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Broodstock development and controlled breeding of cobia *Rachycentron canadum* (Linnaeus 1766) from Indian seas

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ABSTRACT

Cobia, *Rachycentron canadum* has emerged as one of the topmost finfish species for mariculture. In India, cobia broodstock was developed and induced breeding was achieved for the first time at Mandapam Regional Centre of the Central Marine Fisheries Research Institute (CMFRI). The broodstock was developed in sea cages of 6 m diameter and 3.5 m depth. Sexes were separated about two months prior to the onset of breeding season and stocked in separate cages. During March 2010, a female with intra-ovarian egg diameter of 700 µ along with two males were selected for induced spawning. The brooders were induced with human chorionic gonadotropin (hCG) at doses of 500 IU per kg body weight for female and 250 IU per kg body weight for males. Spawning was noted after 39 h of intra-muscular injection. The total eggs spawned were estimated as 2.1 million. About 90% fertilization was recorded (fertilized eggs amounted to 1.9 million). The eggs were collected using a 500 µ mesh net and stocked in incubation tanks at varying densities. The eggs hatched after 22 h of incubation at a temperature range of 28-30 ºC. The percentage of hatching was 80% and the total number of newly hatched larvae was estimated as 1.5 million.

Keywords: Broodstock, Cobia, Induced breeding, *Rachycentron canadum* Sea-cage, Spawning

Introduction

Cobia (*Rachycentron canadum*) is considered as one of the most promising candidate species for warm water marine aquaculture in the world (Franks *et al.,* 2001; Liao, *et al.,* 2004; Benetti *et al.,* 2007, 2010), owing to its extraordinary growth rate, adaptability for captive breeding, low cost of production, good meat quality, high market demand especially for *sashimi* industry and overall aquaculture performance. Cobia, the only member of the family Rachycentridae, is found in the warm temperate to tropical waters of the West and East Atlantic, throughout the Caribbean and in the Indo-Pacific off India, Australia and Japan (Shaffer and Nakamura, 1989; DuPaul *et al.,* 1997). It is often described as Asian salmon since salmon farming has revolutionized cage culture in many temperate countries. During the last decade, several Asian countries have started raising cobia commercially. Being a tropical and subtropical species, the cobia industry is developing fast in south-east Asia. They are found throughout the water column and are caught in both coastal and continental shelf waters, though typically considered to be an offshore species. Research on captive broodstock development of cobia accelerated in the late 1990s with successful spawning (Arnold *et al.,* 2002). The species has protracted spawning season (March to September in Indian seas) and it can spawn in captivity. Sexual maturity is reported in 1-2 years old males and in females of 2-3 years, with females growing larger and faster with maximum sizes up to 60 kg. Under culture conditions, cobia can reach 3-4 kg body weight in one year and 8-10 kg in two years. The number of eggs produced in each spawning by a female weighing 15 kg ranges from 2 to 3 million (Xan, 2005). Hence it is felt that cobia is an ideal species for mariculture in India and research thrust is given on this species. It is well understood that the availability of sufficient quantities of hatchery produced seed is inevitable for the development of farming of the species. The broodstock development and controlled breeding is the first step towards the development of successful seed production technology and it was achieved at Mandapam Regional Centre of Central Marine Fisheries Research Institute (CMFRI), for the first time in India. This paper reports the experimental results on the development of cobia broodstock in open sea cages and induced spawning with hormonal manipulation.

Materials and methods

Fabrication and installation of sea cages

The site for sea cages was selected at a distance of about 300 m from the shore in the Gulf of Mannar region (N 9°16'8.9" to N 9°16'12.6" and E 79°7'87.8" to E 79°7'98.1"). The site was selected after conducting a detailed assessment of environmental parameters such as water currents, wave patterns and water quality. A minimum of 5.5 m depth during low tide was ensured at the site to facilitate efficient water exchange. A total of 5 nos. of circular cages of 6 m diameter and 3.5 m depth were fabricated and launched during November 2008. The collar of the cage was made up of HDPE pipe of 140 mm dia with outer (8 m dia) and inner rings (6 m dia). The outer side of the frame was fitted with HDPE net of stretched mesh size of 80 mm and the inner ring of the cage was fitted with HDPE net of 60 mm stretched mesh size with 5 and 4 mm twine thickness, respectively (Fig. 1).

