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Captive broodstock development, induced breeding and embryonic development of Bengal yellowfin bream *Acanthopagrus datnia* (Hamilton, 1822)

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Abstract

The current study aim to develop the protocol for induced breeding of Bengal yellowfin bream *Acanthopagrus datnia* (Hamilton, 1822) in captivity. Adult male and female fish (weight range: 100-425 g; total length range: 90-260 mm) were implanted with luteinising hormone releasing hormone analog (LHRHa) pellet (dose: 33.33 μg pellet^{-1}) and reared in the brackishwater (4.33-9.67 ppt) for a year. Mature female (oocyte diameter, OD: 400.52-408.78 μm) were administered with different doses of aqueous LHRHa *viz.* 10, 20, 30 and 40 μg LHRHa kg^{-1} body weight. Simultaneously, oozing males were administered with half the dose of LHRHa. Male and female (sex ratio: 2:1) were placed in breeding tanks (salinity: 31±0.50 ppt) (n=3). The fish spawn after the latency period of 55.67±0.47 h. During the course of embryonic development, fertilised eggs (diameter: 859.64±17.21 μm) passes through morula, blastula, gastrula and organogenesis at 2:00, 2:15, 8:00 and 14:30 hours post spawning (hps), respectively. Larvae hatched out after an incubation period of 28±1.5 h (16.20^{0}C) and measured 1.70± 0.08 mm. Results showed that the *A. datnia* broodstock can be developed in brackishwater and spawn successfully with the dose of 30 μg LHRHa kg^{-1} body weight to females and half the dose to males at 31 ppt salinity.

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Introduction

Seabreams or porgies, belonging to the Family Sparidae is widely distributed along the coast of India, Pacific and Atlantic oceans (Sheaves, 2006) and are considered as commercially important aquaculture species in several parts of the world (Rahim *et al.,* 2017). Generally, yellowfin bream ovarian development is of asynchronous type, which spawn intermittently (serial / partial) during spawning season (Cowden, 1995; Black and Black, 2013). Ample research has been conducted to develop the induced breeding protocols of different species of commercially important yellowfin bream. In this line, captive breeding and seed production of *Acanthopagrus latus* (Leu and Chou, 1996), *A. berda* (Abbas *et al.,* 2019; Suresh Babu *et al.,* 2022), *A. australis* (Cowden, 1995; Black and Black, 2013) are well standardised.

Acanthopagrus datnia, senior synonym to *Acanthopagrus longispinnis* is widespread in estuarine and coastal areas of the Bay of Bengal (east coast of India, Bangladesh and Myanmar) and is commonly known as Bengal yellow fin bream (Hasan *et al.,* 2020). *A. datnia* spawns in the winter season (December- January) (Hasan, 2009). Due to the unavailability of hatchery produced seed, farming of *A. datnia* largely depends on wild seed resources. We have noticed that *A. datnia* showed signs of sexual maturation in brackishwater pond environment (salinity: 4.33-9.67 ppt) but does not reach the final stage of maturation, which might be due to a lack of spawning cues (Prem Kumar). These types of reproductive dysfunction of final oocyte maturation, ovulation and spawning can be resolved by environmental and hormonal manipulation (Zohar and Myloans, 2001). Further, researchers have reported that the slow delivery of GnRHa-cholesterol pellets accelerates the maturation and spawning of fish in captivity [Kanemaru *et al.,* 2012 (honeycomb grouper, *Epinephelus merra*), Yazawa *et al.,* 2015 (little tuna, *Euthynnus affinis*) Fakriadis *et al.,* 2019 (greater amberjack, *Seriola dumerili*) and Bera *et al.,* 2021 (milkfish, *Chanos chanos*)].

Quality seed production in captivity is a pre-requisite for the development of aquaculture of this species. With this background the current research work was conducted to develop the protocol for captive maturation of *A. datnia* in brackishwater through LHRHa implantation and induced breeding in normal seawater through the administration of aqueous LHRHa. Current work will serve as a basis for the development and establishment of commercially viable seed production technology for this species.

Materials and methods

Broodstock development

Broodstock development includes collection, quarantine, domestication, captive maturation and spawning (Ranjan *et al.,* 2019). Around three hundred adult *A. datnia* male and female fish (weight range: 100-425 g; total length range: 90-260 mm) were collected from the brackishwater tide fed canal, Kakdwip, West Bengal and transported to Kakdwip Research Centre of ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA) in oxygen packed polythene bags. Before stocking, fish were kept in 5000 l FRP (Fibre-reinforced plastic) tanks, given dip treatment with 50 ml l^{-1} formaldehyde for one minute (Kumar *et al.,* 2021) and transferred to a broodstock holding tank.

Recirculatory Aquaculture System (RAS)

An outdoor recirculatory aquaculture system (RAS) having water holding capacity of 50,000 l, subjected to natural photoperiods and ambient climatic conditions served as the broodstock holding tank . Dissolved oxygen (DO) was maintained between 6-8 ppm by delivering oxygen at the rate of 10 l m^{-1} (oxygen generator). The ideal environment for the captive maturation of *A. datnia* was maintained by the continuous operation of RAS. The components of the RAS were broodstock tank, egg collection chamber (500 l), electric pump (2 HP), rapid sand filter (10, 000 l h^{-1}, Waterco Pvt Ltd., Sydney, Australia) and biological filter (1,000 l, filled with 1,500 numbers of bio-balls and 50 oyster shells). The water flow of nearly 1000 l h^{-1} was maintained by adjusting the suction and outlet valve. Every day broodstock tank bottom was cleaned and the filter was backwashed to maintain ideal water quality of broodstock holding tank. Loss of water due to evaporation and cleaning was compensated by adding disinfected water of similar quality.

Feed and feeding

Fish were fed twice daily (08:00 and 16:00 hrs), first with commercial feed (size, 2.5 mm; protein 38 and lipid 6%) and later with chopped Bombay duck, *Harpadon nehereus* and tilapia (*Oreochromis niloticus*) to satiation. Following the procedure of Ranjan *et al.* (2018), around 500 g commercial feed was supplemented with cod liver oil (2 ml), vitamin A (500 IU), vitamin B-complex (100 mg), vitamin E (200 mg) and vitamin-mineral mix (100 mg) to avoid the possible nutritional deficiencies. All the vitamins and mineral-mix were dissolved in cod liver oil and mixed to feed thoroughly (Ranjan *et al.,* 2018).

Physico-chemical parameters of water

Water samples from broodstock holding tank were collected during morning (08:00 hrs) at monthly intervals to analyse the different physico-chemical parameters [DO, temperature, pH, salinity, total hardness, alkalinity, total ammonia nitrogen (TAN) and nitrite nitrogen (NO_2-N)]. DO, temperature, pH and salinity were measured with water quality measuring probe (HACH-HQ40d). Total hardness, alkalinity, TAN and NO_2-N were estimated following the standard methods (APHA, 1995). Above mentioned physico-chemical parameters were also measured in spawning tank.

Hormone pellet preparation and implantation

The hormone pellet was prepared following the methods of Sherwood *et al.* (1988), with slight modification. Cholesterol 80% (800 mg) and cellulose 20% (200 mg) were mixed thoroughly. One mg of LHRHa [des-Gly^{10}, D-Ala^{6}] (Sigma) was dissolved in 0.5 ml of 80% ethanol, which was then added to the cholesterol-cellulose matrix and mixed thoroughly. To make the mixture sticky, two drops of molten coco butter was added, mixed thoroughly and dried at room temperature. The paste was loaded into hand pelletiser and 30 cylindrical pellets, each weighing around 33.33 mg and measuring 2 mm in diameter and 8 mm in length, were prepared. The approximate calculated amount of LHRHa in each pellet was around 33.33 µg [1000 mg base materials (cholesterol and cellulose) contain one mg (1000 µg) LHRHa and the weight of each pellet was 33.33 mg, which contain approximately 33.33 µg LHRHa]. Prepared pellet was dried in oven at 37^{0}C and stored in air tight glass vials. The first and second implantations were made at inception of maturation, that is during September and November, respectively. As a control, un-implanted fish were marked with caudal fin clipping. Before implantation, fish were anesthetised with phenoxyethanol (dose: 0.3 ml l^{-1}) and implantation site was disinfected with 70% ethanol. After implantation, betadine ointment was applied to heal the implantation injury.

