South Asian J Exp Biol; 12 (2): 203-212; 2022 [DOI: 10.38150/sajeb.12(2).p203-212]

South Asian Journal of Experimental Biology

ISSN: 2230-9799 Vol. 12, Issue 2 Page 203-212 http://www.sajeb.org

REGULAR ARTICLE

The effects of Synthetic gonadotropin releasing hormone analogue (S-GnRHa) on artificial propagation of spotted scat, *Scatophagus argus* (Linnaeus, 1766)

Mizanur Rahman Washim†, Shawon Ahmmed†, AKM Shafiqul Alam Rubel, Debashis Kumar Mondal, Nilufa Begum, Md. Latiful Islam*

Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna-9280, Bangladesh
†First author

ARTICLE INFO

Article History:
Received: 5 Dec 2021
Revised: 14 Feb 2022
Accepted: 17 Feb 2022

**Corresponding Author:*
Email: latiful.bfri@gmail.com
Telephone: +880-01715-645260

Keywords: Spotted scat, S-GnRHa, Induced breeding, Hormone, Latency time

ABSTRACT

Spotted scat *Scatophagus argus* is an important brackishwater species predominantly inhabited in south-west coast of Bangladesh. The aim of this study was to develop a hormonal induced reproduction protocol of farm-domesticated *S. argus* along with larvae rearing techniques. Synthetic gonadotropin releasing hormone analogue (S-GnRHa) was injected at the dose of 60:30 µg/kg BW (Female: Male). In female fish 20% of the total dose was applied as priming dose and 12 hours later 80% as resolving dose. The latency period ranged from 32 to 36 h was determined by observing the time between first injection and ovulation. However, after achieving full oocyte maturation and ovulation, eggs were collected by stripping and fertilization was carried out using pooled sperm from male. The mean ovulation, buoyancy, fertilization and hatching rate were found 70±1.8; 68±7.7; 40±2.3% and 53±5.2% respectively. Over the embryonic development following stages were witnessed in embryos: meroblastic cleavage (2-cell), blastula, gastrula and pharyngula. Maximum embryos hatched within 19 hr post fertilization at 28±2.0°C temperature and 28±2.0 ppt salinity. In this study, we dispense the first information on the reproduction of spotted scat in captive condition using S-GnRHa and the optimal dose limit and larval rearing techniques are established. Further studies are warranted to develop the larvae into juvenile stage under variable environmental conditions to increase the fertilization, hatching rate and survival of embryos.

1. Introduction

Spotted scat is a euryhaline teleost which is locally called “Chitra” or “Bistara” fish and mostly dwelling in near shore waters of south-west coastal Bangladesh; and ubiquitously distributed throughout the brackish and marine habitats of Indo-Pacific zone (Ni and Kwok, 1999; Ghazilou et al., 2011; Sivan and Radhakrishnan, 2011; Gandhi et al., 2014). This species is a popular candidate in brackishwater aquaria (Amarasinghe et al., 2002; Shao et al., 2004); as economically important farmed fish (Wei et al., 2017; Yang et al., 2020) and is also an important food fish in east and South-east Asia (Musikasung et al., 2006; Sivan and Radhakrishnan, 2007; Wongchinawit, 2007).

Experiences suggested that *S. argus* fish fry has

immense demand to our coastal farmers since they have been culturing it with shrimp and other brackish water finfishes from many years back. Therefore, along with increased harvesting of fry and various anthropogenic interferences such as, destructive fishing pressure, habitat degradation, climate change etc. clearly threatens its natural stock (Gupta, 2016). The most effective way to conserve its wild populations by restocking and for sustainable aquaculture, development of artificial mass seed production technology in controlled condition is the primary prerequisite that would certainly minimize the fishing pressure (Rahdari et al., 2014; Xie et al., 2014; Su et al., 2019). This study is the only single successful report on induced spawning of *S. argus* with applying S-GnRHa hormone.

Bangladesh Fisheries Research Institute (BFRI) have already been developed artificial propagation and mass seed production techniques of some commercially important brackishwater finfishes like Long whiskers catfish (*Mystus gulio*) and Green back mullet (*Chelon subviridis*) which has enormously been reinforcing our coastal aquaculture industry. This earlier success has driven us to take endeavor for the development of artificial reproduction of *S. argus*.

In the past scanty efforts were undertaken to bred them artificially in hatcheries (Barry et al., 1993; Chang and Hsieh, 1997; Liao et al., 2001; Cai et al., 2010), but till date most of the seed used in aquaculture are being supplied from the wild capture (Huang et al., 2019). Obviously, obtaining spotted scat offspring via artificial breeding is quite difficult because of the different gonad maturation time between the sexes due to protandry (Chen et al., 2015). More importantly, one of the limiting factors for successful artificial fertilization is post-ovulation oocyte ageing in fish ovaries (Bahre Kazemi et al., 2010). However, prior to initiate artificial fertilization, it is especially crucial to know the peak breeding season, true hormone for its inducement with their effective doses, latency time, the time interval between hormonal injection and stripping, when conducting artificial fertilization (Yu et al., 2017). In our reproductive biology study (2017-2019), on the basis of gonado-somatic index (GSI) the peak breeding season of *S. argus* is marked between May-July (Unpublished data). In line with this, available information has also confirmed that, their peak spawning time is June-July (Barry and Fast, 1992) in Philippines; May -July in China (Ze-ping et al., 2010) and June-August in India (Gandhi et al., 2014). So far, our knowledge, there is acute paucity of information on the complete artificial breeding protocol inducing with salmon gonadotropin-releasing hormone analog (S-GnRHa). A female spotted scat was reported to ovulate using synthetic hormone doses (100 µg/kg luteinizing hormone-releasing hormone analogue (LHRHa) and 5000-6000 IU/kg human chorionic gonadotropin (HCG) on both male and female) with oocyte diameter 424–522 µm in a latency period of 32-38 hours. The author also reported 15-40% fertilization and 10-45% hatching rate in the study (CIBA, 2012). Later, Su et al. (2019) has reported gonadal maturation, spawning and larval rearing techniques in different salinities applying LHRHa in China. Females were induced with a double dose of HCG (2000 IU/kg) and a single dose of LHRHa (400 µg/kg) each at 24 h interval, whereas males received only single dose of LHRHa (200 µg/kg) with a fertilization and hatching rate of 73.67±5.4% and 70.33±9.2% when females with a mean initial oocyte size of 451–500 µm (Mandal et al., 2021). Considering the above perspective and relevant positive information, this study was aimed to establish a controlled reproduction protocol of farm domesticated *S. argus* fish and mass offspring production techniques which will tremendously bolster our coastal aquaculture industry in future.

