Artificial Propagation of *Pomadasys hasta* (Bloch, 1790): A Key to Reach Sustainable Aquaculture

Shawon Ahmmed[1], Mizanur Rahman Washim[1], AKM Shafiqul Alam Rubel[1], Debashis Kumar Mondal[1], Md. Hashmi Sakib[1], Yahia Mahmud[2], Md. Latiful Islam[1]*

[1]Bangladesh Fisheries Research Institute, Brackishwater Station
Paikgacha, Khulna-9280, Bangladesh
[2]Bangladesh Fisheries Research Institute
Mymensingh-2201, Bangladesh
Email: latiful.bfri@gmail.com

Abstract

Reproduction of Pomadasys hasta was studied in captivity to establish an induced breeding protocol and larvae rearing tactics. Three distinct hormones viz., human chorionic gonadotropin (HCG), luteinizing hormone-releasing hormone analogues (LHRHa) and salmon gonadotropin hormone-releasing hormone (S-GnRHa) were injected to matured brooders in treatment 1 (T1), treatment 2 (T2) and treatment 3 (T3) but only 0.9% NaCl used in T4 as a control. In each treatment, three experimental trails, E1 (♀40:♂20 µg.kg^{-1}), E2 (♀50:♂25 µg.kg^{-1}) and E3 (♀60:♂30 µg.kg^{-1}) were conducted along with three replications of each trail in order to optimize the hormone dose for the target species. The obtained results have aroused much attention as this is the first breakthrough on induced breeding of a grunter in the Indian subcontinent. Variation in fertilization rate, latency period, egg output and hatching rate in response to different treatments and trails were revealed here. Spawning was occurred between 33-48 h of injection in all the experiments at 17-25°C water temperature. Above all, the highest fertilization (95.45±2.34) and hatching (75.45±4.07) rates were observed in E2 of T2. After 22-26 h of fertilization, the larvae emerged from the egg membrane and the newly hatched larvae were 1.6-1.8 mm in length. Turning larvae into juveniles was noticed by 40–45 days post-hatch (dph) with scales on the entire body surface. The total length was recorded as 43.5±2.1 mm and average body weight as 0.7±0.2 g at 95 dph. This investigation unlocked a track for producing seed of P. hasta in a commercial hatchery for a sustainable aquaculture without hampering their wild stock.

Keywords: *induce breeding, breeding performance, latency period, spawning, fertilization*

Introduction

Haemulidae represents an important family of tropical fishes as because of their high economic importance (McKay, 2001). In particular, species form Pomadasynae are found in inshore waters of mangrove habitats. A total 35 species were ensiled form the genus, *Pomadasys* (Jawad *et al*., 2014). In Bangladesh, two grunters named *Pomadasys hasta* and *Pomadasys maculatus* are available in marine and brackishwater (Roy *et al*., 2019). However, White grunter, *Pomadasys hasta* (Bloch, 1790) is one of the commercially important marine fishes and this species is popularly known as Datina in all over the country. Generally, local people call the species as Sada Datina which is usually landed with other fishes from brackish and marine waters of this delta (Mustafa and Azadi, 1995).

In recent years, major issues such as overfishing, climate change, heavy pollution, and loss of habitats have made the coastal fisheries vulnerable (Musick *et al*., 2000). Therefore, time-bound actions are essential to mitigate such worries on the ichthyofaunal resources (Borah *et al*., 2020). Aquaculture is known as the quickest growing sector in fish production around the globe which represents as a sustainable and potential substitution of fisheries stock enhancement (Food and Agriculture Organization, 2011). This fisheries sector committed to play a significant role in conservation of numerous extant fish species by reducing extra pressure on depleted stocks (Diana, 2009; De Silva, 2012).

Stock rebuilding of various declined fish species in the wild by captive breeding has brought fruitful results in many aquatic niches of the world (Philippart, 1995). In aquaculture, successful

*) Corresponding author

https://ejournal.undip.ac.id/index.php/ijms
DOI: 10.14710/ik.ijms.28.1.37-48
Received : 01-11-2022
Accepted : 11-02-2023

propagation of fishes requires in detail studies on taxonomy, distribution, feeding, and reproductive dynamics of broodstocks (Meffe, 1990; Maitland, 1995). Information on the reproductive biology of some economically important fish species which include *P. kaakan, P. hasta, P. maculatum, P. jubelini, and P. stridens* of different parts of the world has been reported in few literatures (Iqbal, 1989; Deshmukh, 1973; Khan, 2004; Adebiyi, 2013; Al-Ghais, 1995). However, the reproductive biology of *P. hasta* was not widely reported in the scientific studies.

Studies on reproductive biology, induced spawning and embryonic development of the grunts are very rare in the world. Until now, not a single experiment on breeding of *P. hasta* was reported in the brackishwater regions of Bangladesh. Considering backdrops, this study aimed to establish a standard for induced breeding and larval rearing of *P. hasta* to solve the crisis of fry supply in the local markets for aquaculture.

Materials and Methods

Collection, domestication and broodstock development

P. hasta fries (2.50±0.22 cm; 0.15±0.02 g) were collected from adjacent rivers of the Sundarbans mangrove forest and acclimated with a fish-meal based commercial diet (35% crude protein) to domesticate and to develop the broodstock at the brackishwater station. Females of *P. hasta* grows faster than males when the body size becomes above 12 cm. While reaching 12 cm in length, females and males were reared separately with commercial pellet feed (32% protein) of Tongwei fish feed manufactured by Tongwei Co. Ltd. China, at 5% of body weight (BW) and fed twice a day in the ponds (0.5 ha) for 3 y to avoid food competition and cannibalism. The selected ponds were located in the intertidal zone, and the salinity ranged between 2-17 ppt during the culture period.

Gonad maturation and brood-fish selection

A total of 195 healthy and alive fishes such as 130 males (♂) and 65 females (♀) were chosen for captive rearing and induced breeding of *P. hasta*. Targeting peak breeding season of Datina (December-February), the level of gonad maturation and progress of some potential Datina fish (males: 185.50±25.05 g; females: 325±40.15 g) were observed by rearing them in cemented cistern (8 m × 6 m × 1 m) at 30 ppt saline water for 1.5 months inside hatchery. Moreover, they were reared by providing high protein (35%) containing commercial diet with vitamins (A, B, C, and E) and fed twice a day until sexual maturation. The photoperiod was regulated by keeping them in the light for 14 h and in the dark for 10 h. The ripeness of white grunter was carefully monitored daily whereas water parameters were checked at every two days interval. The healthy and ripened male and female brood fish were selected by investigating physical appearance and secondary sexual characters. The females were relatively larger and possessed a swollen, fluffy and soft abdomen with fleshy, crystalline, and bulging genital papilla including an oviduct. In contrast, the males were smaller in size with a small genital papilla which releases milt with a very gentle push on the posterior part of the abdomen.