Ovarian biopsy

Maturity in males and females were examined from August onwards. Fish were anesthetised with phenoxyethanol (0.3 ml l^{-1}) before examination. Mature females were distinguished from males by their larger size and soft bulged belly. Males were examined for oozing milt by gently pressing the abdomen (Fig. 1a). Oocyte samples were collected from more than 25 females by performing an *in vivo* ovarian biopsy with a sterile polythene cannula (1.2 mm dia.) (Fig.1b). The oocyte diameter (OD) of more than 30 randomly collected oocytes was measured under a trinocular microscope (Radical RXLr-5, India) supported by software (ProgRes Capture 2.7). Based on the microscopic examinations of oocytes, they were classified into five different groups *viz.*, smaller oocytes (oogonia) (Stage I), pre-vitellogenic oocytes (Stage II), vitellogenic oocytes (Stage III), post-vitellogenic oocytes (Stage IV) and ripe oocytes (Stage V) as per earlier reports (Kumar *et al.,* 2020).

(a) (b)

Fig. 1. Maturity assessment of *A. datnia* (a) Oozing male, (b) Ovarian biopsy of female

Standardisation of hormone dose

Standardisation of hormone dose for the induced breeding experiment was conducted during the peak natural spawning season (December to January, 2022). One month before spawning season, oozing males (mean length: 140±12.5 mm; mean weight: 150±2.5 g) and mature females (mean length: 285±5.00 mm; mean weight: 375±10.5 g; OD: 400.52-408.78 µm) were selected from the broodstock tank. Three sets of males and females in a sex ratio of 2:1 (15 ripe females and 30 oozing males) were transferred to five separate spawning tanks (1000 l) having similar water salinity as of broodstock tank (6±0.50 ppt). The salinity of the spawning tank water was increased gradually at the rate of 2 ppt day^{-1} by adding sterile brine (90 ppt) to attain a salinity of around 31±0.50 ppt in 13-14 days. Physico-chemical parameters of spawning tank water were as follows: salinity 31±0.50 ppt; temperature 16±2^{0}C; total ammonia 0.02±0.01 ppm and DO 7.5±0.5 ppm. The water quality in the spawning tank was maintained by continuous operation of RAS and siphoning of 5 to 10% of bottom water daily to remove left-out feed and faecal matter. Fish were fed to satiation with trash fish, and disturbances were kept to a minimum. One day before hormone administration feeding was stopped. The maturity stage of females was confirmed through ovarian biopsy, whereas oozing males were selected by gentle pressure on the abdomen (Figs. 1a, b). Based on published literature on related species (Suresh Babu *et al.,* 2022), females with an OD range of 400.52-408.78 µm were selected for induced breeding. Following the method of Kumar *et al.* (2020), aqueous solution of LHRHa (1 mg) was prepared in 0.9% physiological saline and used for induced breeding of *A. datnia*. Females were randomly injected intramuscularly (i/m) with a single dose of physiological saline (control), 10, 20, 30 and 40 µg LHRHa kg^{-1} of body weight, simultaneously the males were injected with half the dose of females.

Breeding performance

To evaluate the breeding performance, three breeding trials were conducted simultaneously. In each breeding trial, almost similar size of mature brooder (male: female; 2:1) were used. The breeding performance of *A. datnia* was evaluated as per our earlier publications on *Mystus guio* and *Tenualosa ilisa* (Kumar *et al.,* 2018; 2021). Spawning (%) = (Number of spawned fish / Total number of fish injected) x 100; Latency period (h) = Time interval between injection and spawning; Relative fecundity (Number of egg spawned kg^{-1} fish) = Total numbers of egg spawned / Weight of female; Fertilisation rates (%) = (Numbers of fertilised eggs / Total numbers of eggs counted x 100; Incubation period = Time interval between spawning and inception of hatching; Hatching rates (%) = (Numbers of eggs that hatched / Total numbers of fertilised eggs) x 100. Thirty minutes after spawning, around 100 numbers of eggs (n=3) were collected from the incubation tank for calculation of fertilization rates. After 45 minutes from the inception of hatching, the hatching rate was determined from 100 numbers of eggs per sample (n=3). The average incubation time from three incubation tanks was calculated.

Spawning and incubation

With standardised hormone dose, induced spawning was executed in larger spawning tanks (capacity: 8,000 l), which were subjected to natural photoperiod and climatic conditions. The spawning tanks have the provision of RAS, which keep the water clean and maintain a constant water flow of 10 to 12 l min^{-1}. Egg incubation tanks (25 l, conical shape, black colour) were filled with clean, filtered and disinfected seawater of 31 to 32 ppt salinity. After spawning, the eggs were collected from the spawning tank using a fine mesh net (200 µm), disinfected with 20 ppm iodophore for five minutes and gently stocked in incubation tank at the density of 100 eggs l^{-1} (Anil *et al.,* 2019). Unfertilised eggs that settled to the bottom of the incubation tank were siphoned out.

Embryonic development

To study the embryonic development, around 30 eggs were collected from the incubation tanks at regular intervals and observed under the light microscope to examine the different stages of embryonic development *viz.,* fertilised eggs, cell division (2, 4, 8, 16 and 32 cells), morula, blastula, gastrula (epiboly), neurula, organogenesis and hatching. Until the blastomere stage, embryo was observed at 5 min intervals and then at 30 to 45 min intervals. Heart beat and twitching movement of the embryo was also recorded.

Statistical analysis

The mean and standard deviation (SD) for raw data were calculated using MS-Excel. Homogeneity of data was checked before performing statistical analysis by Levene's test (SPSS). Comparison of physico-chemical parameters of water between months was carried out through one-way analysis of variance (ANOVA). Further the comparison of OD value of different months (August to January) was made using ANOVA. All the statistical analyses were performed using SPSS 20.0 for windows. Turkey's HSD test was used for *post-hoc* comparison of the means ($p<0.05$).

Results and discussion

The Bengal yellowfin bream, *A. datnia* is a potential candidate species for brackishwater aquaculture owing to its market acceptance, economic value and euryhaline nature. The current study is the first report on captive broodstock development, induced breeding and embryonic development of *A. datnia*.

Physico-chemical parameters *viz.,* salinity, temperature, hardness, alkalinity, DO, pH, ammonia-N and nitrite-N of water measured in broodstock RAS are given in Figs. 2 to 5. Physico-chemical parameters of water in the broodstock holding tank were within the optimal range for euryhaline fish (Kumar *et al.,* 2023). This could be attributed to the ideal design and efficient working of RAS. A slope of 50 mm in the broodstock holding tank (water holding~ 50,000 l) towards central drainage and entry of water from biological filter in broodstock tank creates a whirling motion of water, which helps in the central collection and cleaning of waste. Variations in water temperature (summer, 30.93±0.43^{0}C; winter 16.20±0.30^{0}C) could be attributed to the seasonal effects. Salinity in broodstock RAS ranged between 9.67±0044 to 4.33±0.04 ppt. Gradual increase in salinity during June, July and August of the source water causes the increase in salinity and hardness (1,600 ppm) in broodstock tanks. Total ammonia-N and nitrite-N values were high during the summer months and low during winter months, which might be due to differences in metabolic rates, during summer and winter, respectively. However, there is no published literature to support this finding. The salinity of the spawning tank water was gradually increased at the rate of 2 ppt day^{-1} by adding sterile brine (90 ppt). The salinity of the breeding tank reached around 31±0.50 ppt in 14 days. Except for temperature (16±20^{0}C), physico-chemical parameters (salinity, 31±0.50 ppt; total ammonia, 0.02±0.01 ppm and DO, 7.5±0.5 ppm) of spawning tank water were within optimal range as reported for other closely related species like *Acanthopagrus* spp. (Mohammadi *et al.*, 2012) and *Acanthopagrus berda* (Abbas *et al.,* 2019). The ideal physico-chemical parameters are effected by the continuous operation of RAS and siphoning out of 5 to 10% of bottom water daily to remove left-out feed and faecal matter. In the current study, wide variation in temperature (lowest, 16.20±0.30 and highest 30.93±0.43) observed could be due to seasonal effect.