Fig. 1. Broodstock sea-cages for cobia

Broodstock development in sea cages

Fishes were obtained from the commercial catches in hooks and line and transported in truck to the Mandapam Regional Centre of Central Marine Fisheries Research Institute. After transporting to hatchery, the fishes were treated with 100 ppm formalin for 2-5 min and quarantined for 48 to 72 h in 5 t capacity FRP tanks before transferring to cages. The fishes were stocked in sea cages without separating sexes. These fishes were fed twice daily at 09 00 hrs and 1530 hrs with sardines, squids and portunid crabs @ 2-5% of body weight. Vitamins and mineral supplements were also given twice in a week along with feed in order to complement any possible nutritional deficiencies in their diet. A total of 40 fishes were stocked in four cages. The length and corresponding weight of brood fishes recorded during April 2009 ranged from 80 to 127 cm and 4 to 20 kg, respectively.

The sexes were identified by gonadal biopsy using a flexible catheter (1 mm dia) and males and females were stocked in separate cages. Thereafter the females were cannulated every fortnight to ensure that the fish had fully developed oocytes which were in the final stages of maturation. A trial for determining the maturity of cobia conducted during the month of April 2009 revealed that the broodstock was with maturing gonads. For induced spawning experiment, female fish with the intra-ovarian eggs of around 700 µ size (Fig. 2) were selected and males were chosen based on the oozing of milt following slight abdominal massage.

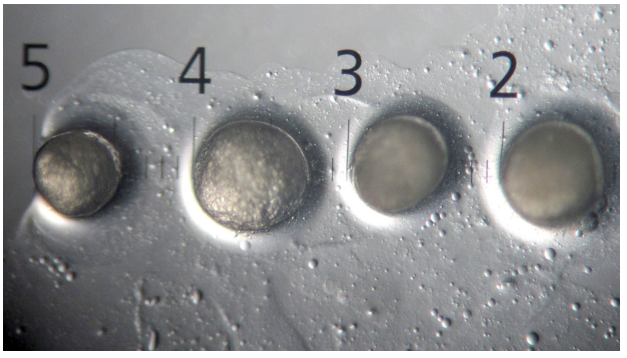

Fig. 2. Intra-ovarian eggs

Induction of spawning

The selected brooders were introduced into a roofed 100 m^3 capacity concrete tank filled with 65 m^3 of seawater. Seawater was initially pumped from the Gulf of Mannar and held under static conditions to allow particulates to settle out for at least 48 h before use. Two males and one female were selected for the experiment. The selected female brooder for induction was 120 cm in length and 23 kg in weight. The selected brood males measured 100 cm and 101 cm in length and weighed 11 kg and 13.5 kg, respectively (Fig. 3). Fish were not fed during the experiment. They were administered with human chorionic

Fig. 3. Brooders of cobia in the spawning tank

gonadotropin (hCG – FERTIGYN, Unimed Technologies Ltd., Gujarat, India) diluted in 0.9% NaCl solution and injected into the dorsal musculature for spawning experiments (Fig. 4). The optimum dosage of hCG was determined after conducting a number of trials on different dosages. A single intramuscular injection of human chorionic gonadotropin (hCG) was administered to the brooders at a dosage of 500 IU per kg body weight for female and 250 IU per kg body weight for males. Fish handling for hormone injections and follicle samplings were done under anesthesia (Aqui-S, New Zealand). The water quality parameters were monitored at regular intervals. The occurrence of spawning was monitored periodically by checking for the presence of eggs in the water column. As soon as spawning was confirmed, the fertilized floating eggs were collected by a net of 500 µ size (Fig. 5). The percentage of fertilized eggs was determined using a stereo microscope by randomly selected floating eggs one hour after spawning when the eggs were at the cleavage stage. The total number of eggs and fertilization rate were determined from three one litre aliquots.

Fig. 4. Administration of hormone

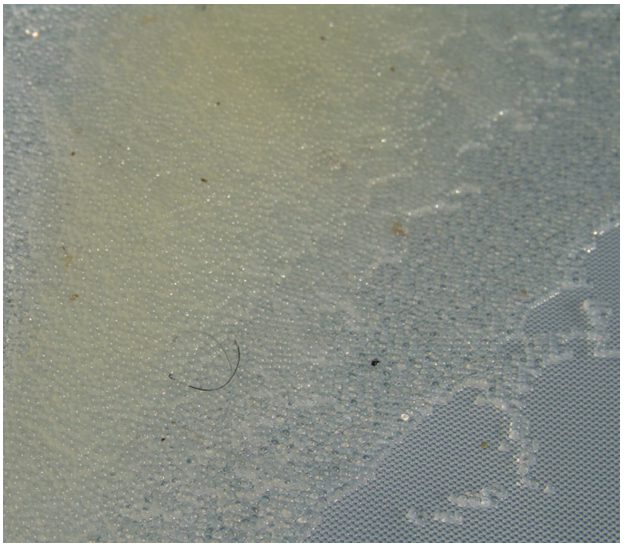

Fig. 5. Fertilized eggs collected from spawning tank

$$\text{Fertilization \%} = \frac{\text{No. of fertilized eggs}}{\text{No. of buoyant eggs}} \times 100$$