Hormone administration for stimulation

For induced breeding of *P. hasta*, the brooders were transferred from rearing tanks to 300 L fiber reinforced plastic (FRP) tanks for acclimatization before injecting hormones. To standardize the potential hormone, hormonal doses, and breeding performances, three different treatments were performed with three different hormones viz., human chorionic gonadotropin (HCG), luteinizing hormone-releasing hormone analogues (LHRHa), and salmon gonadotropin hormone-releasing hormone (S-GnRHa) as T1, T2, and T3 along with T4 as control where only 0.9% NaCl were applied. The three experiments were employed as E1 (40:20 $\mu g.kg^{-1}$), E2 (50:25 $\mu g.kg^{-1}$), and E3 (60:30 $\mu g.kg^{-1}$) of each hormone for female and male, respectively with three replications of each treatment (Table 1.). In addition, whole study was repeated three times to confirm the consistency of the findings.

Before injecting hormones, broods were anesthetized in 50 $mg.L^{-1}$ tricaine methanesulfonate, MS-222 (Argent TR2905, Redmond, WA, USA). Thereafter, doses were calculated and prepared conforming to the body weight of the brooders. During hormonal treatment, 20% of the total dose was injected in females as the first dose (priming dose) for the initiation of oocyte maturation along with ovulation and the rest 80% was injected as a second dose (resolving dose) after 12-15 h of first dose. On the other hand, male fish was given only a single dose at the time of giving female's second dose and a male without any hormonal dose was introduced in the breeding tank after 24 h of female's 1st dose. Injections were given intramuscularly under the soft base of the pectoral fin with an angle of 45° of the body. For the control group (T4), injections of physiological saline solution (0.9% NaCl) were given. Females were regularly observed in every single hour up to a duration of 30-40 h of post-injection. After

hormonal injection, the broods were kept in a 300 L circular fiber reinforced plastic tank (diameter: 1m) maintaining a sex ratio of 1:2 (♀: ♂) with the provision of continuous air and water flow system.

Breeding performance (spawning, fertilization and hatching)

Generally, *P. hasta* takes 36-40 h for ovulation. Immediately after ovulation, a small amount of milt with good motility was sprinkling over the released eggs for ensuring successful fertilization. Immediately after 5 mins of incubation, only the buoyant eggs from the surface water were collected and shifted to the hatching tank (250 L fiber reinforced plastic tank) holding seawater (30 ppt salinity) for additional incubation. The fertilized eggs were found floating on the water surface. Microscopic investigation revealed that the fertilized eggs were translucent with an unbroken nucleus whereas the unfertilized were opaque and broken nucleus. The effective fecundity of each spawning female was estimated from the released eggs through random sampling (Ruensirikul and Chiayvareesajja, 2020). The number of fertilized eggs was determined by computing the normal embryos which started the cleavage division (Unuma *et al*., 2004; Behera *et al*., 2010; Oliveira and Hable, 2010). Fertilization rate and hatching rate was counted visually following the formula:

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

Developmental stages of fertilized eggs and embryos were monitored continuously and documented under LEICA DM1000 LED microscope from zero hour post-fertilization (hpf) to larval stage. After reaching to the hatching phase, they were nourished in circular fiber reinforced plastic (FRP) tanks.

Rearing of larvae

Normally, the required average hatching time was between 22 and 26 h, at 24-26ºC ambient temperature. The larvae rearing tanks were placed in a separate experimental compartment by maintaining a natural photoperiod of 12 h light and 12 h dark. Small size rotifer was filtered with 45 µm mesh net and supplied in the larvae rearing tank as live weaning feed at a density of 15-20 rotifers.ml^{-1}. Rotifers were provided four times daily at 6 h intervals and adjusted accordingly to meet the requirement. From 3rd day of post-hatch (dph), siphoning was performed to remove the debris, feces, and dead larvae from the tank bottom. However, water was exchanged partially (15%) every day. Then, newly hatched artemia nauplii were introduced into the larval diet on day twelve. After 30 d, a commercial powder feed was also incorporated into the larval diet for 45 days old post-hatch larvae (dph). Just after 45 d, the fry of *P. hasta* were stocked in the nursery pond for protecting them from cannibalism.

Statistical analysis

The collected data were primarily analyzed and tabulated by MS Excel to generate the research findings. Comparison between the different experimental observations were tested by one-way ANOVA and DMRT (Duncan, 1955). Both computer programs Microsoft Office Professional Plus 16 and SPSS (Statistical Product and Service Solutions) ver. 20 were used to perform all statistical analysis.

Result and Discussion

The necessity for the controlled production of *P. hasta* has increased in recent years as the availability of fish fry from the natural environment has declined dramatically. However, the propagation of fish under controlled conditions is difficult and the evaluation of breeding success in captive conditions is substantially related to the breeding performance of fish under hormone treatments (Dou *et al*., 2004; Burgerhout *et al*., 2011; Kucharczyk *et al*., 2016; Nowosad *et al*., 2016). 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 (Butts *et al*., 2009; Targońska *et al*., 2012; Nowosad *et al*., 2015).

Breeding behavior

The length and weight of brood fish used in the breeding trials, latency period, egg output, fertilization, and hatching rate in response to different treatments are summarized in Tables 2, 3, & 4. No ovulation and fertilization took place in the T1 and control (without hormonal stimulation) set of experiments. After 12-15 h of hormonal administration, the brooders of *P. hasta* showed various mating behavior in all three treatments (T1-T3) except control (T4). Each female was paired with a male and the breeding was continued by showing active courtship. The male followed the female when it swims around the breeding tank. Before spawning, females were swimming up frequently at the water surface. The latency period varied significantly against different hormonal doses and in the different experimental setup (Figure 1.). Spawning took place between 33 to 48 h of injection in different experiments as shown in Tables 2, 3, & 4.