Results of the current study showed that the two successive LHRHa pellet (average weight: 0.02 g, diameter: 2 mm, length: 8 mm, hold approximately 33.33 µg of LHRHa) implantation at the dose of 33.33 µg implant^{-1} at stage I and II, respectively trigger the oocyte growth to stage V (OD: 400.52-408.78 µm) of *A. datnia* in brackishwater (4.33±0.04 to 9.67±0044 ppt), whereas un-implanted female of *A. datnia* does not grow beyond stage III. Similar to current finding, GnRHa pellet implantation induced maturation and spawning of many commercial fishes (*A. australis,* Cowden, 1995; honeycomb grouper, *Epinephelus merra* Kanemaru *et al.,* 2012; greater amberjack, *Seriola dumerili,* Fakriadis *et al.,* 2019; milkfish, *Chanos chanos*, Bera *et al.,* 2021). Oocyte development of captive reared *A. datnia* is shown in Figs. 6 and 7. In captivity, there were five stages of oocytes development *viz.* Stage I (oogonia cells, mean OD 57.47-86.85 µm, during August, September and October) (Fig. 7A), Stage II (pre-vitellogenic, mean OD 281.17 µm, during November) (Fig. 7B), Stage III (vitellogenic oocytes, mean OD 281.17 µm, during early December) (Fig. 7C), Stage IV (post-vitellogenic oocytes,

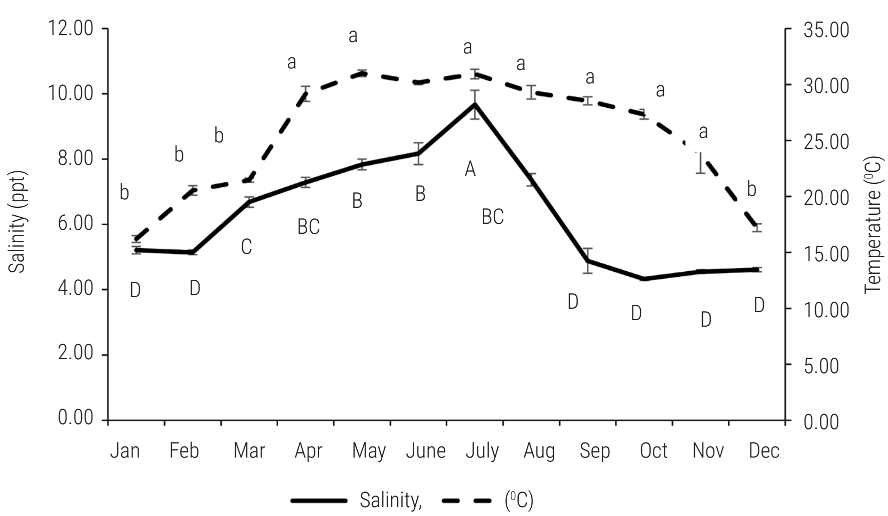

Fig. 2. Year-round variation in salinity (ppt) and temperature (^{0}C) in recirculatory aquaculture system (RAS) stocked with broodstock of *A. datnia*

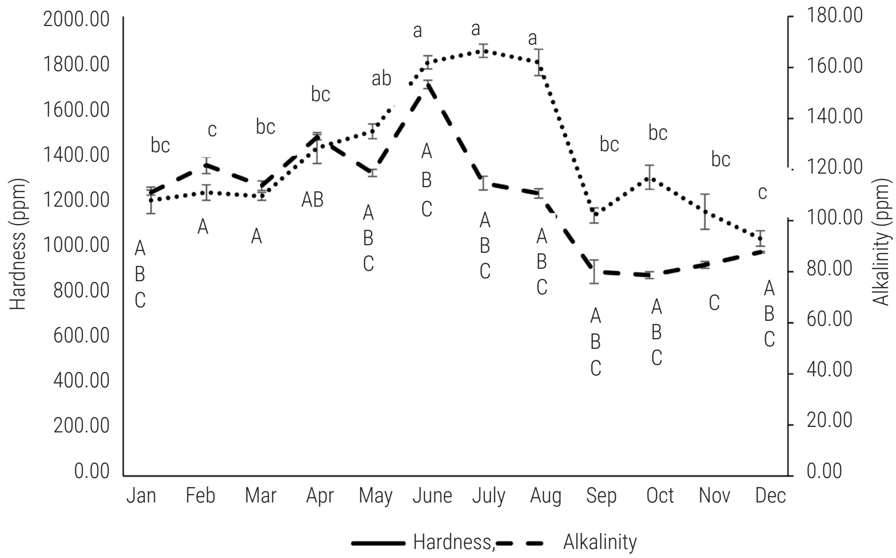

Fig. 3. Year-round variation in water hardness (ppm) and alkalinity (ppm) in RAS stocked with broodstock of *A. datnia*

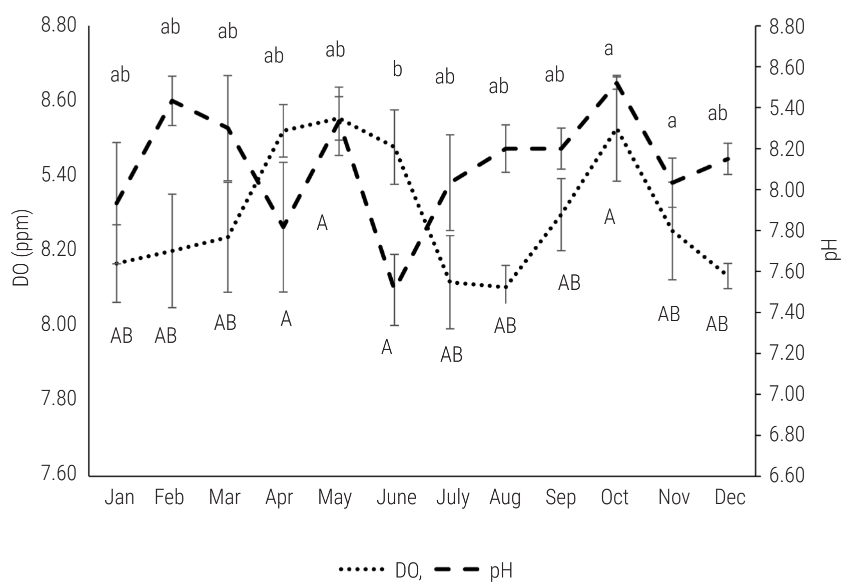

Fig. 4. Year-round fluctuation in dissolved oxygen (ppm) and pH in RAS stocked with broodstock of *A. datnia*

mean OD 385.15 µm, during mid-December) (Fig. 7D) and Stage V (ripe oocytes, mean OD 405.20-408.78 µm, during late-December to January end) (Fig. 7E). Ovulated oocytes about to release from oviduct are depicted in Fig. 7F. The current recorded OD for induced breeding of *A. datnia* was in the range of 400.52-408.78 µm, which is very close to the mature OD in *A. australis* (400 µm) (Cowden *et al.,* 1995) and *A. berda* (400-450 µm) (Suresh Babu *et al.,* 2022). Asynchronous type of oocyte development and multiple spawning is common in seabreams (Wallace and Selman, 1981) such as *A. latus* (Abou-seedo *et al.,* 2003) and *A. berda* (Suresh Babu *et al.,* 2022). In the current observation, spawning season of *A. datnia* coincides with low water temperature and short photoperiod, which is similar to the observation made in *A. australis* (Cowden, 1995), *A. datnia* (Hasan, 2009) and *A. berda* (Suresh Babu *et al.,* 2022).

Optimisation of hormonal dose for spawning of fish is essential for captive breeding, as any sub or supra-optimal dose of hormone leads to the failure of spawning in fish. In general, a lower dose leads to failure of spawning and a higher dose causes' counter reproductive effect (Kumar *et al.,* 2021). Different species of seabreams are bred in captivity with the administration of different types and doses of hormones. For example, *A. berda* spawns successfully with the administration of commercial hormones, containing gonadotropin releasing hormone analogues (GnRHa) (Abbas *et al.,* 2019, 0.25 ml kg^{-1}; Suresh Babu *et al.,* 2022, 0.25 ml kg^{-1}). *A. australis* spawned successfully with the administration of LHRHa (75 µg kg^{-1}) (Black and Black, 2013). *A. latus* spawned positively with the administration of ovaprim (0.5 ml kg^{-1}) (Leu and Chou, 1996).