2. Materials and methods

2.1. Domestication and broodstock development

Prior to domesticate and development of broodstock, wild fry (0.5 g) and sub-adult (40 g) of *S. argus* were collected from surrounding river of Sundarban mangrove area and brought them in the hatchery facility of brackishwater station. After acclimatization for 2 weeks, they were released in to four on-station ponds (22°35′46.59″N, 89°18′28.54″E; 22°35′49.09″N, 89°18′27.92″E; 22°35′46.21″N, 89°18′26.02″E; 22°35′48.66″N, 89°18′25.76″E) of 0.5 ha maintaining a stocking density @ 10 fish/decimal. Primarily commercial diet *e.g.* powder form and starter crumble with 35% protein content @ 15% body weight (BW) was applied to accustomed with. After 2.5 months of culturing most fish reached approximately 8 cm in length. Then males and females were separated and transferred to broodstock ponds to avoid food competition and cannibalism. At this condition, they were reared for about 3 years as because *S.*

argus becomes sexually mature between 2.5 and 3 years. During this course of time, commercial pellet feed containing 32% protein (Mega feed manufactured by Spectra Hexa Feeds Company Limited) was fed @ 5% BW twice a day. The water quality variables were monitored fortnightly and the salinity fluctuation was recorded between 1and 18 ppt.

2.2. Admission of fish into higher salinity for promotion of gonadal maturation

Targeting peak breeding season of Chitra (May-June), the level of gonad maturation and progress of some potential Chitra fish (males: 191.28±39 g; females: 196±63.13 g) were observed by rearing them at 30 ppt salinity in rectangular cemented tank (Length: 3.65 m x Width: 1.6 m x Depth: 1 m) inside hatchery facility since the salinity level of broodstock ponds were lower (14 ppt) due to heavy rainfall. Along with these, they were intensively cared by applying proper amount of vitamin (A, B, C and E) mixing with commercial feed (Tongwei feed- manufactured by Tongwei Feed Mill Bangladesh Ltd.) containing high protein level (35%) twice daily to prepare them sexually mature. Gonadal maturation was carefully monitored at daily basis whereas physico-chemical characteristics of water was checked at every 2 days interval. The water temperature was kept up from 25-28 ^{o}C, pH at 7.0-8.5, dissolved oxygen (DO) at 5.5-6.6 mg/L and the photoperiod was maintained at 14 hrs light and 10 hrs darkness. Under above management and facilities, brood fish was reared for approximately 3 weeks with 10-15% water was exchanged daily.

2.3. Procedures of hormone injection for inducement

Before injection, eggs were taken from the female genital pore with an infant feeding tube and gamete quality was tested (fullness of vitellogenic oocytes) with a LEICA DM1000 LED microscope (Leica, Glattbrugg, Switzerland). In parallel, sperm were collected by gently pressing on the surface gonadal muscle of the males and spermiation stage was measured on a scale. Before hormonal injection fish were anesthetized in 50 mg/L tricaine methanesulfonate (MS-222) (Argent TR2905, Redmond, WA, USA). In the treatment groups, stimulation was accomplished with two hormonal injections of salmon gonadotropin-releasing hormone analog (S-GnRHa (OVUPIN), SANSHENG Biological Technology, China). During injection 20% of total hormonal dose was inserted in female as first dose (priming dose) for the initiation of oocyte maturation along with ovulation, then rest 80% injected as second dose (resolving dose) after 12-15 hours. Contrarily, male fish was given a single dose only during second dose of female. In different treatment groups several hormonal doses were administered for male and female fish (Table 1) following the standardized dosage by U.S Fish & Wildlife Service, 2016 (10–75 µg sGnRHa/kg body weight).

Treatment	1 (♀:♂)	2 (♀:♂)	3 (♀:♂)	4 (control) (♀:♂)
Inducing agent	S-GnRHa	S-GnRHa	S-GnRHa	0.9% NaCl
Hormonal dose (µg/ kg BW)	50:25	60:30	70:35	-

Table 1: Hormonal doses used for the stimulation of *S. argus* in different treatment.

Injections were given intramuscularly under the soft base of the pectoral fin (Figure 1). For the control group, injections of physiological saline solution (0.9% NaCl) were substituted. At every 2 h between 26 and 36 h post injection females were periodically inspected. When the females swollen abdomen became soften, some eggs were examined from the genital pore again. Eggs bearing a spherical shape, limpid and greasy instead of collapsed and flabby characteristics, was considered to be the right moment to strip eggs. A few females were selected randomly for dissections to scrutinize the ovaries status, before and after injection. After hormonal injection the broods were kept in a 300 L circular fiber glass cistern (diameter: 1m) maintaining a sex ratio of 2:3/4 (♀:♂)

2.4. Stripping and fertilization

Artificial fertilization was carried out during the pinnacle phase of oestrus manner or acute courtship behavior. At the same time, suitable and productive males were chosen after gentle abdominal stripping, with expressible milt present. However, females were then stripped by gentle abdominal pressure and the expelled eggs were collected in a 3000 ml circular manger (Figure 2a). The eggs from two females were artificially fertilized by sprinkling over the milt with good motility from three males in each replication group. Eggs and sperm were gently jolted with a sterilized duck feather for 30 s along with gradual addition of 2 L seawater and then washed with a physiological