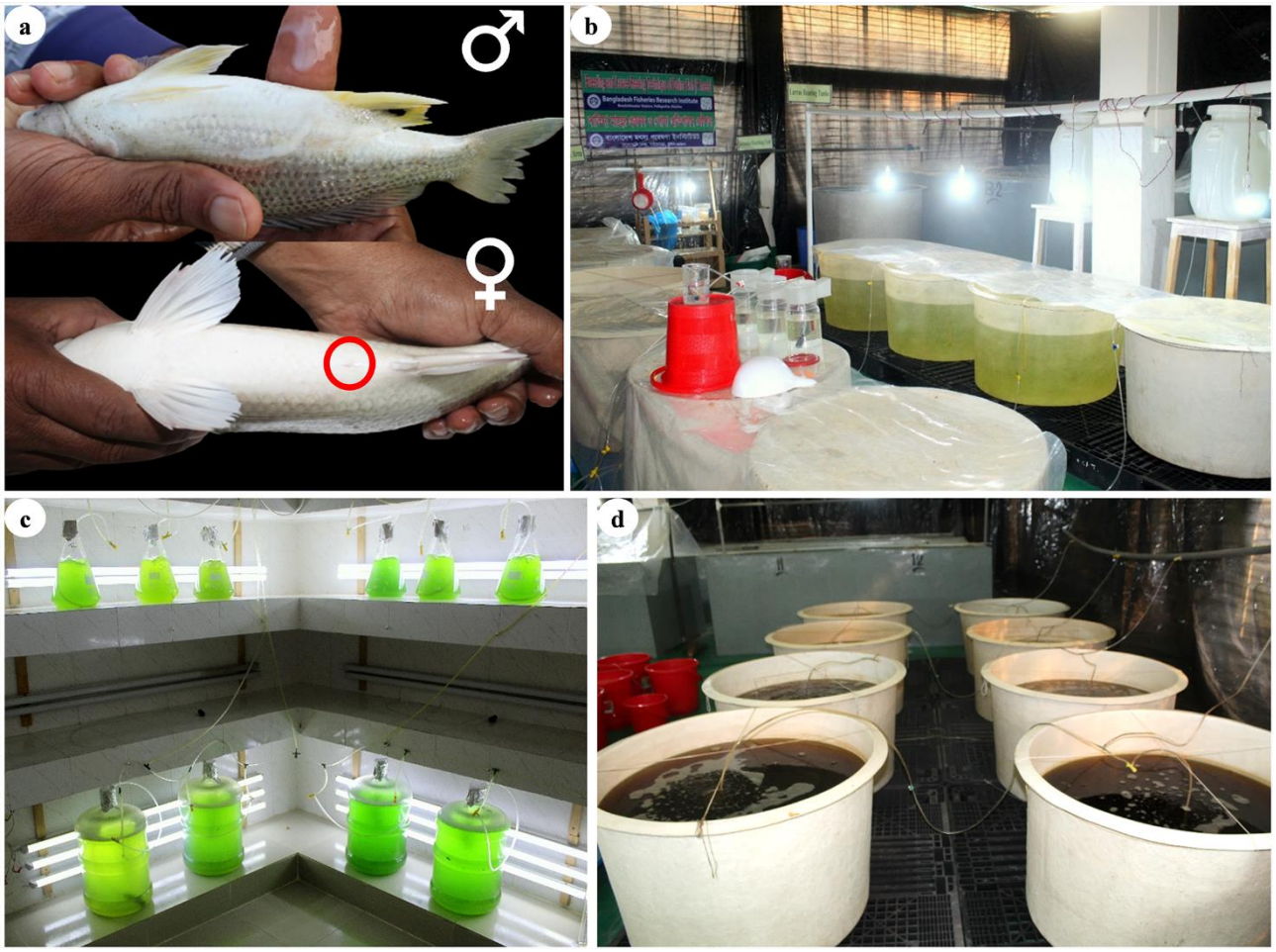

Figure 1. Experimental set up (a) mature male and female (b) larval rearing tank (c) microalgae culture setup (d) rotifer culture setup

Table 1. Doses of hormones to stimulate *Pomadasys hasta* in different treatments and experiments

Treatments	Inducing agent	Hormone doses (µg.kg^{-1} body weight)		
		E1(♀: ♂)	E2 (♀: ♂)	E3 (♀: ♂)
T1	HCG	40:20	50:25	60:30
T2	LHRHa	40:20	50:25	60:30
T3	S-GnRHa	40:20	50:25	60:30
T4	0.9% NaCl	40:20	50:25	60:30

Fertilization and hatching rate

The fertilization rate in E1 was 78.50±1.89 of T2 and no fertilization took place in all the other treatments of E1 (Table 2.). In E2, a significantly higher fertilization rate was observed in T2 (95.45±2.34) and the lowest was in T3 (70.50±0.90) and no fertilization took place in T1 and T4 (control) (Table 3.). Alike E2, the fertilization in E3 was observed only in T2 and T3, wherein T2, the fertilization rate was highest (87.17±1.38) (Table 4.). Among all of the experiments, the highest fertilization rate was observed in T2 (95.45±2.34) of E2 ($P<0.05$). The fertilization rate of E2 is significantly higher ($P<0.05$) in all the treatments compared to E1 and E3. A curled movement of the embryos was observed within 16-18 h of spawning and the hatching took place within 22-24 h of fertilization. The calculated hatching rate was significantly higher ($P<0.05$) in E2 compared to E1 and E3, as presented in Tables 2, 3, & 4. The highest hatching rate was observed in T2 (75.45±4.07) with LHRHa hormonal injection at the rate of 50:25 µg.kg^{-1} body weight of female and male brood fish. In this breeding program, LHRHa hormone

Table 2. Captive breeding in E1 of *Pomadasys hasta* (♀: ♂ = 40:20 µg.kg^{-1})

Brooders size				Treatments	Latency Period (h)	FR (%)	HR (%)
Female		Male					
W (g)	TL (cm)	W (g)	TL (cm)				
558.33±29.67	28.83±2.05	251.33±21.73	15.50±1.44	T1	-	-	-
551.67±15.57	27.80±0.40	263.33±15.57	15.97±1.02	T2	44.5±2.0	78.50±1.89	55.83±1.63
517.67±25.79	27.13±0.47	244.67±20.03	14.83±0.95	T3	-	-	-
394.33±15.18	21.43±0.91	231.33±14.19	14.23±0.61	T4	-	-	-

Note: W = Weight; TL = Total Length; FR = Fertilization Rate; HR = Hatching Rate

Table 3. Captive breeding in E2 of *Pomadasys hasta* (♀:♂ = 50:25 µg.kg^{-1})

Brooders size				Treatments	Latency Period (h)	FR (%)	HR (%)
Female		Male					
W (g)	TL (cm)	W (g)	TL (cm)				
570.67±42.48	29.87±2.29	255.76±14.57	15.47±1.04	T1	-	-	-
582.33±32.65	29.63±1.85	259.67±25.32	15.80±1.60	T2	36.0±1.0	95.45±2.34	75.45±4.07
567.33±33.65	29.33±1.95	260.33±20.40	15.77±1.33	T3	46.0±2.0	70.50±0.90	52.08±1.66
560.33±39.58	28.47±1.04	232.33±23.71	14.43±1.21	T4	-	-	-

Note: W = Weight; TL = Total Length; FR = Fertilization Rate; HR = Hatching Rate

Table 4. Captive breeding in E3 of *Pomadasys hasta* (♀:♂ = 60:30 µg.kg^{-1})

Brooders size				Treatments	Latency Period (h)	FR (%)	HR (%)
Female		Male					
W (g)	TL (cm)	W (g)	TL (cm)				
596.33±24.38	30.03±1.57	232.33±23.12	14.43±1.17	T1	-	-	-
579.67±22.55	29.27±1.61	233.67±19.22	14.40±1.11	T2	33.8±0.8	87.17±1.38	69.08±1.23
495.33±17.01	26.63±0.50	252.67±13.50	15.47±1.04	T3	41.8±1.6	67.45±3.34	48.45±5.65
523.33±17.24	27.17±0.51	236.33±17.16	14.53±1.07	T4	-	-	-

Note: W = Weight; TL = Total Length; FR = Fertilization Rate; HR = Hatching Rate

Table 5. Water physicochemical parameters different larval rearing tanks (LRT)

Parameters	Larval Rearing Tank (LRT)			
	T1	T2	T3	T4
Salinity (ppt)	26-30	26-30	26-30	26-30
Temperature (°C)	18-25	17-25	17-25	18-25
pH	7.4-8.3	7.3-8.4	7.5-8.2	7.3-8.5
Ammonia (ppm)	0.0-1.5	0.0-1.3	0.2-2.0	0.1-1.6
Dissolved oxygen (ppm)	5.5-7.2	5.4-7.5	5.6-7.7	5.4-7.6
Total alkalinity (ppm)	110-160	120-170	116-168	126-156

most successfully invoked the OM, ovulation, and spawning for fertilization. Alike, many authors showed that, artificial insemination (ovulation and spawning) of perciformes accelerated exclusively by LHRHa individually or mixing it with other hormone (Cai *et al*., 2010; Su *et al*., 2019; Ruensirikul and Chiayvareesajja, 2020). In red seabream (*Pagrus major*) application of LHRHa promoted precocious puberty (Kumakura *et al*., 2003). Reproductive technologies conjoining salinity acclimation and stimulation of luteinizing hormone-releasing hormone analog (LHRH-A2) resulted in a fertilization rate of 83.2–91.3% and embryonic survival rates is above 90% (Su *et al*., 2019).

