropriate management of live feeds in suitable quantities and also taking into consideration the nutritional requirements of the larvae. The larvae were stocked in FRP tanks of 5 t capacity for larviculture (Fig. 11). The intensive larviculture tanks were provided with green water at a density of about 1 x 10^5 cells/ml and rotifers enriched with DHA SELCO at a density of 6-8 nos./ml from 3 to 9 dph. The critical stage for the larvae was 5 to 7 dph when they entirely resorted to exogenous feeding from yolk sac feeding. During this period, large scale mortality (about 80%) was noted. Thereafter, the mortality rate was moderate. From 9 to 21 dph, the larvae were fed four times daily with enriched *Artemia* nauplii by maintaining a nauplii concentration of 2-3 nos./ml.

Fig. 11. Larvae in the rearing tank

During this period, co-feeding with rotifers was also continued due to the presence of different size groups of larvae. Green water was also maintained in appropriate densities in the larval tanks. From 18 dph onwards, the larvae were fed with newly hatched *Artemia* nauplii and weaning to larval inert feeds was also started as per details given below:

Stage of larvae (dph)	Size of larvae (cm)	Size of feed (μ)
18 - 19	2.3 - 2.6	100 - 200
20 - 23	2.5 - 3.5	300 - 500
23 - 30	3.5 - 8.0	500 - 800
31 onwards	> 4.0	800 - 1200

From 25 dph, grading of larvae was started. The shooters were fed exclusively with the artificial feed of size 500-800 μ and 800-1200 μ. On 30 dph, three size groups of juveniles were noted with mean sizes of 10 cm (10%), 6 cm (25%) and 4 cm (65%). The juveniles measuring 10 cm length were ready for stocking in hapas and the other two size groups would be ready for stocking in hapas after rearing for another two to three weeks. All the fingerlings (Fig. 12) of 10 cm length and above were stocked in hapas in the sea for nursery rearing for about a month before transferring them to the grow-out cages.

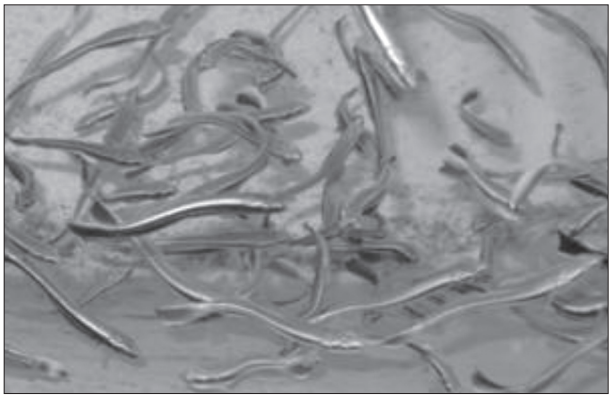

Fig. 12. Fingerlings of cobia

Cobia farming - general aspects

Nursery rearing

Nursery rearing of cobia generally comprises three phases. In first phase, 0.2 - 2g to 5g fry grow rapidly to fingerlings of 8-10 cm (20 to 45 dph). In the second nursery phase, cobia fingerlings are reared from 2 -5 to 30 g (45 to 75 dph) in large ponds with green water or in hapas in the sea. Artificial feed

are provided manually to satiation, 5 to 6 times daily. The size of the pellet feeds is increased gradually as the fish grows. Even during this phase, grading should be undertaken. The third nursery phase is from 30 to 600 - 1000 g (75-150 dph to 180 dph). Nursery rearing is either in outdoor ponds or inshore cages. Grading is undertaken only once during this stage. It is not advisable to stock cobia juveniles smaller than 30 g size in offshore cages, because of their weak resistance to strong water currents and also due to the necessity of occasional grading to prevent cannibalism (Liao *et al.,* 2004).

Grow-out farming

Cobia is cultured in offshore grow-out cages until they reach marketable size. Culture period ranges between 6-8 months. Small scale family owned cage farms and commercial cage farms are employed for cobia grow-out farming. Usually most of the cage farms integrate nursery and grow-out culture in one area for convenient transfer of fish stock from nursery to grow-out cages. Sinking and floating pellet feeds are used in grow-out cages. Cobia juveniles are cultured in smaller cages for 4-5 months until they reach a size of about 800 g and then they are transferd to larger cages (Liao and Leano, 2007).

Nutritional aspects

Nutrition is paramount to production success and the design of specific diets for all stages including broodstock, larvae, juveniles and adults. Rotifers and *Artemia* enriched with *Isochrysis galbana* or with commercial products along with green water culture provided best survival for cobia larvae (Faulk and Holt, 2005). Survival of cobia larvae was improved by addition of *I. galbana* or *N. oculata* in rearing tanks. Growth and survival rate can be improved if *Artemia* feeding schedule is reduced, since cobia larvae could take larger food size by 14 dph. Recent work (Chou *et al.,* 2001) has reported optimum dietary protein and lipid levels in juvenile cobia as 45% and 5-15% dry weight respectively. However, there is limited information on amino acid and essential fatty acid requirements of cobia. There is no information available on vitamin or mineral requirements of cobia. At present, commercial cobia feeds are based on those for Asian seabass or grouper and achieve acceptable growth with feed conversion ratio ranging from 1.5-1.8 (Chou *et al*., 2004). In areas such as Taiwan, where cobia culture is popular, cobia are fed once a day at feeding rate of 0.5-0.7% body weight on a pellet diet consisting of 42-45% crude protein with 15-16% fish oil and achieve FCRs of around 1.5 during the grow-out stage (Liao *et al.,* 2004).

Diseases

Diseases caused by bacteria, viruses and parasites occur in all stages of culture of cobia. During larval stage, *Epistylis* and *Nitzchia* infestation is very common. During the nursery stage, a viral disease *viz*., *Lymphocystis* is common, but not fatal as long as good water and feed management are employed. *Amyloodinium ocellatum* also cause problem which can result in high mortality if left uncontrolled. *Trichodina* infestations are also common during the nursery stage. Mixosporidian infections are also reported among cobia nursery phases causing mass mortality (Chen *et al.,* 2001). In grow-out stage, the ectoparasite *Neobenedenia* sp. Is common which together with secondary bacterial infection causes blindness to cobia juvenilies. Pasteurellosis caused by *Photobacterium damsela* is one of the most common diseases during cobia juvenile phases which can cause mass mortality. Vibriosis affects fingerlings, juveniles and adults and the causative agent is *Vibrio anguillarum*. Another bacterial problem in sea cages is caused by *Vibrio alginolyticus* which result in hemorrhages and subsequent mass mortality.

Prospects

Cobia is recognised as a finfish with emerging global potential for mariculture. Following successful development of cobia culture in Taiwan, this activity expanded very fast in south-east Asia, the Americas and the Caribbean regions. Cobia has all the qualities required for a successful species for aquaculture. The global aquaculture production of cobia has been increasing from 2003 onwards and the major contributors are China and Taiwan. It has been noted that rapid growth rate and good flesh quality of cobia make it one of the best species for future expansion of production. Increasing the supplies from aquaculture combined with effective marketing can substantially enhance cobia production in the near future. The present success in the captive breeding

and seed production in India can be considered as a milestone towards the development of lucrative cobia farming in the country. However, this is only a first step and standardisation of technologies for seed production and farming of cobia to suit our environmental conditions have to be further pursued on a priority basis so that India can also emerge as a contributor for cobia production in the near future.