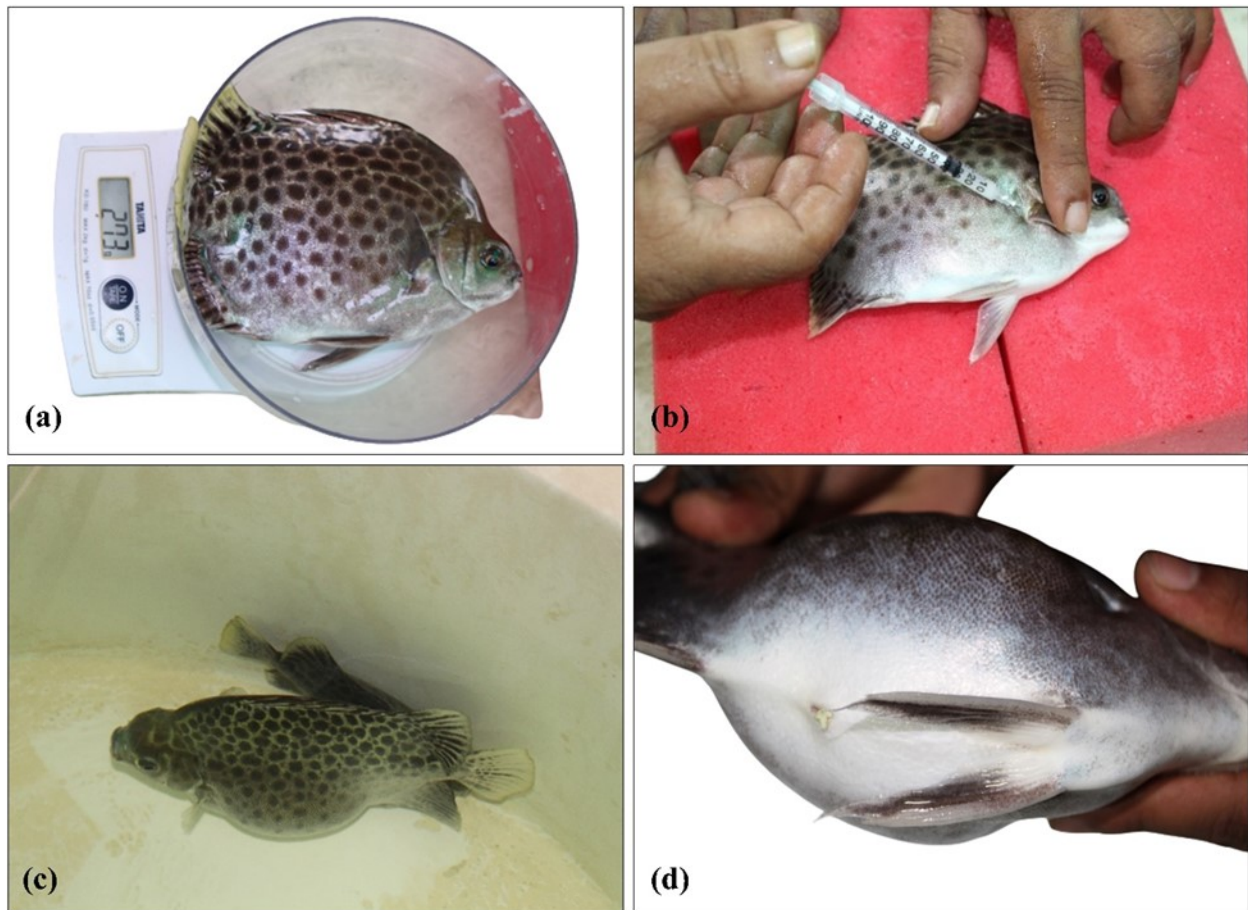

Figure 1: Artificial breeding of *S. argus* (a) Chitra (*S. argus*); (b) Hormone injection; (c) Courtship; (d) Swollen abdomen after ovulation.

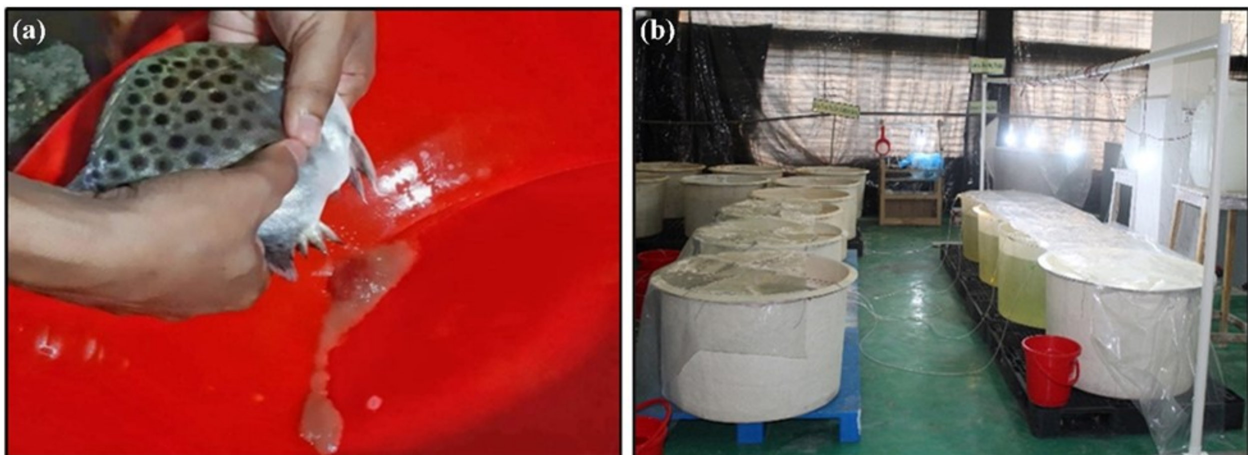

Figure 2: (a) Stripping of puffy abdomen after ovulation; (b) Incubation and larvae rearing set-up.

solution (0.9% NaCl). Immediately, after 5 minutes of incubation, only the buoyant eggs from upper layer were gathered and shifted to hatching cistern holding 250 L sea water for accessory incubation (Fig. 2b) till hatching. The ovulation and buoyancy rate (%) was calculated following the equation stated by Ruensirikul and Chiayvareesajja (2020). The number of fertilized eggs was reckoned by enumerating the ordinary embryos that enrolled in the cleavage division (Unuma et al., 2004; Oliveira & Hable, 2010).

2.5. Hatching and rearing of larvae

A continuous water flow in each hatching cistern with gentle turbulence was generated by aeration pump connected with air-stones to keep the eggs in suspension. The environmental parameters in all consecutive trials were as follows, water temperature: 28.0±2.0°C; salinity: 28±2.0 ppt, pH: 7.3-8.4; DO: 5.1-6.5 mg/L and ammonia 0.04-0.30 mg/L. Embryonic development was observed continuously under LEICA DM1000 LED microscope

from the beginning of fertilization to mature hatchling/larvae stage. However, the average hatching time was between 32-36 hrs. The larvae rearing cisterns were placed in a separate experimental compartment by maintaining a natural photoperiod: 12 hr light and 12 hr darkness. On the 48-hour post hatching (hph) rotifers were administered in the larvae rearing tank as live weaning feed at a density of 15-20 rotifers/ml (filtered with 45-µm mesh) and EZ larvae (premium liquid larval diet manufactured by ZEIGLER; USA) at the rate of 1ml/1000L water twice daily. Rotifers was provided 4 times daily at 6 a.m., 12 p.m., 6 p.m. and 12 a.m. and was adjusted accordingly to meet the requirement. From 3 days post hatch (dph), fractional water was exchanged (15%) every day. Regular siphoning was done to draw off detritus, faeces and dead fish from the tank bottom. At day 6 newly hatched artemia nauplii were incorporated into the larval diet.