Results

Gonadal biopsy conducted during March and April 2009 revealed that the broodstock consisted of 31 males and 9 females. The weights of the brooders ranged from 8 to 25 kg for males and 6 to 23 kg for females. In the first experiment conducted during the month of June, one female with intra-ovarian egg diameter of 500 µ was selected for induced breeding experiment. Subsequent experiments on induced spawning were conducted using females with ova diameter of 600-700 µ. The average size of eggs released during spawning were 0.8 to 1.1 mm in one experiment and 1.0 to 1.1 mm in the other, but they all were unfertilized. In all the experiments, chasing and pairing behaviour were noted. The ranges of water quality parameters recorded in the spawning experiments were : water temperature 28.5-30 ºC; salinity 33-38 g l^{-1}; dissolved oxygen 4.67-5.72 mg l^{-1} and pH 8.0 - 8.3. The details of experiments and the results obtained are summarised in Table 1.

The sixth experiment conducted was successful, resulting in the production of fertilized eggs. In this experiment, intense chasing and pairing behaviour were noted prior to spawning. The female was followed by the two males in a swimming position which were closely below the lower abdomen of the female. The chasing became very intense 5-6 h before the actual time of spawning. The belly of the female became visibly enlarged as the spawning approached owing to the final maturation of oocytes. The spawning occurred at about 39 h after the administration of hormone.

The freshly spawned eggs measured 1.0 – 1.1 mm in dia meter. They were yellowish brown in colour with one prominent oil globule. All the fertilized eggs were found floating. The total number of eggs spawned was estimated to be around 2.1 million and the fertilized eggs were around 1.9 million with fertilization rate of 90%. The diameter of incubated eggs measured 1.2 to 1.3 mm.

Discussion

The capacity to produce large and dependable quality of cobia seeds is the key for establishing reliable and sustainable cobia aquaculture. One of the bottlenecks in the development of commercial aquaculture and a prerequisite for providing bio-secure and quality certified fry is the control of reproductive processes of fish in captivity (Zohar and Mylonas, 2001). Aquaculture research on cobia was first reported in 1975 with the wild collected cobia eggs off the coast of North Carolina. Research continued and by 1997 the technology to raise large

Table 1. Details of cobia breeding experiments at the Mandapam Regional Centre of CMFRI

Exp. No.	Month	Sex ratio M:F	Weight of females and males (kg)	Hormones and dosage (IU kg^{-1})	Time of spawning (h)	Result
1	June 2009	2: 1	F: 13.5 M: 17 and 18	hCG Femal e: 1000 Male: 500	No Spawning	No response even after 72 h of injection
2	August 2009	2:2	F: 16 and 18 M: 10 and 16	hCG Female: 1000 Male: 500	54	Eggs were released; ova diameter ranged from 0.8-1.1mm and with fully developed oil globule. Eggs were not fertilized.
3	August 2009	2:1	F: 11 kg M: 20.5 and 24	hCG Female: 500 Male: 275	48	Eggs were released; ova diameter ranged from 1.0-1.1mm and with fully developed oil globule; signs of fertilization noted but further embryonic development was arrested.
4	August 2009	2:1	F: 12.5 M: 14 and 18	hCG Female: 500 Male: 275	No Spawning	No response even after 72 h of injection
5	October 2009	2:1	F: 19 M: 16 and 20	LHRH*a* Female: 20 µg kg^{-1} Male: 10 ìg kg^{-1}	No Spawning	No response even after 72 h of injection
6	March 2010	2:1	F: 23 M: 11 and 13.5	hCG Female: 500 Male: 250	39	2.1 million eggs were spawned

quantities of cobia fry was developed and Taiwan Province of China is currently producing juvenile fish for grow-out mostly in near-shore cage systems (Su *et al.,* 2000; Liao *et al*. 2004; Yeh, 2000; Liao and Leano, 2005). Cobia production is also reported in the United States, Puerto Rico, Bahamas, Martinique, Belize, Brazil and Panama (Benetti *et al.,* 2008).