Water physicochemical parameters

The recorded physicochemical parameters viz. salinity, temperature, pH, total ammonia and dissolved oxygen of water during the larvae rearing

period in this experiment were presented in Table 5. Generally, all the hydrological parameters of the experimental treatments were within the acceptable ranges for Datina larvae rearing in hatchery conditions except rarely exceeds an acceptable limit of ammonia. The mean values of water parameters were not significantly (P<0.05) different among the different experimental treatments. Temperature is one of the key physical factors that affect the growth, energy flow, and biology of *P. hasta* larvae. There were found high-temperature fluctuations in the larval rearing tank (LRT) under different treatments between day and night due to sudden weather changes. The temperature of different treatments ranges between 17°C and 25°C. Salinity is considered one of the key factors for Datina breeding and larval rearing. The salinity levels were kept between 26 ppt and 30 ppt in different experimental treatments. The recorded pH of this study ranged from 7.3 to 8.5. Dissolved oxygen (DO) and total ammonia levels were varied between 5.4 to 7.7 ppm and 0.0 to 2.0 ppm, respectively. Jin-cong (2007) described the experimental studies on embryonic and larval development of *P. hasta* with artificial fertilization method with the conformation characters of larvae were also observed. The embryonic development from fertilized eggs to the earliest larvae took 16 h and 45 mins with the temperature at 25.1-26.8°C, salinity at 30 ppt. Jin-cong (2009) also reported preliminary research on artificial breeding of *P. hasta* and found that larval fish developed into juvenile in 27-39 d on the condition of water temperature 23-30.5°C, salinity 27-32 ppt and pH 7.8-8.9. The length of juvenile fish was 24-46 mm.

Embryonic development

The embryonic development phases of *P. hasta* were categorized into eight steps viz. zygote, cleavage, blastula, gastrula, segmentation, pharyngula, hatching, and larval stages were described as follows (Figure 2, 3, 4, & 5.).

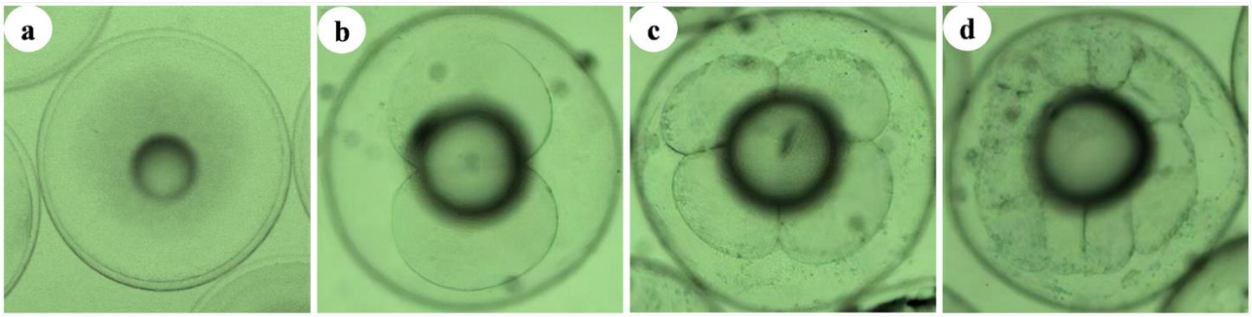

Figure 2. Cleavage stage of *Pomadasys hasta*. (a) Zygote stage; (b–d) meroblastic cleavage stage, comprising with (b) 2-cell stage, (c) 4-cell stage, (d) 16-cell stage

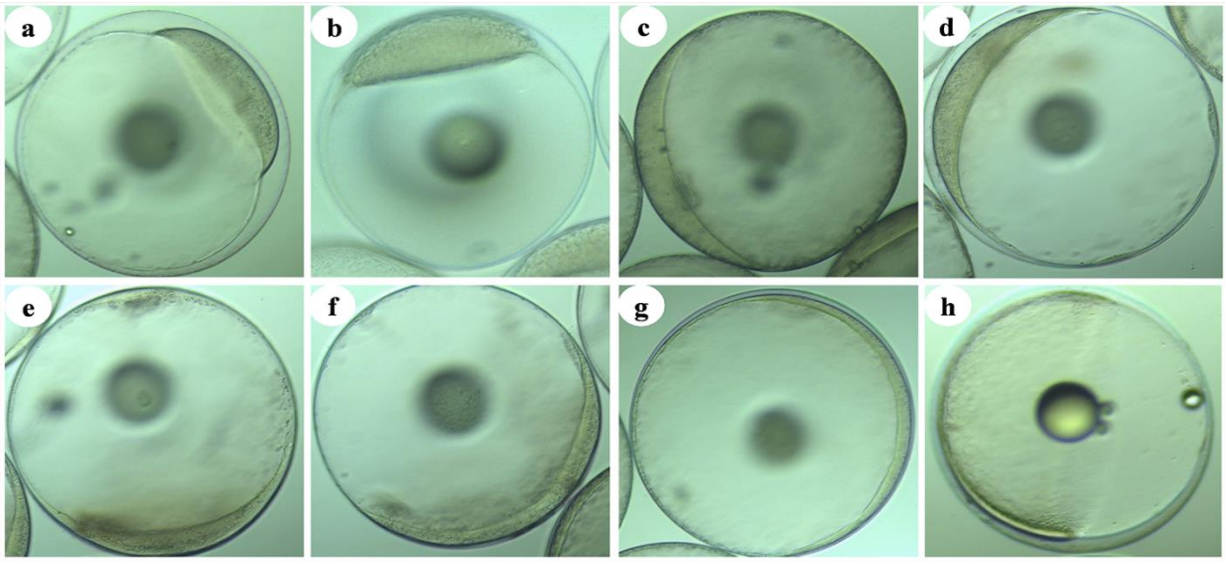

Figure 3. Blastula stage of *Pomadasys hasta*, blastula stage (a–h). Cell cycle length increased, and epiboly was approximately 50% complete (h)