3. Results

The average age of parent fishes employed for the artificial propagation in this study was near about 3 years and the fishes were acclimatized for twenty one days in the water of 30 ppt salinity before hormonal inducement. The incubation of fertilized eggs and larval rearing process was executed thoroughly using 30 ppt saline water. However, the onset of ovulation, spawning, and viability of eggs, was greatly dependent on female gonadal maturation prior to injection, hormone dosage, and the portion of the month during which the study was performed. Latency period considered for this study is defined as the time between first injection and ovulation. The oocyte maturation (OM) and ovulation were successfully occurred in treatment 2 only by incorporating S-GnRHa at the dose of 60:30 **(**♀:♂**)** µg/kg BW. The average latency period in three consecutive trials were from 32-36 hrs then eggs were taken out by stripping and fertilization was executed using pooled sperm from male. The ready to spawn females had pinkish genital papilla before hormonal injection (Fig 3a). The mature ovary contained whitish and tiny eggs connected with each other alike cohesive substance (Fig 3b). Even after full latency period, the oocyte from few females were gotten hard to strip easily in some replication. In spite of very inflated bellies, the abdomens has not softened. The eggs were still white inside and linked to each other, howbeit the color of some eggs changed from white to semi-transparent (Fig 3c). These females had trouble with spawning and were incapable to strip. The ovulation and buoyancy rate (%) was 70±1.8 and 68±7.7 respectively. However, the fertilization rates were between 37.6±1.3% and 41.5±3.3% and the hatching rates of different trials were from 47.3±4.8% to 58±5.5% (Table 2).

Treatment	1	2	3	4
Latency period (hours)	-	32-36	-	N/A
Ovulation rate (%)	-	70±1.8	-	N/A
Buoyancy rate (%)	-	68±7.7	-	N/A
Fertilization rate (%) ± SD	-	37.6±1.3-41.5±3.3	-	N/A
Hatching rate (%) ± SD	No	47.3±4.8-58.0±5.5	No	N/A

Table 2: Fertilization and hatching profiles.

The embryonic development encompassed following distinctive phases, zygote stage ((Fig 4a-b): newly fertilized eggs moved to the first cell division and attained meroblastic cleavage (Fig 4c-d) stage: at approximately 1 hour post fertilization (hpf). After 5 hpf, blastula stage (Fig 4e-f) appeared with 30% epiboly (Blastoderm extension) which then reached immediately at optic lobe (Fig 4g) and heart-bit structure respectively (5.3 hpf; Fig 4h). Later at gastrula stage, it undergone in to 50, 80 and 90% epiboly and finally expansion and development was completed over the duration of 8

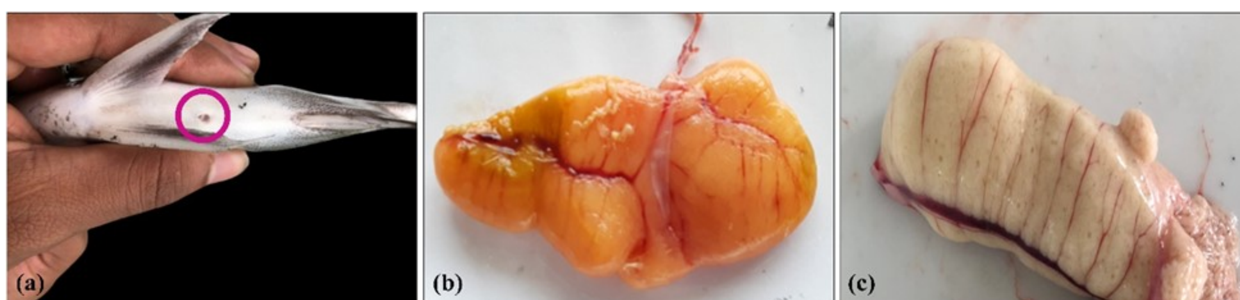

Figure 3: Maturation status off eggs (a) Pinkish genital papilla; (b) Mature oocyte; (c) Immature oocyte after injection.