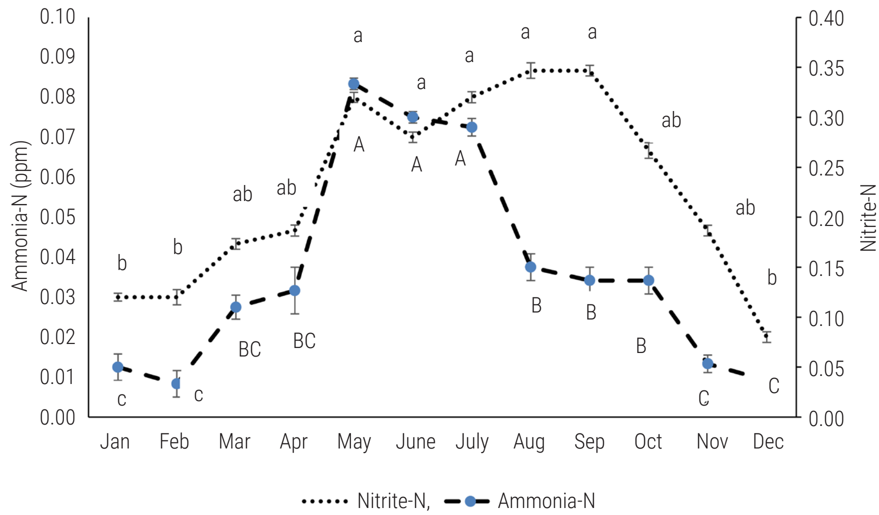

Fig. 5. Year-round changes in ammonia-N (ppm) and nitrite-N (ppm) in RAS stocked with broodstock of *A. datnia*

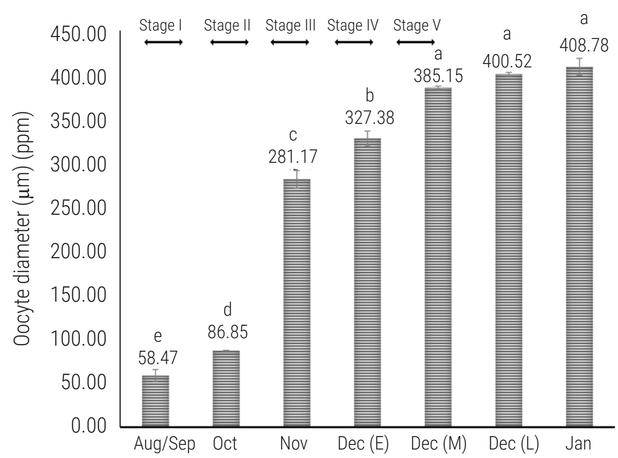

Fig. 6. Growth of *A. datnia* oocyte from August (inception of oogenesis) to January (final maturation)

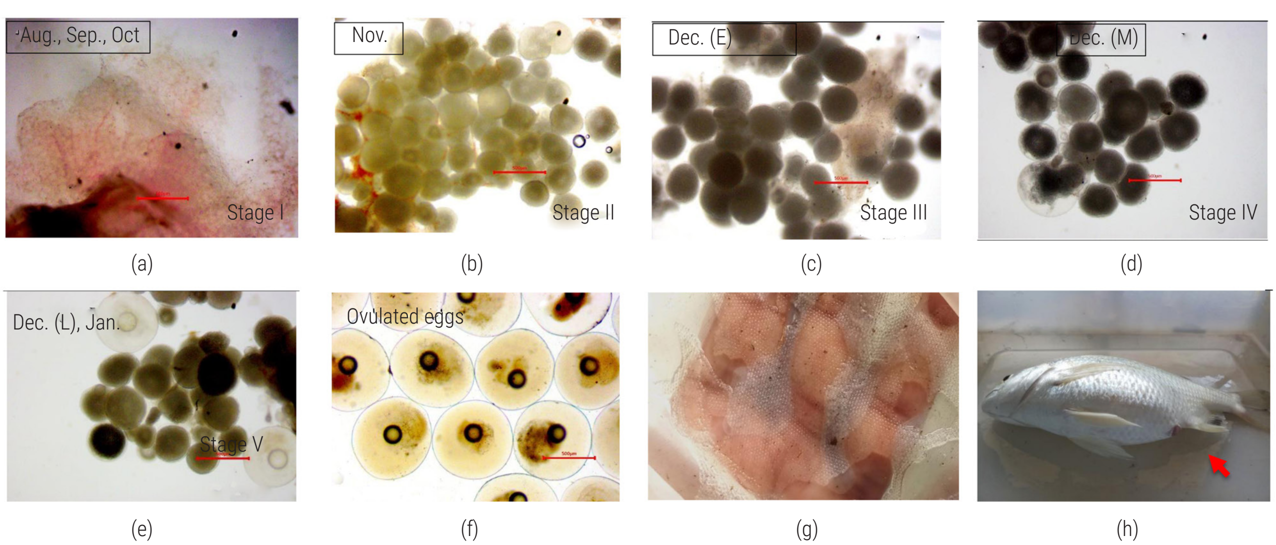

Fig. 7. Stages of *A. datnia* oocyte growth and development. (A) Stage I (oogonia cells, mean oocyte diameter 57.47-86.85 µm, (B) Stage II (pre-vitellogenic, mean OD 281.17 µm), (C) Stage III (vitellogenic oocytes, mean OD 281.17 µm), (D) Stage IV (post-vitellogenic oocytes, mean OD 385.15 µm), (E) Stage V (ripe oocytes, mean OD 405.20-408.78 µm), (F) Ovulated oocytes, (G) Spawned eggs, (H) Female with clogged vent

A. butcheri and *A. schlegeli* spawned with the administration of HCG (1000 IU kg^{-1}) (Haddy, 2000 and Huang *et al.,* 2000). GnRHa is found to be the most commonly used inducing hormone for the captive breeding of sparids (Black and Black, 2013; Abbas *et al.,* 2019; Suresh Babu *et al.,* 2022). In the present study, *A. datnia* did not respond to sub-optimal dose of LHRHa (10 μg kg^{-1}), which might be due to insufficient quantity of LHRHa. Mortality of males (100%) and females (66.66%) was high at a supra-optimal dose of LHRHa (40 μg kg^{-1}). The supra-optimal dose of LHRHa, would have accelerated the egg hydration due to a sharp increase in plasma gonadotropin. These hydrated oocytes would have clogged the genital pores and inhibited the spawning of *A. datnia*. Similar observation was made in *M. gulio* (Kumar *et al.,* 2021). In the current study, different doses of hormone (0, 10, 20, 30 and 40 μg LHRHa kg^{-1} body weight) used for induced breeding of *A. datnia* showed that the fish does not respond to a lower dose of 10 μg LHRHa kg^{-1} body weight. Partial spawning of 11.11% was observed at the dose of 20 μl LHRHa kg^{-1} body weight and 100% spawning was observed at the dose of 30 μg LHRHa kg^{-1} body weight (Fig. 7G). At a higher dose of 40 μg LHRHa kg^{-1} body weight, female did not spawn and died with a bulged vent (Fig. 7H). At this dose, around 66.66% mortality of females and 100% mortality of males were noticed. This optimal dose is slightly higher (15-20 μg kg^{-1}; Cowden, 1995) and lower than the dose required for spawning of closely related species, *A. australis* (75 μg kg^{-1}, Black and Black, 2013). The current optimal dose of LHRHa would be sufficient to stimulate the pituitary gland to secrete an adequate amount of gonadotropin that is needed for ovulation and spawning of *A. datnia,* and any sub and supra optimal dose causes poor spawning performance. The ideal salinity for breeding of *A. butcheri* reported was 10-35 ppt (Sherwood and Backhouse, 1982; Haddy and Pankhurst, 1998) and 11 to 35 ppt (Haddy and Pankhurst, 2000). In the present study, induced breeding and incubation were successful at the salinity of 31±0.50 ppt.