Broodstock management generally comprises collection, selection and domestication of brooders; control of maturation as well as spawning, egg collection and incubation (Liao *et al.,* 2001). Prior to 1980s, broodstock of finfishes were grown mainly in indoor concrete tanks. Since 1980s, wild-caught broodstock has been reared either in outdoor earthen ponds or in sea cages. It has been proved that cultivation of broodstock in sea cages is highly effective in improving gonadal development for most species like cobia, groupers, pompano, red seabream, Japanese flounder and yellow croaker (Hong and Shang, 2003). The present experiments on development of broodstock in sea cages were found to be successful, resulting in development of broodstock within a course of about one year. In general, many fish exhibit reproductive dysfunctions when reared in captivity, most commonly, females fail to undergo final oocyte maturation (FOM), and thus ovulation and spawning (Zohar, 1988, 1989a, b; Peter *et al.,* 1993). Several factors, including hormone dose, administration method and degree of ovarian development, have recently been shown to influence the efficacy of gonadotropic agents in stimulating ovulation (Mylonas and Zohar, 2001).

Human chorionic ginadotropin (hCG) was used for induction as it appeared more appropriate because it acts much faster, *via* direct stimulation of the gonad, in inducing FOM, spermiation and spawning (Hodson and Sullivan, 1993). The dosage of hCG in the breeding experiments was determined after conducting trials with different dosages. hCG is often given in a single dose, which ranges between 100 and 4000 IU per kg body weight (Zohar and Mylonas, 2001). A single and relatively low dose of hCG (275 IU per kg body weight) was also enough to induce ovulation in fish with post-vitellogenic oocytes (Caylor *et al.,* 1994). In the present study, LHRHa was also tried in one of the experiments, but there was no response and eventually the brooders died after the experiment. This may be attributed to the longer response time required for LHRHa. The long latency period between treatment and spawning may result in pre-spawning mortalities, due to stress induced by the capture of gravid broodstock, or the transport of cultured broodstock from cages to the hatchery (Hodson and Sullivan, 1993). A significant difference in fertility was observed between hCG and LHRHa, hCG being advantageous over LHRHa (Denson *et al.,* 2007).

In the breeding experiments conducted during the month of June 2009, spawning did not occur. The final maturation and spawning did not take place which may be attributed to the non-attainment of optimum size of oocytes. The suitability for spawning induction in females was found to be correlated with ovarian follicle size in *Pangasius*

bocourti (Cacot *et al.,* 2002). In the other two experiments, eventhough spawning occurred, the eggs were unfertilized. It might be due to the fact that the males would have failed to respond synchronously with the females. The absence of spawning in the last two experiments could be attributed to the sub-optimal size of the oocytes. The hormone would have failed to induce the final oocyte maturation (FOM) which might be due to specific receptor insufficiency.

In the sixth experiment, the cannulated eggs almost detached from ovarian follicle and the average diameter ranged from 700 to 800 µ. Hence, it can reasonably be assumed that the diameter of the egg plays a crucial role in the final oocyte maturation and the response to hormone for induction of spawning. In addition, the broodstock rearing in cages with adequate feeding seems to provide suitable conditions for maturation and a large number of brooders can be stocked in a limited water volume (Cacot *et al.,* 2002).

The present success in the broodstock development and fertilized egg production can be considered as a major step towards the development of commercial level seed production technology. It is well understood that the ready availability of cobia seed can only lead to the development of cobia aquaculture. The success in development of broodstock from in-shore facilities depend on the sophistication of the system with recirculation and raceway systems as practiced in the USA (Holt *et al.,* 2007). However, it is a capital intensive programme and hence the development of broodstock in cages which is widely practised in China, Vietnam and Taiwan is comparatively more economical. The present study also corroborates the same. However, for developing bio-secure broodstock, sophisticated land based recirculation systems may be required in future. The global aquaculture production of cobia has been increasing from the year 2003 onwards and the major contributors are China and Taiwan. The current status of cobia culture in Asia clearly indicates that farming of this species has a bright future. In this context, it is felt that development of cobia culture has good potential in India which can enhance mariculture production. Hence, development and standardization of technologies for seed production and farming of cobia by modifying the available technologies elsewhere to suit our environmental and socio-economic conditions have to be pursued on a priority basis so that India can also emerge as a major contributor for cobia production in the near future. In this context, the present success in the broodstock development, induced spawning and production of fertilized eggs is a major step forward to promote marine finfish farming.

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