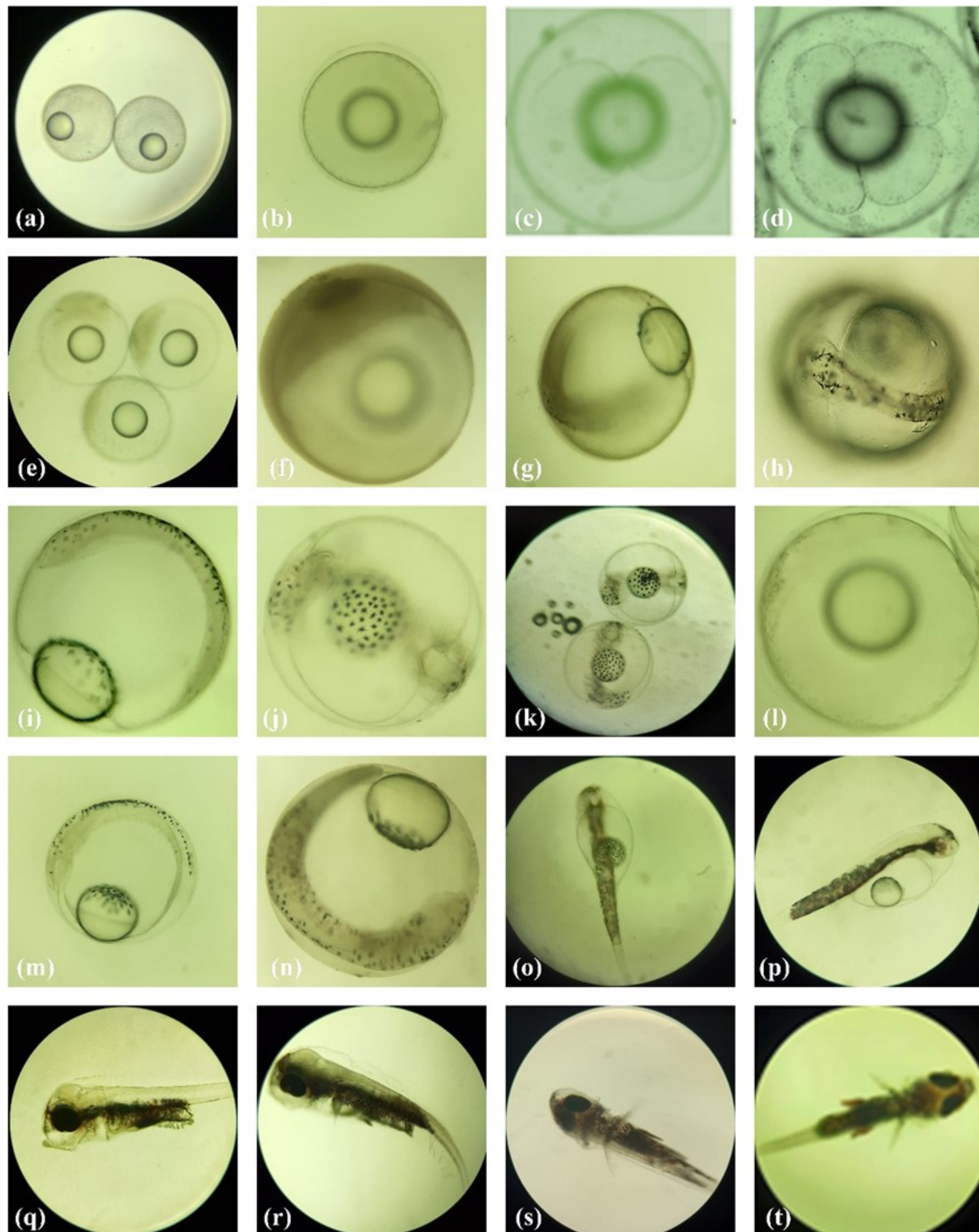

Figure 4: Embryonic development stage and larval maturation of *Scatophagus argus* (a-b) zygote stage; (c-d) meroblastic cleavage (2-cell, 4-cell) stage; (e-f) blastula stage (blastoderm extension); (g) Optic lobe; (h) heart-bit; (i-k) gastrula stage (50%, 80% and 90% epiboly respectively); (l) segmentation (germ ring); (m) pharyngula stage; (n) embryo; (o) newly hatched larvae with hatching gland; (p) larvae with yolk sac; (q) 5 days old larvae with clear mouth gap; (r) 6 days old larvae with intestinal tract and developing fins; (s) 7 days old larvae; (t) 8 days old larvae.

hpf (Fig 4i-k). The germ ring began to develop in the segmentation stage (8-10 hpf; Fig 4l), manifesting the inception of embryonic axis formation (the embryonic shield). In the pharyngula phase (11 to 15 hpf), the embryo inhabited the classic vertebrate body plan, and tiny asteroid pigments emerged over the trunk and the oil globule (Figure 4m). In particular, formation of fins commenced in this period and gill arches were observable as well. In line with this, at hatching stage, complete embryo (Fig 4n; 15-16 hpf) with developing gill rakers and muscles were observed. The morphogenesis of many fontal organ was almost complete.

After that, over the duration of 16-22 hpf, newly hatched larvae emerged from eggs consisting of

hatching gland (Fig 4o) and then converted into larvae with yolk sac, a tiny pre-anal fin fold and an elongated fin fold from the dorsal region to the tail, and the mouth gap was still under developed and nonfunctional. In the meantime, the development of jaws and the gills proceeded rapidly. Melanophores came across the head and scattered along the trunk. Additionally, stellate black pigments were seen on the ventral surface of the yolk (Fig 4p). On the third day after hatching, the morphogenesis of the larvae was totally accomplished. Numerous modifications were evident, encompassing the upheaval of the swim bladder and the incessant anterior-dorsal protrusion of the mouth. The yolk sac was slowly reduced and absorbed within 3 days post hatching (dph). At 5 dph a clear mouth gap formed (Fig 4q) and within 6 dph the larvae transformed into mature hatchling with developing gastrointestinal tract and fins (Fig 4r). Meanwhile, they started to take external feed (*viz*. Liquid larval diet, rotifers). By 7-8 dph, the larvae were observed somewhat opaque; black pigments were dispersed on the abdomen, the ventral part, and the jaw, and the rudimentary basis of caudal fin was viewable (Fig 4s -t).

4. Discussion

The evaluation of breeding success in captive conditions is substantially related to the breeding performance of fish. Therefore, in order to achieve best breeding output in artificial propagation, determination of true hormone in combination with optimal dose and latency period is desirable. The mean age of parent *S. argus* used in this breeding program was ≥2.5 year which was perfect because Cai et al. (2010) have postulated that, a two year biological age of male and female fish promptly adapts to artificial breeding. For both marine and brackishwater migratory fish such as spotted scat, environmental salinity, may play a profound role in the gonadal maturation to the timing of spawning, embryonic development (Nissling et al., 2002; Leu et al., 2018) and the rate of embryo/larval survival (Butts et al., 2009). Hering (2000) has reported that, to breed adults *S. argus* full strength seawater is required (Nowosad et al., 2015; Pedrazzani et al., 2014) and, alteration from optimal salinity can cause lethal and sub-lethal effects on fish gametes and embryos. In another study of *S. argus*, higher ovulation rate were obtained within the range of salinities between 15 and 25 ppt (Ruensirikul and Chiayvareesajja, 2020). The results of a similar study by Su et al. (2019) observed a fertilization rate of 87.27% at 25ppt salinity and the highest embryonic survival and hatching rate (about 92%) at 15 ppt salinity in *S. argus* which is much higher than the present study. Therefore, during the entire breeding process (brood acclimatization up to larval rearing), we preferred to use a consistent water salinity (28±2.0 ppt), thus obtained a ovulation, buoyancy, fertilization and hatching rate of 70±1.8; 68±7.7; 40±2.3% and 53±5.2% respectively, which corresponds to the achievement of artificial propagation of Waigieu sea perch in high salinity >28 ppt (Pham et al., 2010). Water temperature largely influence the hatching time for fertilized oocytes, the development of larvae and directly affects organogenesis from the embryological aspects (Byun et al., 2007). The latency time is mostly associated to the water temperature and the duration that decreases the temperature increases (Kucharczyk et al., 2005), but in our study the latency time was investigated at same temperature (28.0**±**2.0°C) with indifferent dose of hormonal injections which ranged from 32-36 hrs. The possible reason for variable latency time might be related to the absence of synchronization in attainment of readiness for spawning by the fish.