Breeding performance in terms of the latency period, relative fecundity, fertilisation rate (%), incubation period and hatching rate (%) of *A. datnia* was evaluated with an ideal hormonal dose of 30 μg LHRHa kg^{-1} body weight (Figs. 7G). After 24 h of post-injection, bulged bellies in female and darker anal fin in male were deciphered. Male chased female at interrupted time interval. Both male and female remained at the bottom of the spawning tank. Fish spawn during the night hours (21:00 h) after a latency period of around 55.67±0.47 h. Relative fecundity was 2.03±0.16 lakhs eggs kg^{-1}. The fertilisation rate (%) was 85.67±5.13. The incubation period ranged from 26 to 30 h with a mean value of 28±1.5 h. The hatching percentage ranged between 61 to 63% with a mean of 63±2.0%. The breeding performance of *A. datnia* in terms of the latency period, fertilisation rate and hatching rate was very close to that reported for *A. berda* (Abbas *et al.,* 2019; Suresh Babu *et al.,* 2022). In this study, *A. datnia* spawned after the latency period of 55.67±0.47 h, which is higher than the earlier finding of Black and Black (2013) in *A. ausralis* (36 h) and Suresh Babu *et al.* (2022) in *A. berda* (42 h). This slight variation in the latency period might be due to the difference in the size of mature oocytes and the physico-chemical parameters of water. The fertilisation rate depends on the maturation stage and OD of fish (Kumar *et al.,* 2020). In the current study, a fertilisation rate of 85.67±5.13% was recorded, which is slightly lower than the earlier report in *A berda* (Abbas *et al.* (2019, 92%) and Suresh Babu *et al.* (2022, 88%) and higher than the in *A. australis* Black and Black (2013, 70%). The incubation period recorded in sparids varied from 20 to 120 h, depending on water temperature and an inverse relation was found between the incubation period and temperature (Yufera *et al.,* 2011). Temperature is an important environmental parameter influencing embryonic development and the incubation period in *A. latus* (Bahmani et al., 2009). They observed that the difference of 3^0C makes a five hour difference in the incubation period. The incubation period recorded in the current study (28±1.5 h, 16.20^0C) was slightly longer than the record of Suresh Babu *et al.* (2022) (18-20 h at 28^0C) and shorter than the report of Abbas *et al.* (2019) (42 h, 22-24^0C) in *A. berda*. The hatching percentage of the fish egg depends on the physico-chemical parameters of water, especially temperature (Kumar *et al.,* 2019, 2020). At higher water temperatures, the incubation period is shorter, the embryo hatches faster and larval survival is greater (Olaniyi and Omitogun, 2013). In the present study, the hatching rate was 63±2.0%, which is slightly lesser than the earlier reports in *A. berda* (Abbas *et al.,* 2019, 42 h; Suresh Babu *et al.,* 2022, 80%) and *A. australis* (Black and Black, 2019). The longer incubation period (28±1.5 h) in the current study might be due to a lower water temperature of 16.20 ^{0}C. Relative fecundity of *A. australis* ranged between 7, 55, 000-1, 52, 000 kg^{-1} (Black and black, 2013) and *A. berda* 2, 50, 000 kg^{-1} (Suresh Babu *et al.,* 2022). In the current study, the relative fecundity of *A. datnia* was 2, 03, 000 kg^{-1} body weight of the fish. It is reported that *A. latus* needs more males to female ratio for breeding (Eskandari *et al.,* 2013), whereas *A. berda* needs a similar number of male and female (1:1) (Suresh Babu *et al.,* 2022). In the current study, male to female sex ratio was maintained at 2:1, which is the most commonly used sex ratio for captive breeding of fish.

The details of embryonic development from fertilised eggs to hatching with the respective time taken for each stage are summarised in Table 1 and Fig. 8. Knowledge of embryonic and larval development and organogenesis has an important role in understanding the basic biology of species (Borcato *et al.,* 2004). Embryonic development is a process in which cells differentiate and proliferate at different rates. In general, the process of fertilisation, division and morphological changes during embryonic development follows the common basic pattern in most of the teleosts. However, significant differences exist in yolk sac size, oil globules number, yolk composition, timing of organogenesis, incubation period and hatching process (Gomathi *et al.,* 2021). In the current study, the ontogeny of embryonic development of *A. datnia* was almost similar to the other breams such as *A. schlegeli* (Fukuhara, 1987), *A. berda* (Abbas *et al.,* 2019) and *A. latus* (Mohammadi *et al.,* 2012). The fertilised eggs of *A. datnia* were spherical (859.64±17.21 μm), pelagic, transparent and non-adhesive, with a narrow perivitelline space and single oil globule (190.55±3.84 μm) (Fig. 8a). Above attributes of fertilised egg of *A. datnia* are quite similar to other described sparids like *A. australis* (fertilised egg: 786.8±19.7 μm; oil globule: 186.2±7.4 μm) (Cowden, 1995), *A. latus* (fertilised egg: 739.35±0.0081 μm) (Bahmani *et al.,* 2009), *A. latus* (fertilised egg: 740.33±0.012 μm; oil globule diameter: 185±0.005 μm) (Mohammadi *et al.,* 2012) and *A. berda* (fertilised egg: 760±6 μm; oil globule diameter: 180±12 μm (Suresh Babu *et al.,* 2022). In *A. datnia,* the first cleavage was noticed after 0.45 hps at the animal pole (Figs. 8b, c, d and e). The cleavage produces almost equal size of many blastomere cells and give rise to the morula stage (2:0 hps) (Fig. 8f). Like other fishes, in the current study, the size of blastomere reduces with progress in division. The morula stage was characterised by the formation of a mulberry or half berry-like shape at the animal pole. These cleavage patterns of *A. datnia* are similar to other teleosts (Olaniyi and Omitogun, 2013). In the current study, the blastula stage of *A. datnia* initiated at 2:15 hps, which was characterised by the formation of yolk syncytial layer (YSL), blastodisc, blastoderm and blastocoel (Figs 8g, h and i). The above characters of blastula are similar to other teleosts like *Mystus gulio* (Kumar *et al.,* 2018) and *Tenualosa ilisha* (Kumar *et al.,* 2019).

In teleosts, the beginning and end of gastrula is characterised by the inception of epiboly and closure of blastopore, respectively. In current observation, during gastrulation, blastoderm cells proliferated and expanded towards the vegetal pole (epiboly). In this stage, epiboly of 30, 50 and 90% was deciphered in Figs. 8j, k and l, respectively. At the end of epiboly, a germ ring forms and blastopore closed, which marks the end of gastrulation (Fig. 8m). Neurula was characterised by the appearance of neural keel, polster and tail bud in the embryo (Fig. 8n). In *A. datnia,* epiboly starts at 8:00 hps and blastopore gets closed at 12:30 hps. Appearance of polster and tail bud are signs of inception of neurulation in teleosts. During neurulation, the polster and tail bud formed the cephalic and caudal parts, respectively (Olaniyi and Omitogun, 2013). In this study, formation of the tail bud and poster were noticed during 13:00 hps, which is similar to the report of Suresh Babu *et al.* (2023) in *A. berda* (12h at 28^{0}C). During somatogenesis, somite blocks formed and progressed to the caudal end. At 3-5 somites stage (14:30 hps), brain vehicle (neurocoele cells), kupffer cells, neural keel and caudal bud were clear (Fig. 8o). At 10-15 somites stage (16 hps), development of optic cups (optic vesicles) and notochord were perceived (Fig. 8p). At 16-18 somites stage (17 hps), formation of primitive heart, muscle and chromatophores were noticed (Fig. 8q). At this stage, inception of heart beats was recorded (20-25 min^{-1}). At 30 somites stage (19 hps), optic lance was formed, muscle twitched at the rate of 9-10 min^{-1}, and caudal region detached from yolk sac (Fig. 8r). After 20:15 hps, both heart beat (20-30 min^{-1}) and muscle twitching rate (15-20 min^{-1}) increased. At this stage, a well-distinguished tail, otolith bone, heart and enlarged optic lance were observed (Figs. 8s, t). After 22 hps, embryo muscle twitched at the rate of 40-60 min^{-1} and heart beat at the rate of 40-50 min^{-1}. At this age, two chamber hearts and tail fins were developed (Figs. 8u, v). After 24 hps, muscle twitched vigorously form the caudal end and heart beat at the rate of 90-100 min^{-1} (Fig. 8w). Hatching commenced after an incubation period of 28 h and almost all larvae hatched out of eggs within 15 min (Fig. 8x). Due to muscular twitching force, larvae broke the egg chorion and came out with head first (Fig. 8y). Newly hatched larvae ranged in total length between 1.68-1.71 mm (average 1.70± 0.08 mm) (Fig. 8z). In teleosts, during the early segmentation stage, somite block, polster, cephalic vehicle, auditory vehicle and Kupffer cells formed, and at the later segmentation stage, otolith, myotome and heart appeared (Kumar *et al.,* 2018, 2019). An almost similar observation was made in *A. datnia,* early-segmentation (before heart formation, 14:30 hps), which is characterised by the formation of somite blocks, brain vehicle, polster, optic vesicles, notochord