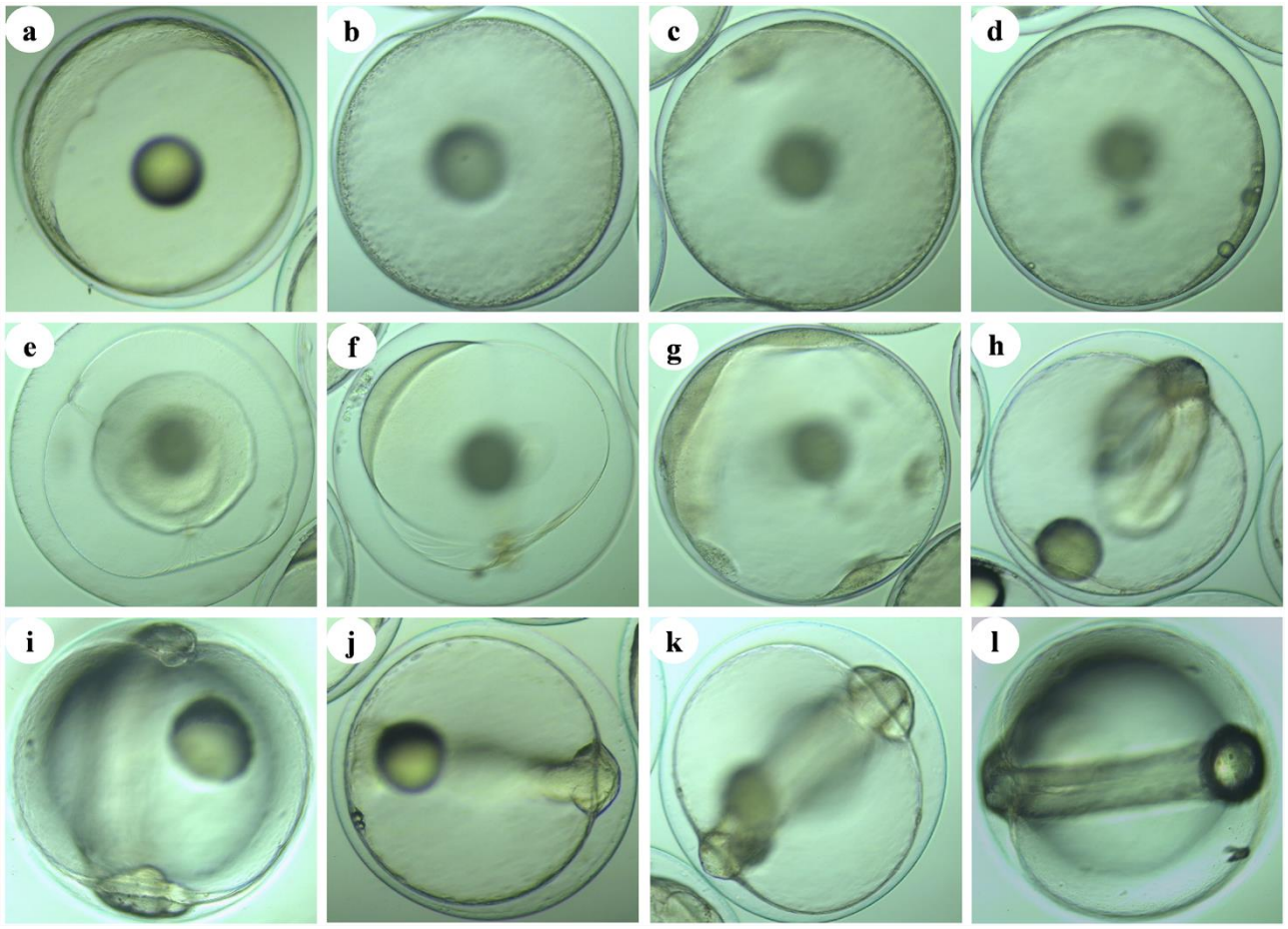

Figure 4. Embryonic development of *Pomadasys hasta*, gastrula stage (a–g). Epiboly gradually expanded, and development was complete by the end of this stage (g); germ ring (h); optic lobe (i-j); 15 somites (k); heartbeat (l).

The zygote period initiated with newly fertilized eggs by completing a stage of the first cell division. The fertilized eggs were transparent, spherical, glazy, and whitish and the diameter of eggs measured between 1.1 and 1.3 mm. Cytoplasmic movements were noticed after the completion of fertilization.

The cleavage period included the second to the seventh cleavage, which occurred rapidly and chronologically. The first cleavage stage i.e., blastomere formation was observed between 20-30 mins after fertilization. The blastomeres were simultaneously divided at an interval of 25-40 mins and completed 4-cell containing stage within 1.0-1.5 h. The egg reached at 16-cell containing stage by 1.5-2.0 h (Figure 2.).

The blastula stage was started with the entire formation of 128-cell of the fertilized egg. At this stage, the cell was rearranged and the duration of this period was observed between 3-6 h and completed with the initiation of the gastrula stage. The blastoderm later developed into a cup-shaped structure with no discernible cells. The blastula period extended as 10, 20, 30, 40, and 50% epiboly at the time interval of every 30-60 mins, respectively (Figure 3.). The embryo was extended up to 50% epiboly stage after the completion of this phase.

In gastrula stage, substantial cell movements were observed simultaneously with convergence, involution, and extension producing three germs' layers and embryonic axis. At this period, the blastoderm underwent 50% to 100% epiboly. Gastrula period ranged 10-13 h. When the germ ring started to develop, signifying the beginning of embryonic axis formation (the embryonic shield). The brain rudiment was thickened and the tailbud was prominent during the late stages of this phase (Figure 4.).

The first morphological cells differentiated and the first body movements become visible in the segmentation period. Sequential formation of the somites was also observed in this stage and this period continued before hatching (Figure 5.). The segmentation period was observed between 12-18 h.