In this breeding program, S-GnRHa successfully invoked the OM and ovulation, and the ovulated eggs were stripped manually for fertilization. Albeit, many authors (Cai et al. 2010; Su et al. 2019; Ruensirikul and Chiayvareesajja, 2020) has been shown that, artificial induction (Ovulation and spawning) in *S. argus* can be accelerated solely by using LHRHa individually or mixing it with others. Yom-din et al. (2016) found a significant increase of FSH levels and more advanced follicle stages in Russian Sturgeon ovary by stimulation with GnRHa+T (Testosterone). In red seabream (*Pagrus major*) application of GnRH agonist individually promoted precocious puberty (Kumakura et al., 2003). Additionally, a recent molecular study (Chen et al., 2020) identified three subtypes of GnRH in the spotted scat and their *In vivo* experiments have proven that, during ovarian maturation it significantly upgraded the expression of *fsh* (follicle stimulating hormone) and *lh* (luteinizing hormone) genes. Therefore the results of this study is strongly suggesting that, S-GnRHa can play a vital role in the artificial reproduction of *S. argus*, which is concomitant with the findings in salmon and chum salmon (Ando et al. 2006; Park et al., 2007), gilthead seabream (Holland et al., 1998), sea bass

(*Dicentrarchus labrax*) (Mateos et al., 2002), rainbow trout (Vazirzadeh et al., 2008), gray mullet (*Mugil cephalus*) (Aizen et al., 2005), European seabass (Mylonas et al., 2010), and snow trout (Rahdari et al., 2014). Similarly, the recommended doses of GnRH analogues for aquaculture vary between 10 and 50 µg/kg BW depending on the fish species, and mode of its insertion (Zohar et al., 2010) which is closely analogous with the dose applied in our study: 60 and 30 µg/kg BW for female and male respectively. Some variable dosage have also been documented for other fish species of the same order such as *Anabas testudineus* (F:M = 15:7.5 µg/kg BW); *Sander lucioperca* (F:M = 50:50 µg/kg BW) and *Ompok pabda* (F:M = 1000:1000 µg/kg BW) breed successfully with highest fertilization rate when treated with sGnRHa (Mandal et al., 2016; Zarski et al., 2019; Roy et al., 2021). Before administration of GnRHa, the full hormonal dose should be divided in to two part *e.g.* in a priming dose (5-10%) and a resolving dose (95-90%) after a particular time interval (Mehdi and Ehsan, 2011). It is important to mention that, we administered two hormonal injection in every breeding episode which is evidently explained by the fact that, a repeated GnRHa injection in Senegalese sole (*Solea senegalensis*) was more effective than a single injection for stimulating egg production by stripping, beyond compromising egg quality (Rasines et al. 2012). In another study, Yu et al. (2017) found that, a double hormonal injection concomitantly decreased the percentage of poorly matured females and upgraded the fertilization and hatching rate. Following subsequent phases were observed during the embryonic development of spotted scat embryos: meroblastic cleavage (2-cell), blastula, gastrula, pharyngula and embryo. However, the average hatching time (16-22 hpf) of the present study was investigated somewhat lower than the reported range 18-21 hpf by Su et al. (2020) using LHRHA2 under diversified environmental conditions. Other spawning hormone may be evaluated to compare the response and results in future. In the first six days after hatching, about 30 percent larval mortality was observed. Again, 8 days later all the larvae unexpectedly passed away. The possible reason of this sudden downfall can be clarified as, the stress and injury evolved during the larval transfer from the spawning tank to the larval rearing tank may be attributed to high larval mortality, and high mortality cannot be usually unavoidable at this period (Madhu et al., 2016). One more cause was appeared as, the starvation of larvae because of higher aeration speed which could hamper the larvae to reach and catch rotifer as their food for survival.

So far our knowledge goes, yet not a single research on artificial propagation of *S. argus* have been performed in the world using S-GnRHa rather than LHRHa. It is therefore plausible to assert that, the information generated in this study will be a cornerstone to proceed further research and refinement of larvae rearing process as well.

5. Conclusion

The findings of the present research includes, development of *S. argus* broodstock in captive condition; identification of true hormone together with proper dose for the successful inducement and development of management techniques for their larval rearing. Now the research challenge is to develop accurate and complete management techniques for the upheaval of larvae survival up to juvenile stage. Therefore, further study should be continued to fulfill the gap and shortcomings regarding larval rearing of this attractive fish.

Acknowledgements

This work was supported by the corresponding core research project (Code no. BFRI/BS/01) entitled “Breeding and seed production of brackishwater fin fishes (*Scatophagus argus* and *Pomadasys hasta*)” financed by Bangladesh Fisheries Research Institute (BFRI), Ministry of Fisheries and Livestock, Peoples Republic of Bangladesh.

References

Aizen J, Meiri I, Tzchori I, Levavi-Sivan B, Rosenfeld H (2005) Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition. General and Comparative Endocrinology 142: 212-221.

Amarasinghe US, Amarasinghe MD, Nissanka C. (2002) Investigation of the Negombo Estuary (Sri Lanka) brush park fishery, with an emphasis on community-based management. Fisheries Management and Ecology 9: 41-56.

Ando H, Luo Q, Koide N, Okada H, Urano A. (2006) Effects of insulin like growth factor I on GnRH induced gonadotropin subunit gene expressions in masu salmon pituitary cells at different stages of sexual maturation. General and Comparative Endocrinology 149:21-29. DOI: 10.1016/j.ygcen.2006.04.013

Bahre Kazemi M, Soltani M, Matinfar A, Abtahi B, Pusti I, Mohagheghi Samarin A, Mojazi A.B. (2010) Biochemical and histological studies of over-ripened oocyte in the Caspian brown trout (*Salmo trutta caspius*) to determine biomarkers for egg

quality. Iranian Journal of Fisheries Sciences 9(1):33-48.

Barry TP, Castanos MT, Fast AW (1991) Induced spermiation in the male spotted scat (*Scatophagus argus*) by long-term administration of 17α-methyltestosterone followed by LHRHa. Asian Fisheries Science 4 (2): 137-145.

Barry TP, Fast AW (1992) Biology of the spotted scat (*Scatophagus argus*) in the Philippines. Asian Fisheries Sciences 5: 163-179.

Butts IAE, Trippel EA, Litvak MK (2009) The effect of sperm to egg ratio and gamete contact time on fertilization success in Atlantic cod *Gadus morhua* L. Aquaculture 286: 89-94. DOI: 10.1016/ j.aquaculture.2008.09.005

Byun SG, Lee BI, Lee JH, Ku HD, Park SU, Yun SM, Hwang SY, Kim YC, Han HG (2007) Egg development and morphological change of larvae and juveniles of the starry flounder, *Platichthys stellatus*. Korean Journal of Ichthyology 19:350-359.

Cai ZP, Wang Y, Hu JW, Zhang JB, Lin YG (2010) Reproductive biology of *Scatophagus argus* and artificial induction of spawning. J. Trop. Oceanogr., 29 (5): 180-185. (in Chinese with English abstract).