Table1. Key morphological characters of embryonic development in *A. datnia*

Stages	Average time [hours post-spawning (hps)]	Features of embryo
Spawning/fertilised egg	0	Transparent and floating
Two cells	00.45	First cleavage gave 2- blastomeres
16 cells	00:55	16- blastomeres
32 cells	01:15	32-blastomeres
Many cells	01:30	More than 64 blastomere cells
Morula	02:00	Many blastomere cells
Blastula		
Early blastula	02:15	Clear blastodisc with YSL
Mid blastula	03:30	Blastoderm appeared and spread
Late blastula	04:00	Blastocoel formed
Gastrula		
30% epibolly	08:00	Germ ring epiboled 30% of embryo
50% epiboly	10:00	Germ ring epiboled 50% of embryo
90% epiboly	12:00	Germ ring epiboled 90% of embryo
Germ ring	12:30	Closer of blastopore
Neurula	13:00	Polster (cephalic) and tailbud seen
Somatogenesis and organogenesis		
3-5 somites	14:30	Brain vehicle (neurocoele cells)
10-15 somites	16:00	Optic vehicle and notochord seen
16-18 somites	17:00	Heart beat started
30 somites	19:00	Optic lance appeared
20: 15 h old embryo	20:15	Otolith bone seen
22 h old embryo	22:00	Two chambered heart
24 h old embryo	24:00	Fast muscle twitching
28 h old embryo	28:00	Hatching
Hatching completion	28:15	Hatching completed

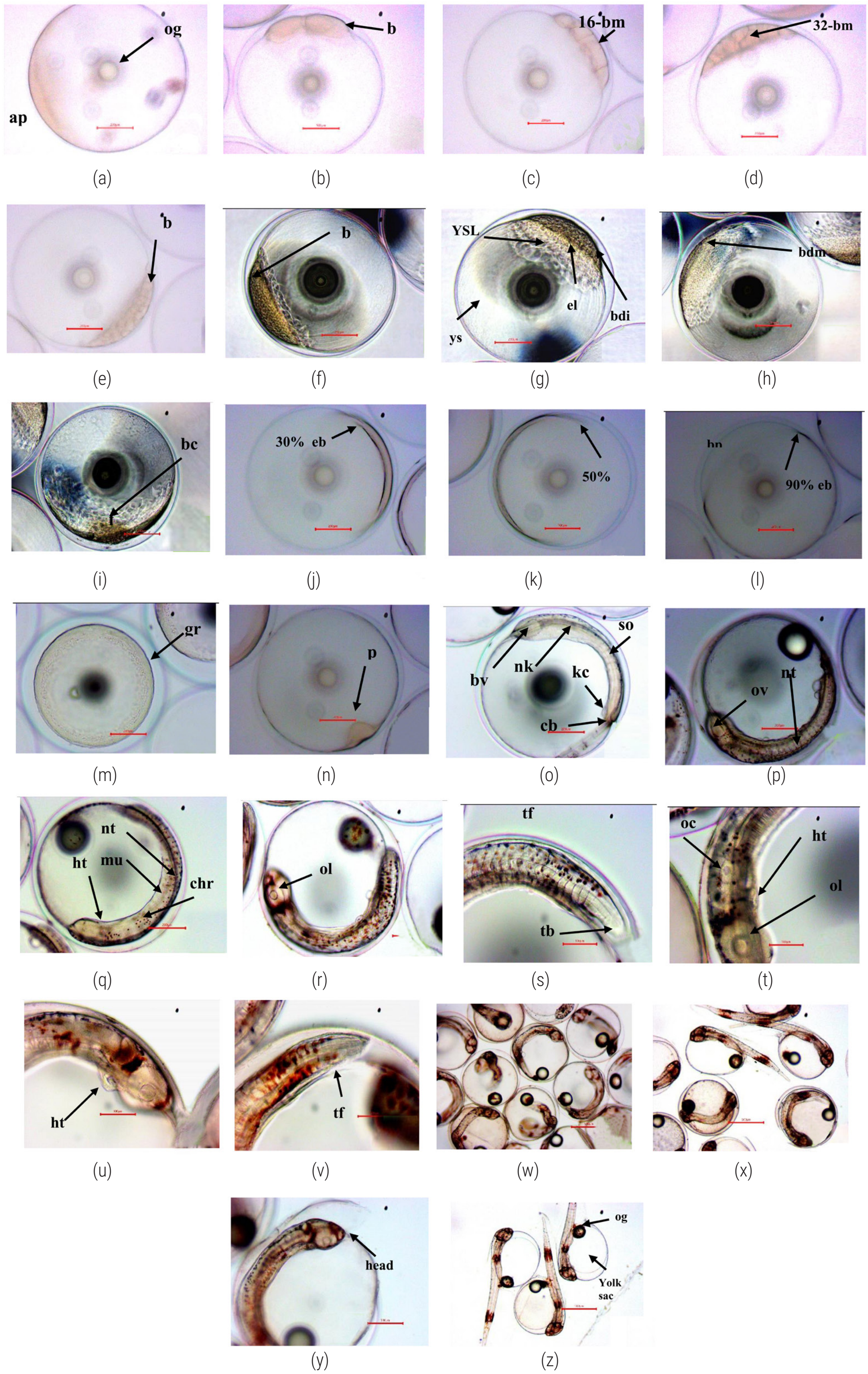

Fig. 8. Embryonic development of *A. datnia* at 16.20 ^{0}C. (a) Fertilised egg, (b) Two cells stage, (c) 16 cells stage, (d) 32 cells stage, (e) Many blastomeres cells, (f) Morula, (g) Early blastula, (h) Mid blastula, (i) Late blastula, (j) 30% epiboly, (k) 50% epiboly, (l) 90% epiboly, (m) End of gastrulation, (n) Neurula, (o) 3-5 somites, (p) 10-15 somites, (q) 16-18 somites, (r) 30 somites, (s) Tail bud, (t) Heart and otolith cup, (u) Developed heart, (v) Tail fin, (w) Vigorous twitching, (x) Hatching, (y) Head come out, (z) Newly hatched larvae. (ap - Animal pole; og - Oil globule; bm - Blastomeres; YSL - Yolk syncytial layer; ys - Yolk sac; el - Envelope layer; bdi - Blastodisc; bdm - Blastoderm; bc - Blastocoel; eb - Epiboly; gr - Germ ring; p - Polster; bv - Brain vesicle; nk - Neural keel; kc - Kupffer cells; so - Somites; cb - Caudal bud; ov - Optic vesicle; nt - Notochord; ht - Heart; mu - Musculature; chr - Chromatophores; ol - Optic lance; tb - Tail bud; oc - Optic cups; tf - Tail fin; og - Oil globule)