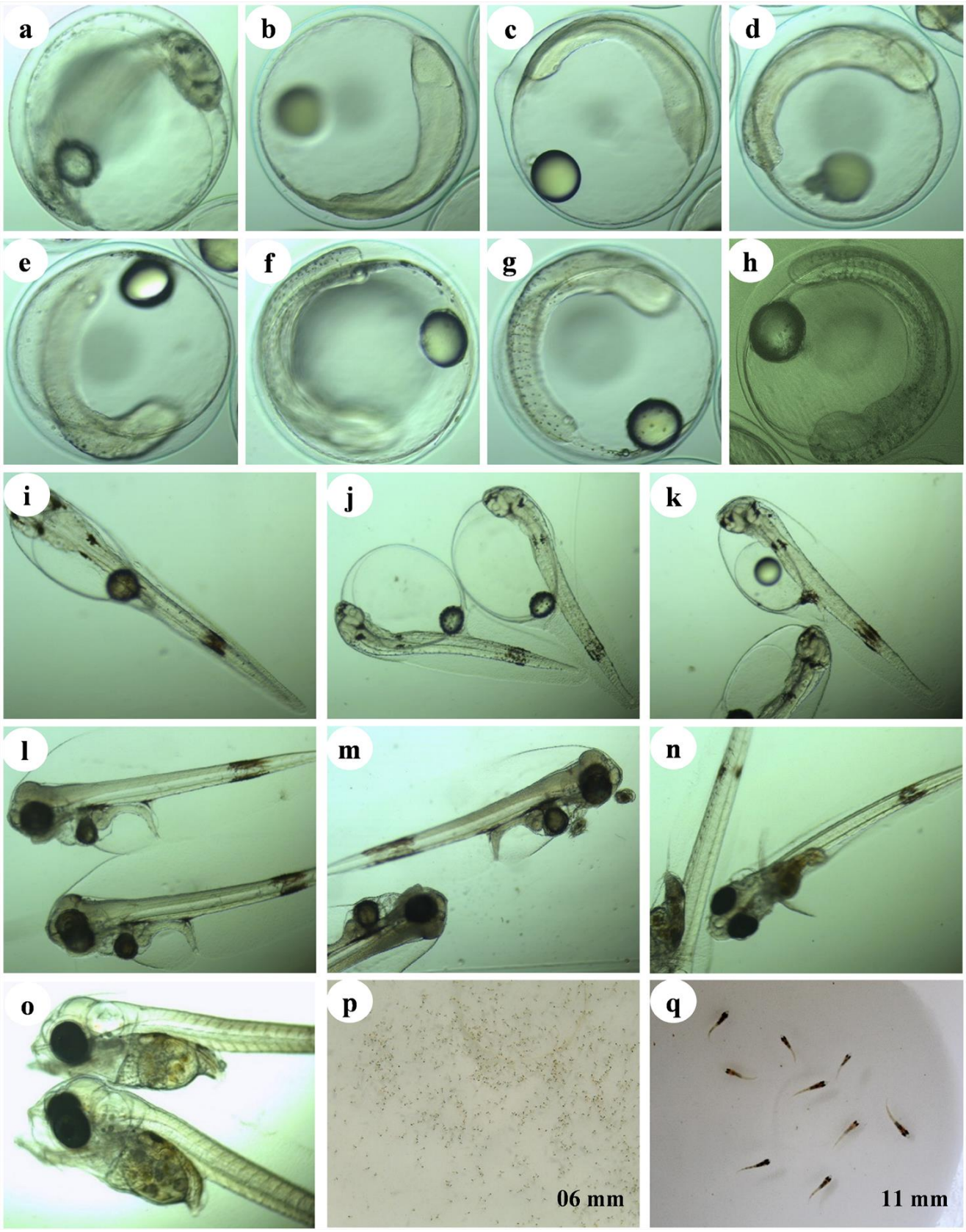

Figure 5. Embryonic development of *Pomadasys hasta*, segmentation (a); pharyngula stage and embryo elongation (b–h); hatching (i-k); larvae with developing fins and intestine (l-o); 20 days larvae (p); and 45 days larvae (q).

In the pharyngula period (16.0 to 24.0 h after fertilization), the embryo possessed the classic vertebrate body plan, and tiny stellate pigments appeared over the trunk and the oil globule (Figure 5.b–h). Gill arches were recognizable. The heart began to beat at the onset of this period and formed well-delineated chambers. Behavioural development was observed at the end of this phase. After 22-26 h of fertilization, the morphogenesis of many organ rudiments was almost complete. The jaws and the gills developed rapidly. At this time, the larva emerged from the egg membrane and the newly hatched larva was 1.6-1.8 mm in length. The hatchling was appeared slender and transparent with a voluminous yolk with large oil globules (Figure 5. i-k). About half of the body of the hatchling was covered by the yolk sac. The newly hatched larva was not very active and remained in resting condition within the water column, adjacent to the wall and bottom of the larval tank. After 10-12 h, they became very active and came to the surface of the water. The heart is not prominent but the heartbeat was observed as 110-120 beats.min^{-1}.

The yolk globule was absorbed within 33-36 hrs post-hatch (hph) and immediately after the end of yolk globule rotifer was provided as a first feeding. Between 54 and 60 hph, a clear mouth gap was witnessed (Figure 5.l-m). Then the larvae were observed as morphological progress with developing fins and gastrointestinal tract with a duration of 80-84 hph (Figure 5.n). The total length of the larvae increased from 2.0–2.5 mm (0 dph) to 3.0–3.5 mm (3 dph). At 7-9 dph, the larvae were less transparent; black colors on the ventral region, and the jaw, and the caudal fin anlage were visible (4.0-4.4 mm). The dorsal and anal-fin anlagen appeared simultaneously when the larvae were 5.2–5.5 mm long, with a gap between the anal-fin origin and the anus. Notochord flexion occurred when larvae were 6.2–6.8 cm long from 20 dph onward, and the girth of the larvae increased after flexion. The head became arcuate from the late preflexion stage, and the eyes were large and round. All dorsal and caudal principal fin rays formed from 25 to 30 dph (larval length: 7.5–8.4 mm). By 40–45 dph when the larval length was >11 mm, the body was thoroughly covered with scales, and the metamorphosis from larvae to juveniles was complete (Figure 5.q). At this stage the larvae have well developed digestive system, ready to accept the commercial feed along with live feed and able to escaping themselves from the predators. Therefore, become ready to rear in the nursery pond. At nursery pond, the fry (60 dph) attains 30.3±3.2 mm length and 0.2±0.07 g body weight (Figure 6.a). However, total length was recorded as 43.5±2.1 mm and average body weight as 0.7±0.2 g for 95 dph (Figure 6.b).

This study described the embryonic development of Grunter fish (*P. hasta*) under captive conditions and the results were similar to those reported in other studies on species from the same order. The egg diameter of European sea bass *Dicentrarchus labrax* were 1.162±0.004 mm (Saka *et al*., 2001) and gilthead sea bream *Sparus aurata* were 1.001±0.005 mm (Kamacı *et al*., 2005) which is similar with the present study (1.1-1.3 mm). In relation to *P. hasta* newly hatched larvae (1.6-1.8 mm) there were slightly larger than those reported for pond perch *Diplectrum radiale* 1.33±0.02 mm (López *et al*., 2002), *H. bonariense* 1.42 + 0.05 mm (Cuartas *et al*., 2003) and somewhat smaller than colorado snapper *Lutjanus colorado* 1.9 mm of length (Abdo-de la Parra *et al*., 2014). Newly hatched larvae (1.5-1.7 mm) of *S. argus* fish in the same order was concomitant with the present study (Su *et al*., 2019).