Chang SL, Hsieh CS (1997) Studies on the early development and larval rearing of spotted scat (*Scatophagus argus*). J. Taiwan Fish. Res., 5: 41-49.

Chen H, Cui X, Wang Y, Li Z, Tian C, Jiang D, Zhu C, Zhang Y, Li S, Li G (2020) Identification, functional characterization, and estrogen regulation on gonadotropin-releasing hormone in the spotted scat, *Scatophagus argus*. Fish Physiol Biochem. DOI: 10.1007/s10695-020-00825-5

Chen JH, He MX, Yan, BL, Zhang, JB, Jin, SC, Liu L (2015) Molecular characterization of dax1 and SF-1 and their expression analysis during sex reversal in spotted scat, *Scatophagus argus*. J. World Aquac. Soc., 46 (1): 1-19. DOI: 10.1111/jwas.12165

CIBA, 2012. Annual Report 2011–2012. Central Institute of Brackishwater Aquaculture, Chennai (ISSN 0976-5336).

Gandhi V, Venkatesan V, Ramamoorthy N (2014) Reproductive biology of the spotted scat *Scatophagus argus* (Linnaeus, 1766) from Mandapam waters, south-east coast of India. Indian Journal of Fisheries 61(4): 55-59.

Ghazilou A, Chenary F, Morovvati H, Zolgarneine H (2011) Time course of saltwater adaptation in spotted scat (*Scatophagus argus*) (Pisces): A histomorphometric approach. Italian Journal of Zoology 78: 82-89.

Gupta S (2016) An overview on morphology, biology, and culture of spotted scat *Scatophagus argus* (Linnaeus 1766). Rev. Fish. Sci. Aquac., 24 (2): 203-212.

Hering W (2000) *Scatophagus argus*- how long can you keep them? Calypso Fish and Aquaria Club, Aquarticles, London, England. http://www.aquarticles.com/articles/breeding/ hering_Scats.html, accessed on 1 October 2021.

Holland M, Gothilf Y, Meiri I, King J, Okuzawa K, Elizur A, Zohar Y (1998) Levels of the native forms of GnRH in the pituitary of the gilthead seabream, *Sparus aurata*, at several characteristic stages of the gonadal cycle. General and Comparative Endocrinology 112:394-405. DOI: 10.1006 /gcen.1998.7138

Huang Y, Jiang D, Li M, Mustapha UF, Tian C, Chen H, Huang Y, Deng S, Wu T, Zhu C, Li G (2019) Genome Survey of Male and Female Spotted Scat (*Scatophagus argus*). Animals 9 (12), 1117. DOI: 10.3390/ani9121117

Kucharczyk D, Kujawa R, Mamcarz A, Targonska-Dietrich K, Wyszomirska E (2005) Induced spawning in bream (*Abramis brama* L.) using pellets containing GnRH. Czech J Anim Sci. 50:89-95.

Kumakura N, Okuzawa K, Gen K, Kagawa H (2003) Effects of gonadotropin-releasing hormone agonist and dopamine antagonist on hypothalamus-pituitary-gonadal axis of pre-pubertal female red seabream (*Pagrus major*). General and Comparative Endocrinology 131: 264-273.

Leu MY, Tai KY, Meng PJ, Tang CH, Wang PH, Tew KS (2018) Embryonic, larval and juvenile development of the longfin batfish, *Platax teira* (Forsskål, 1775) under controlled conditions with special regard to mitigate cannibalism for larvi-culture. Aquaculture 493: 204-213. DOI: 10.1016/j. aquaculture.2018.05.006

Liao CI, Su HM, Chang EY (2001) Techniques in finfish larviculture in Taiwan. Aquaculture 200: 1-31.

Madhu K, Madhu R, Retheesh T (2016) Spawning, embryonic development and larval culture of redhead dottyback *Pseudochromis dilectus* Lubbock, 1976 under captivity. Aquaculture 459: 73-83. DOI: 10.1016/j.aquaculture.2016.03.017

Mandal B, Kailasam M, Bera A, Sukumaran K, Hussain T, Biswas G, Vijayan KK (2021) Standardization of oocyte size during artificial fertilization and optimization of stocking density during indoor larval and outdoor nursery rearing of captive spotted scat (*Scatophagus argus*) for a viable juvenile production system. Aquaculture 534: 736262. DOI: 10.1016/ j.aquaculture.2020.736262

Mandal B, Kumar R, Jayasankar P (2016) Efficacy of exogenous hormone (GnRHa) for induced breeding of climbing perch *Anabas testudineus* (Bloch, 1792) and influence of operational sex ratio on spawning success. Animal Reproduction Science 171, 114-120. DOI: 10.1016/j.anireprosci.2016.06.006

Mateos J, Mananos E, Carrillo M, Zanuy S (2002) Regulation of follicle stimulating hormone (FSH) and luteinizing hormone (LH) gene expression by gonadotropin releasing hormone (GnRH) and sexual steroids in the Mediterranean Sea bass. Com Biochem Physiol B. 132:75-86. DOI: 10.1016 /s1096-4959(01)00535-8

Mehdi Y, Ehsan MS (2011) A review of the control of reproduction and hormonal manipulations in finfish species. African Journal of Agricultural Research 6(7): 1643-1650. DOI: 10.5897/AJAR10.1080

Musikasung W, Danayadol Y, Songsangjinda P (2006) Stomach content and ecological feature of *Scatophagus argus* (Linnaeus) in Songkhla Lake. Technical Paper No. 47, Coastal Aquaculture Research Institute, Coastal Fisheries Research and Development Bureau, 30 pp.

Mylonas CC, Fostier A, Zanuy S (2010) Broodstock management and hormonal manipulations of fish reproduction. General and Comparative Endocrinology 165, 516-534.

Ni IH, Kwok KY (1999) Marine fish fauna in Hong Kong waters. Zool. Stud. 38: 130-152.

Nissling A, Westin L, Hjerne O (2002) Reproductive success in relation to salinity for three flatfish species, dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), and flounder (*Pleuronectes flesus*), in the brackish water Baltic Sea. ICES Journal of Marine Science 59: 93-108. DOI: 10.1006/jmsc.2001.1134

Nowosad J, Kucharczyk D, Targonska K, Wyszomirska E, Chwaluczy R, Kupren K (2016) The synergistic effect of temperature and hormonal stimulation on spawning efficiency of common barbel, *Barbus Barbus* L. Turkish Journal of Fisheries and Aquatic Sciences

16: 517-524.

Oliveira K, Hable WE (2010) Artificial maturation, fertilization, and early development of the American eel (*Anguilla rostrata*). Canadian Journal of Zoology 88: 1121-1128.