and Kupffer cells, and late-segmentation (after heart formation, 17:00 hps) characterised by the appearance of heart, myotome, ear bone (otolith) and chromatophores. In *A, datnia* the first somite was formed after completion of epiboly (14:30 hps). Similar observation has been made in *A. berda* (14 h, 28^{0}C) by Suresh Babu *et al.* (2022). In the current study, 30 somites were counted at 19 hps, whereas only 19 somites formation were reported by Abbas *et al.* (2019) at 19:30 h post fertilisation. In the present study, heart beat and muscular twitching increased with the progress of incubation, which is the same as reported in *M. gulio* (Kumar *et al.,* 2018) and *T. ilisha* (Kumar *et al.,* 2019). In the current study, muscle twitched at the rate of 90-100 min^{-1}. Hatching of larvae in fish is facilitated by muscular twitching and vigorous movement of the tail (Kumar *et al.,* 2018; 2019). In this study, vigorous twitching of the tail and muscle broke the egg chorion and larvae came out head first, which is similar to other teleosts (Langeland and Kimmel, 1997; Bera *et al.,* 2021) but contrary to *M. gulio* (Kumar *et al.,* 2018) and *T. ilisha* (Kumar *et al.,* 2021). Hatching commences after 28 h of incubation period and gets completed around 28:15 hps at a water temperature of 16.20±0.30, which is slightly more than the incubation period (26:15 h at 24^{0}C) reported in *A. latus* (Mohammadi *et al.,* 2012) and *A. berda* (20 h at 28^{0}C) (Suresh Babu *et al.,* 2022). This might be due to a lower water temperature of 16^{0}C. Bahmani *et al.* (2009) reported that the egg incubation in *A. latus* was significantly influenced by water temperature. They reported incubation period of 31:15 h and 26:15 h at 20±1^{0}C and 23±1^{0}C, respectively. Newly hatched larvae of *A. datnia* had a single large yolk sac and a smaller oil globule at the posterior end of the yolk sac. The larvae were pigmented with presence of chromatophores. The total length of newly hatched larvae ranged between 1.68-1.71 mm (average 1.70± 0.08 mm). Similar features of larvae were reported in *A. berda* (total length, 1.70±0.14 mm Suresh Babu *et al.* (2022) and in striped seabream, *Lithognathus mormyrus* (1.74±0.03) by Firat *et al.* (2005). In the current study, larvae could not survive beyond two days which might be due to low water temperature caused by severe cold waves. Further detailed investigation is required to standardise the larval rearing of *A. datnia*.

The present study is the first report on captive broodstock development, gonad maturation, induced spawning and embryonic development of *A. datnia*. The results of the study clearly indicated that brackishwater reared broodstock of *A. datnia* can be triggered to spawn administering LHRHa at the dose of 30 μg kg^{-1} to females and half the dose to males in seawater (31ppt).

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References

Abbas, G., Kasprzak, R., Malik, A., Ghaffar, A., Fatima, A., Hafeez-ur-Rehman, M., Kausar, R., Ayub, S. and Shuaib, N. 2019. Optimised spawning induction of blackfin sea bream, *Acanthopagrus berda* (Forsskal, 1775) in seawater ponds using Ovaprim hormone, with general remarks about embryonic and larval development. *Aquaculture*, 512: 734387. https://doi.org/10.1016/j.aquaculture. 2019.734387.

Abou-seedo, F. S., Dadzie, S. and Al-Kanaan, K. A. 2003. Sexuality, sex change and maturation Patterns in the yellow fin sea bream, *Acanthopagrus latus* (Teleostei: Sparidae) (Hostuyn, 1782). *J. Appl. Ichthyol.*, 19: 65-73. https://doi.org/10.1046/j.1439-0426.2003.00355.x.

Anil, M. K., Gomathi, P., Sugi, V. V., Raheem, P. K., Raju, B., Ambarish P. Gop., Santhosh, B., Philipose, K. K., Gopakumar, G. and Gopalakrishnan, A. 2019. Captive maturation, breeding and seed production of Pink ear emperor, *Lethrinus lentjan* (Lacepede, 1802) (Family: Lethrinidae) in recirculating aquaculture system (RAS). *Aquaculture*, 503: 207-216 https://doi.org/10.1016/j.aquaculture.2018.12.084.

APHA 1995. *Standard methods for the examination of water and wastewater*, 19th edn. American Public Health Association and Water Pollution Control Federation, Washington Dc, USA.

Bahmani, M. A. H. M. O. U. D., Sarvi, E., Kazemi, R. and Sarvi, F. 2009. A study on embryonic development of yellow fin seabream (*Acanthopagrus latus*). *Iran. J. Fish. Sci.*, 18(1), 33-42.

Bera, A., Kailasam, M., Mandal, B., Padiyar, A., Ambasankar, K., Sukumaran, K., Makesh, M., Kumararaja, P., Subburaj, R., Thiagarajan, G. and Vijayan, K. K. 2021. Maturity induction and extended spawning kinetics of milkfish (*Chanos chanos*) administered with combined GnRHa and 17α-methyl testosterone pellet at varied frequencies. *Aquaculture,* 543: 736993. https://doi.org/10.1016/j.aquaculture.2021.736993.

Black, B. J. and Black, M. 2013. Efficacy of two exogenous hormones (GnRHa and hCG) for induction of spontaneous spawning in captive yellowfin bream, *Acanthopagrus australis* (Sparidae) and influence of sex ratio on spawning success. *Aquaculture*, 416: 105-110. https://doi.org/10.1016/j.aquaculture.2013.08.036.

Borcato, F. L., Bazzoli, N. and Sato, Y. 2004. Embriogenesis and larval ontogeny of the 'Piau-gordura', *Leporinus piau* (Fowler) (Pisces, Anostomidae) after induced spawning. *Revista Brasileira de Zoologia*, 21: 117-122. https://doi.org/10.1590/S0101.

Cowden, K. L. 1995. *Induced spawning and culture of yellowfin bream, Acanthopagrus australis (Günther, 1859) and mangrove jack, Lutjanus argentimaculatus (Forsskal, 1775)*.Doctoral dissertation, James Cook University, Australia.

Eskandari, G. H., Saghavi, H., Najafabadi, M. Z., Madiseh, S. D. and Koochaknejad, E. 2013. Effect of salinity on reproductive performance of *Acanthopagrus latus* (Houttuyn) in spawning tanks. *Aquac. Res.*, 44: 588-595. https://doi. org/10.1111/j.1365-2109.2011.03062.x.

Fakriadis, I., Lisi, F., Sigelaki, I., Papadaki, M. and Mylonas, C. C. 2019. Spawning kinetics and egg/larval quality of greater amberjack (*Seriola dumerili*) in response to multiple GnRHa injections or implants. *Gen. Comp. Endocrinol.*, 279: 78-87. https://doi.org/10.1016/j.ygcen.2018. 12.007.

Firat, K., Saka, S. and Kamaci, H. O. 2005. Embryonic and larval development of striped sea bream (*Lithognathus mormyrus* l 1758). *Isr. J. Aquac. Bamidgeh*, 57(2): 131-140. https://doi. org/10.46989/ 001c.20399.

Fukuhara, O. 1987. Larval development and behavior in early life stages of black sea bream reared in the laboratory. *Nippon Suisan Gakkaishi*, 53: 371-379.

Gomathi, P., Siju, R., Anil, M. K., Ambarish, G. P., Surya, S., Raju, B., Raheem, P. K., Ignatius, B. and Gopalakrishnan, A. 2021. Embryonic and larval development of Pink ear emperor, *Lethrinus lentjan* (Lacepede, 1802) under captive conditions., *Aquac. Res.,* 52(11): 5857-5869. https://doi.org/10.1111/are.15461.

Haddy, J. A. and Pankhurst, N. W. 2000. The effects of salinity on reproductive development, plasma steroid levels, fertilisation and egg survival in black bream *Acanthopagrus butcheri*. *Aquaculture*, 188(1-2): 115-131. https://doi.org/10.1016/S0044-8486(00)00326-4.

Haddy, J. A. and Pankhurst, N. W. 1998. Annual change in reproductive condition and plasma concentrations of sex steroids in black bream, *Acanthopagrus butcheri* Munro. Sparidae. *Mar. Freshw. Res.*, 49: 389-397.

Hasan, M. E., Durand, J. D. and Iwatsuki, Y. 2020. *Acanthopagrus datnia* (Hamilton, 1822), a senior synonym of *Acanthopagrus longispinnis* (Valenciennes,1830) (Perciformes: Sparidae). *Zootaxa*, 4750(2): 151-181. https://doi.org/10.11646/ zoota xa.4750.2.1.