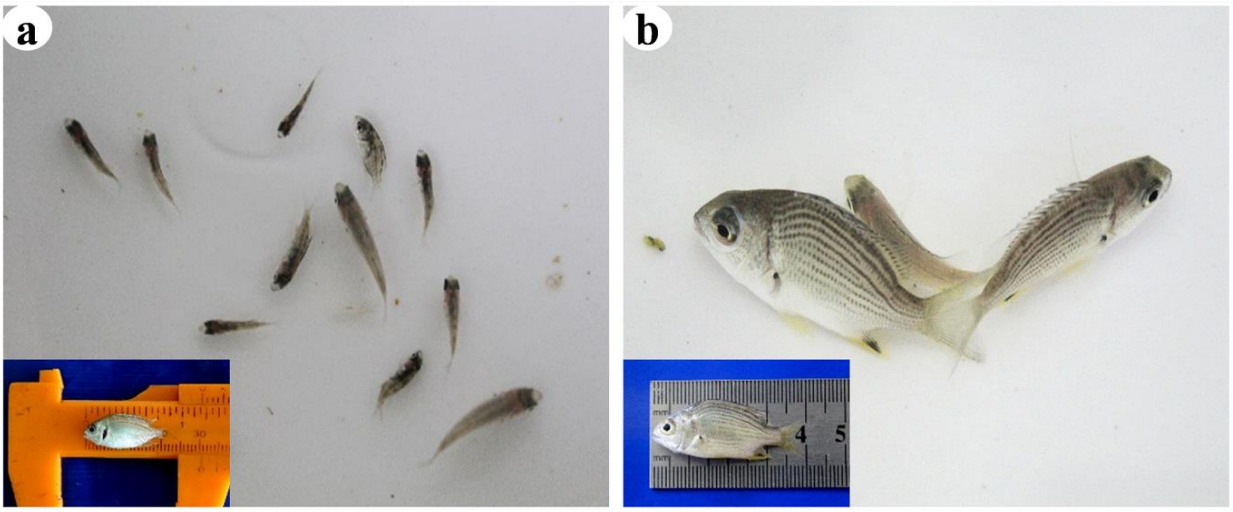

Figure 6. Fry of *Pomadasys hasta* at nursery pond, 60 days fry (a); 95 days fry (b)

The embryonic development of Grunter fish followed the same pattern as the one described for zebra fish *Danio rerio*. The authors defined each stage or period as a reference to identify part of the continuum development and named the stages as zygote, cleavage, blastula, gastrula, segmentation, pharyngula, hatching and early larvae (Kimmel *et al*., 1995). Similar phases were used to describe the embryonic development of spotted scat (Su *et al*., 2019) spotted rose snapper (Boza-Abarca *et al*., 2008) and colorado snapper (Abdo-de la Parra *et al*., 2014). The duration of each phase, stage or period is species-specific and had a close relationship with incubation temperature (Radonic *et al*., 2005, Peña *et al*., 2014). These results showed that the time elapsed in between fertilization and hatching phase is in line with the reproductive biology of spotted scat (Su *et al*., 2019). Such knowledge will enable the refinement of current breeding techniques and facilitate controlled reproduction of *P. hasta* for adequate seed production for the aquaculture industry.

Conclusion

Embryonic development of *P. hasta* was morphologically similar to other species of the same order. After 22-26 h of fertilization, the larva emerged from the egg membrane and the newly hatched larva was 1.6-1.8 mm in length. By 40–45 dph when the larval length was >11 mm, the body was thoroughly covered with scales, and the metamorphosis from larvae to juveniles were completed. At this stage the larvae have well developed digestive system, ready to accept the commercial feed along with live feed and able to escaping themselves from the predators. The present investigation could be used as a tool for the quality assessment of future batches of eggs and as a basis for future advanced study by considering environments. Finally, the description of embryological stages has established an important reference for artificial propagation of *P. hasta*.

Acknowledgement

The authors appreciate the endless effort of Khondoker Shakir Hossain, Field Assistant and Md. Al-Mamun Hossain, Lab Attendant of Brackishwater Station, BFRI during every step of the breeding and larval rearing of *P. hasta* in the hatchery.

References

Abdo-de la Parra, M.I., García-Aguilar, N., RodríguezIbarra, L.E., Velasco-Blanco, G. & Ibarra-Castro, L. 2014. Embryonic Development of Pargo Colorado *Lutjanus colorado* (Jordan & Gilbert, 1882). *Int. J. Morphol.,* 32(3): 902-908. doi: 10.4067/S0717-95022014000300025

Adebiyi, F.A. 2013. The sex ratio, gonadosomatic index, stages of gonadal development and fecundity of sompat grunt, *Pomadasys jubelini* (cuvier, 1830). *Pak. J. Zoo.,* 45(1): 41 - 46.

Al-Ghais, S.M. 1995. Aspects of the biology of *Pomadasys stridens* (Forskaal, 1775) from the West coast of the United Arab Emirates. *Arab Gulf J. Sci. Res.,* 13: 401- 419.

Behera, B.K., Das P., Singh, N.S. & Sahu, A.K. 2010. Captive breeding of an endemic medium carp Pengba, *Osteobrama belangeri* (Val.) with Wova-FH in Manipur. *J. Aqua.,* 18(2010): 23-29.

Borah, R., Sonowal, J., Nayak, N., Kachari, A. & Biswas, S.P. 2020. Induced breeding, embryonic and larval development of *Macrognathus pancalus* (Hamilton, 1822) under captive condition. *Int. J. Aquat. Biol.,* 8(1): 73-82. doi: 10.22034/ijab.v8i1.746

Boza-Abarca, J., Calvo-Vargas, E., Solis-Ortiz, N. & Komen, J. 2008. Induced spawning and larval rearing of spotted rose snapper, *Lutjanus guttatus*, at the Marine Biology Station, Puntarenas, Costa Rica. *Cienc. Mar.,* 34(2): 239–252. doi: 10.7773/cm.v34i2.1246

Burgerhout, E., Brittijn, S.A., Kurwie, T., Decker, P., Dirks, R.P., Palstra, A.P., Spaink, H.P. & Thillart, G.E.V. 2011. First artificial hybrid of the eel species *Anguilla australis* and *Anguilla anguilla*. *BMC Dev. Biol.,* 11(16): 1-9. doi: 10.1186/ 1471-213X-11-16

Butts, I.A.E., Trippel, E.A. & Litvak, M.K. 2009. The effect of sperm to egg ratio and gamete contact time on fertilization success in Atlantic cod *Gadus morhua* L. *Aquaculture*, 286(1-2): 89-94. doi: 10.1016/j.aquaculture.2008.09.005

Cai, Z.P., Wang, Y., Hu, J.W., Zhang, J.B. & Lin, Y.G. 2010. Reproductive biology of *Scatophagus argus* and artificial induction of spawning. In Chinese with English abstract. *J. Trop. Oceanogr.,* 29: 180-185.

Cuartas, A., Rosas, J., Velásquez, A. & Cabrera, T. 2003. Hatching induction, embryonic and larval development of grunt *Haemulon bonariense* Cuvier, 1830 (Pisces: Haemulidae). *Rev. Biol. Mar. Oceanogr.,* 38(1): 27-37. doi: 10.4067/ S0718-19572003000100003

De Silva, S.S. 2012. Aquaculture: a newly emergent food production sector and perspectives of its

impacts on biodiversity and conservation. *Biodivers. Conserv.,* 21(12): 3187-3220. doi: 10.1007/s10531-012-0360-9

Deshmukh, V.M. 1973. Fishery and biology of *Pomadasys hasta* (Bloch). *Indian J. Fish.* 20: 497- 522.