Park WD, Lee CH, Lee CS, Kim DJ, Kim JH, Tamaru CS, Sohn YC (2007) Effects of gonadotropin-releasing hormone analog combined with pimozide on plasma sex steroid hormones, ovulation and egg quality in fresh water-exposed female chum salmon (*Oncorhynchus keta*). Aquaculture 271: 488-497.

Pedrazzani AS, Pham NK, Lin J, Neto AO (2014) Reproductive behavior, embryonic and early larval development of the red head goby, *Elacatinus puncticulatus*. Animal Reproduction Science 145: 69-74. DOI: 10.1016/j.anireprosci.2013.12.013

Pham HQ, Kjorsvik E, Nguyen AT, Nguyen MD, Arukwe A (2010) Reproductive cycle in female Waigieu seaperch (*Psammoperca waigiensis*) reared under different salinity levels and the effects of dopamine antagonist on steroid hormone levels. Journal of Experimental Marine Biology and Ecology 383:137-145.

Rahdari A, Gharaei A, Ghaffari M (2014) Spawning Latency Period in Hormonal Induced Reproduction of Snow trout (*Schizothorax Zarudnyi* (Nikolskii, 1897)). Iran J Biotech. 12(1): e10268.

Rasines I, Gomez M, Martin I, Rodriguez C, Mananos E, Chereguini O (2012) Artificial fertilization of Senegalese sole (*Solea senegalensis*): hormone therapy administration methods, timing of ovulation and viability of eggs retained in the ovarian cavity. Aquaculture 326-329:129-135.

Roy D, Sarker AK, Abedin AMMK, Sarker S, Begum KN, Latifa GA (2021) Some biological aspects of cultured *Ompok pabda* (Hamilton, 1822) collected from a local fish farm in Mymensingh, Bangladesh. Aquaculture Studies, 21, 149-159. DOI: 10.4194/2618-6381-v21_4_02

Ruensirikul J, Chiayvareesajja S (2020) Effects of water salinity on reproductive performance of the female hatchery-reared spotted scat, *Scatophagus argus* (Linnaeus, 1766) broodstock. Songklanakarin J. Sci. Technol. 42(1): 95-100.

Shao YT, Hwang LY, Lee TH (2004) Histological observations of ovotestis in the spotted scat *Scatophagus argus*. Fisheries Science 70: 716-718.

Sivan G, Radhakrishnan C (2007) Studies on Venom of the fish *Scatophagus argus* from Cochin estuary- a biochemical approach, PhD Dissertation, Cochin University of Science and Technology, Cochin, India.

Sivan G, Radhakrishnan CK (2011) Food, feeding habits and biochemical composition of *Scatophagus argus*. Turkish Journal of Fisheries and Aquatic Sciences 11: 603-608.

Su M, Duan Z, Shi H, Zhang J (2019) The effects of salinity on reproductive development and egg and larvae survival in the spotted scat *Scatophagus argus* under controlled conditions. Aquaculture Research 50 (7): 1782-1794. DOI: 10.1111/are.14056.

U.S Fish & Wildlife Service (2016) Fish and Aquatic Conservation. https://www.fws.gov/fisheries/aadap/inads-available/spawning-aids/ovaplantsgnrha/index.html . Cited by 07 Aug 2017.

Unuma T, Kondo S, Tanaka H, Kagawa H, Nomura K, Ohta H (2004) Determination of the rates of fertilization, hatching and larval survival in the Japanese eel, Anguilla japonica, using tissue culture microplates. Aquaculture 241: 345-356. DOI: 10.1016/j.aquaculture.2004.08.005.

Vazirzadeh A Hajimoradloo AM, Esmaeili HR, Akhlaghi M (2008) Effects of emulsified versus saline administration of GnRHa on induction of ovulation in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 280: 267-269.

Wongchinawit S, Sailasuta A, Paphavasit N (2007) Histological studies of the alimentary system in the adult spotted scat, *Scatophagus argus* Linnaeus, in mangrove forests of the Pak Phanang Estuary, Nakhon Si Thammarat Province. In: 11th BRT Annual Conference, October 15-18th 2007 Udonthani. pp: 241.

Xie ZZ, Xiao L, Wang DD, Fang C, Liu QY, Li ZH, Liu XC, Zhang Y, Li SS, Lin HR (2014) Transcriptome analysis of the *Trachinotus ovatus*: identification of reproduction, growth and immune-related genes and microsatellite markers. PLoS One 9 (10). DOI: 10.1371/journal.pone.0109419.

Yang W, Wang Y, Jiang D, Tian C, Zhu C, Li G, Chen H (2020) ddRADseq-assisted construction of a high-density SNP genetic map and QTL fine mapping for growth-related traits in the spotted scat (*Scatophagus argus*). BMC Genomics 21:278.

Yom-Din S, Hollander-Cohen L, Aizen J, Boehm B, Shpilman M, Golan M, Hurvitz A, Dagani G, Levavi-Sivan B (2016) Gonadotropins in the Russian Sturgeon: Their Role in Steroid Secretion and the Effect of Hormonal Treatment on Their Secretion. PLoS ONE 11(9): e0162344. DOI: 10.371/journal.pone.0162344

Yu KL, Lin XW, da Cunha Bastos J, Peter RE (1997) Neural regulation of GnRH in teleost fishes. In: Parhar, I.S., Sakuma, Y. (Eds.), GnRH Neurons: Gene to Behavior. Brain Shuppan, Tokyo pp. 277-312.

Yu X, Wu C, Gjoen HM (2017) Artificial fertilization and generating families for a selective breeding programme of large yellow croaker (*Larimichthys crocea*). Int Aquat Res. 9: 161-167. DOI: 10.1007/s40071-017-0164-3.

Zarski D, Fontaine P, Roche J, Alix M, Blecha M, Broquard C, Krol J, Milla S (2019) Time of response to hormonal treatment but not the type of a spawning agent affects the reproductive effectiveness in domesticated pikeperch, *Sander lucioperca*. Aquaculture 503: 527-536. DOI: 10.1016/j.aquaculture.2019.01.042

Ze-ping C, Yi W, Jiawei H, Junbin Z, Yueguang L (2010) Reproductive biology of *Scatophagus argus* and artificial induction of spawning. J. Trop. Oceanogr. 9(5):180-185.

Zohar Y, Muñoz-Cueto JA, Elizur A, Kah O (2010) Neuroendocrinology of reproduction in teleost fish. General and Comparative Endocrinology 165: 438-455.