Hasan, M. E. 2009 *Some aspects of the reproductive biology and ecology of a sparid, yellowfin seabream (Acanthopagrus latus) in two estuaries of the south-east coast of Bangladesh*. M. Phil Thesis, Institute of Marine Sciences and Fisheries, University of Chittagong, Chittagong, *Bangladesh*, 100 p.

Huang, W. B., Chiu, T. S. and Shih, C. T. 2000. Effects of salinity on eggs and early larval characteristics of the black porgy *Acanthopagrus schlegeli*. *Isr. J. Aquac. Bamidgeh.* 52: 61-69.

Kanemaru, T., Nakamura, M., Murata, R., Kuroki, K., Horie, H., Uchida, K., Senthilkumaran, B. and Kagawa, H. 2012. Induction of sexual maturation of the female honeycomb grouper, *Epinephelus merra*, in the non-breeding season by modulating environmental factors with GnRH analogue implantation. *Aquaculture*, 358: 85-91. https://doi.org/10.1016/j.aquaculture.2012.06.026.

Kumar, P., Behera, P., Biswas, G., Ghoshal, T. K., Das, S., Kailasam, M. and Jithendran, K. P. 2023. Gonad recrudescence and annual sex steroid patterns in female goldspot mullet, *Liza parsia* reared in brackishwater pond. *Anim. Reprod. Sci.,* 248: 107161. https://doi.org/10.1016/j.anireprosci.2022.107161.

Kumar, P., Biswas, G., Ghoshal, T. K., Christina, L., Behera, P. and Kailasam, M. 2020. Effect of salinity acclimatisation and administration of hormones on oocyte maturation, ovulation and spawning of goldspot mullet *Liza parsia* (Hamilton, 1822) reared in brackishwater pond. *Indian J. Fish.,* 67(1): 56-63. https://doi.org/10.21077/ijf.2019.67.1.90246-08.

Kumar, P., Biswas, G., Ghoshal, T. K., Kailasam, M., Christina, L. and Vijayan, K. K. 2019. Current knowledge on the biology, captive breeding and aquaculture of the brackishwater catfish, *Mystus gulio* (Hamilton, 1822): A review. *Aquaculture*, 499: 243-250. https://doi.org/10.1016/j.aquaculture.2018.09.045.

Kumar, P., Biswas, G., Ghoshal, T. K., Kailasam, M. and Vijayan, K. K. 2018. Embryonic and larval developments of brackish water catfish, *Mystus gulio* (Hamilton and Buchanan, 1822) induced with human chorionic gonadotropin and consequent larval rearing. *Aquac. Res.,* 49(7): 2466-2476. https://doi.org/10.1111/are.13706.

Kumar, P., Kailasam, M., Biswas, G., Christina, L., and Ghoshal, T. K. 2021. Effect of different inducing hormones, sex ratio and oocyte diameter on breeding performance of brackishwater catfish, *Mystus gulio*. *Aquaculture,* 530: 735821. https://doi.org/10.1016/j.aquaculture.2020.735821.

Langeland, J and Kimmel, C. B. 1997. The embryology of fish. In: Gilbert, S. F. and Raunio, A. M. (Eds.), *Embryology: Constructing the organism.* Sinauer Associates, Sunderland, Massachusetts, USA, pp. 383-407.

Leu, M. Y. and Chou, Y. H. 1996. Induced spawning and larval rearing of captive yellowfin porgy, *Acanthopagrus latus* (Houttuyn). *Aquaculture,* 143(2): 155-166. https://doi.org/10.1016/0044-8486 (96) 01272-0.

Mohammadi, G., Ghiasabadi, E. S., Bahmani, M. and Khodadadi, M. 2012. Study of embryonic development and evolution stages of Yellow fin sea bream (*Acanthopagrus latus*). *Int. J. Agric. Crop sci.,* 4(20): 1499-1503.

Olaniyi, W. A. and Omitogun, O. G. 2013. Stages in the early and larval development of the African catfish *Clarias gariepinus* (Teleostei, Clariidae). *Zygote*, 22: 314-330. https://doi.org/10.1017/S0967199413000063.

Rahim, A., Abbas, G., Ferrando, S., Gallus, L. and Ghaffar, A. 2017. Assessment of growth performance and meat quality of black fin sea bream, *Acanthopagrus berda* (Forsskal, 1775) reared in brackishwater ponds: A preliminary investigation. *Pak. J. Zool.*, 49: 869-876. https://doi.org/10.17582/journal.pjz/2017.49.3.869.876.

Ranjan, R., Megarajan, S., Xavier, B., Ghosh, S., Santhosh, B. and Gopalakrishnan, A. 2018. Broodstock development, induced breeding and larval rearing of Indian pompano, *Trachinotus mookalee*, (Cuvier, 1832). A new candidate species for aquaculture. *Aquaculture,* 495: 550-557. https://doi.org/10.1111/are.13409.

Ranjan, R., Megarajan, S., Xavier, B., Raju, S. S., Ghosh, S. and Gopalakrishnan, A. 2019. Design and performance of recirculating aquaculture system for marine finfish broodstock development. *Aquac. Eng.*, 85: 90-97. https://doi.org/10.1016/j.aquaeng.2019.03.002.

Sheaves, M. J. 2006. Is the timing of spawning in sparid fishes a response to sea temperature regimes? *Coral Reefs,* 25: 655-669. https://doi.org/10.1007/s00338-006-0150-5.

Sherwood, J. E. and Backhouse, G. N. 1982. Hydrodynamics of salt wedge estuaries implications for successful spawning in black bream (*Acanthopagrus butcheri*). *Research Report*, 82/3: 1-18.

Sherwood, N. M., Crim, L. W., Carolsfeld, J. and Walters, S. M. 1988. Sustained hormone release. I. Characteristics of *in vitro* release of gonadotropin-releasing hormone analogue (GnRH-A) from pellets. *Aquaculture,* 74(1-2): 75-86. https://doi.org/10.1016/0044-8486(88)90088-9.

Suresh Babu, P. P., Anuraj, A., Shilta, M. T., Asokan, P. K., Vinod, K., Praveen, N. D., Srinivasa Rao, K., Vaidya, N. G., Sonali, S. M., Pal, M. and Loka, J. 2022. Broodstock development, breeding and larval rearing of *Acanthopagrus berda* (Forsskal, 1775), a suitable species for farming in tropical waters. *Aquac. Res.,* 53(18): 6439-6453. https://doi.org/10.1111/are.16114.

Suresh Babu, P. P., Anuraj, A., Shilta, M. T., Asokan, P. K., Vinod, K., Praveen, N. D., Srinivasa Rao, K., Vaidya, N. G., Sonali, S. M., Pal, M. and Loka, J. 2022. Broodstock development, breeding and larval rearing of *Acanthopagrus berda* (Forsskal, 1775), a suitable species for farming in tropical waters. *Aquac. Res.,* 53(18): 6439-6453. https://doi.org/10.1111/are.16114.

Wallace, R. A. and Selman, K. 1981. Cellular and dynamic aspects of oocyte growth in teleosts. *Am. Zool.,* 21: 325-343. https://doi.org/10.1093/icb/21.2.325.

Yazawa, R., Takeuchi, Y., Amezawa, K., Sato, K., Iwata, G., Kabeya, N. and Yoshizaki, G. 2015. GnRHa-induced spawning of the eastern little tuna (*Euthynnus affinis*) in a 70 m^3 land-based tank. *Aquaculture,* 442: 58-68. https://doi.org/10.1016/j.aquaculture.2015.01.016.

Yufera, M., Conceicao L. E. C, Battaglene, S., Fushimi, H. and Kotani, T. 2011. Early development and metabolism In: Pavlidas A., Mylonas. C. C. (Eds.), *Sparidae: Biology and aquaculture of Gilthead seabream and other species,* 1st edn. Blackwell Publishing Ltd., Oxford, UK. pp.133-168. https://doi.org/10.1002/9781444392210.ch5.

Zohar, Y. and Mylonas, C. C. 2001. Use of GnRHa-delivery systems for the control of reproduction in fish. *Rev. Fish Biol. Fish*., 10: 463-491. https://doi.org/10.1023/A:1012279814708.