Diana, J.S. 2009. Aquaculture production and biodiversity conservation. *Bioscience*, 59(1): 27–38. doi: 10.1525/bio.2009.59.1.7

Dou, S.Z., Masuda, R., Tanaka, M. & Tsukamoto, K. 2004. Size hierarchies affecting the social interactions and growth of juvenile Japanese flounder, *Paralichthys olivaceus*. *Aquaculture,* 233(1-4): 237-249. doi: 10.1016/j.aquaculture .2003.09.054

Food and Agriculture Organization. 2011. World aquaculture 2010. FAO Fisheries and Aquaculture Technical Paper 500/1, Food and Agriculture Organization of the United Nations. 105 pp.

Iqbal, M.A. 1989. A note on the population dynamics of *Pomadasys kaakan* (Haemulidae) from Pakistan. *Fishbyte,* 7(3): 4-5.

Jawad, L.A., Al-Badri, M.E. & Fricke, R. 2014 New records of thicklips and grunts from the marine waters of Iraq (Teleostei: Haemulidae). *J. Ocean Sci. Foun*., 12: 18-24.

Jin-cong, Y.E. 2007. The Observation on Embryonic and Larval Development of *Pomadasy hasta*. *J. Fuj. Fish.,* 2007(4): 9-11.

Jin-cong, Y.E. 2009. Research on Artificial Breeding of *Pomadasy hasta* in Earth Ponds. *J. Fuj. Fish*. 2009(4): 27-31.

Kamacı, H.O., Saka, Ş. & Fırat, K. 2005. The cleavage and embryonic phase of Gilthead Sea Bream (*Sparus aurata* Linnaeus, 1758) eggs. *E. U. J. Fish. Aqua. Sci*., 22(1-2): 205-209.

Khan, M.A. 2004. Studies on the fecundity and Sex ratio of the Saddle grunt fish *Pomadasys maculatum* (Bloch, 1797), (Family: Pomadasyidae) from Karachi coast. *Int. J. Biol. Biotech*., 1(1): 111-115.

Kimmel, C., Ballard, W., Kimmel, S., Ullmann, B. & Schilling, T. 1995. Stages of embryonic development of the zebrafish. *Dev. Dyn.,* 203(3): 253-310. doi: 10.1002/aja.1002030302

Kucharczyk, D., Nowosad, J., Luczyński, M.J. & Targońska, K. 2016. New technique for fertilizing eggs of burbot, asp and ide under hatchery conditions. *Anim. Reprod. Sci.,* 172: 143–147. doi: 10.1016/j.anireprosci.2016.07. 012.

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*). *Gen. Comp. Endocrinol*., 131(3): 264-273. doi: 10.1016/S0016-6480(03)00012-1

López, P., Rosas, J., Velásquez, A., Cabrera, T. & Maneiro, C., 2002. Embryonic and larval development of the Caribbean sand perch *Diplectrum radiale* Quoy and Gaimard, 1824 (Pisces: Serranidae). *Rev. Biol. Mar. Oceanogr*., 37(2): 127-137. doi: 10.4067/S0718-19572 002000200002

Maitland, P.S. 1995. The conservation of freshwater fish: past and present experience. *Biol. Conserv*., 72(2): 259-270. doi: 10.1016/0006-3207(94)00088-8

McKay, R.J. 2001. Haemulidae (=Pomadasyidae). Grunts (also sweetlips, hotlips, and velvetchins). *In:* Carpenter K.E. & Niem V.H. (Eds). FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Food and Agriculture Organization of the United Nations. Rome. p: 2961-2989

Meffe, G.K. 1990. Genetic approaches to conservation of rare fishes: examples from North American desert species. *J. Fish. Biol.,* 37(SA): 105-112. doi: 10.1111/j.1095-8649. 1990.tb05026.x

Musick, J.A., Harbin, M.M., Berkeley, S.A., Burgess, G.H., Eklund, A.M., Findley, L., Gilmore, R.G., Golden, J.T., Ha, D.S., Huntsman, G.R. & McGovern, J.C. 2000. Marine, estuarine, and diadromous fish stocks at risk of extinction in North America (exclusive of Pacific salmonids). Fisheries, 25(11): 6-30. doi: 10.1577/1548-8446(2000)025<0006:MEADFS>2.0.CO;2

Mustafa, G. & Azadi, M. 1995. Population Dynamics of White Grunter *Pomadasys hasta* from The Bay of Bengal. *Chit. Univ. Stud.* 19(1): 19-22.

Nowosad, J., Kucharczyk, D., Luczyńska, J., Targońska, K., Czarkowski, T. K., Biłas, M., Krejszeff, S., Horváth, L. & Müller, T. 2015. Changes in European eel ovary development and body and ovary chemistry during stimulated maturation under controlled conditions:

Preliminary data. *Aqua. Int.,* 23: 13-27. doi: 10.1007/s10499-014-9794-2.

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 J. Fish. Aquat. Sci.,* 16: 523-530. doi: 10.4194/ 1303-2712-v16_3_04

Oliveira, K. & Hable, W.E. 2010. Artificial maturation, fertilization, and early development of the American eel (*Anguilla rostrata*). *Can. J. Zool*., 88(11): 1121–1128. doi: 10.1139/Z10-081

Peña, R., Dumas, S., Zavala-Leal, I. & Contreras-Olguín, M. 2014. Effect of incubation temperature on the embryonic development and yolk-sac larvae of the Pacific red snapper *Lutjanus peru* (Nichols & Murphy, 1922). *Aqu. Res.,* 45(3): 519-527. doi: 10.1111/j.1365-2109.2012.03255.x

Philippart, J.C. 1995. Is captive breeding an effective solution for the preservation of endemic species? *Biol. Con.,* 72(2): 281-295. doi: 10.1016/0006-3207(94)00090-D

Radonic, M., López, A.V., Oka, M. & Aristizábal, E.O. 2005. Effect of the incubation temperature on the embryonic development and hatching time of eggs of the red porgy *Pagrus pagrus* (Linne, 1758) (Pisces: Sparidae). *Rev. Biol. Mar. Oceanogr*., 40(2): 91-99.

Roy, B.J., Singha, N.K., Rahman, M.G., Mohanta, S.K., Nazrul, K.M.S., Mamun, A. & Haroon, M.I. 2019. Production trends of different group of industrial marine fishes in Bangladesh. *Int. J. Adv. Res. Biol. Sci.,* 6(3): 28-43. doi: 10.22192/ijarbs. 2019.06.03.004

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.

Saka, Ş., Fırat, K. & Kamacı, H.O. 2001. The development of European sea bass (*Dicentrarchus labrax* L., 1758) eggs in relation to temperature. *Turkish J. Vet. Anim.,* 25(2): 139-147.

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. *Aqua. Res.,* 50(7): 1782-1794. doi: 10.1111/ are.14056.

Targońska, K., Perkowski, T., Żarski, D., Krejszeff, S., Mamcarz, A., Kujawa, R., *et al*. 2012. Method of evaluation of wild common tench, *Tinca tinca* (L.), female suitability for artificial reproduction during the spawning season. *Ital. J. Anim. Sci*., 11(2): 164-168. doi: 10.4081/ijas.2012.e30

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(1-4): 345-356. doi: 10.1016/j. aquaculture .2004.08.005.