Heliyon 10 (2024) e24995

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Effects of dietary squid oil on breeding performance and embryonic and larval development of butter catfish *Ompok pabda*

Md Saddam Hossain [a,1], Mohammad Moniruzzaman [b,1], Shahanaj Parvin Rumki [a], Tutul Kumar Saha [a], Mohammad Matiur Rahman [a], Sungchul C. Bai [c,d], Taesun Min [e,*], Zakir Hossain [a,**]

[a] *Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh*
[b] *Department of Animal Biotechnology, Jeju International Animal Research Center (JIA), Sustainable Agriculture Research Institute (SARI), Jeju National University, Jeju, Republic of Korea*
[c] *Feeds & Foods Nutrition Research Center, Pukyong National University, Busan, Republic of Korea*
[d] *FAO World Fisheries University Pilot Program, Busan, Republic of Korea*
[e] *Department of Animal Biotechnology, Bio-Resources Computing Research Center, Sustainable Agriculture Research Institute (SARI), Jeju National University, Jeju, Republic of Korea*

ARTICLE INFO

Keywords:
Polyunsaturated fatty acids
Breeding performance
Larval development
Fecundity
Spawning
Squid oil
Butter catfish

ABSTRACT

The present study was conducted to determine the effects of squid oil on the endangered butter catfish, *Ompok pabda* to confer enhanced breeding with embryonic and larval development for promoting its aquaculture. A total of 360 fish were obtained from the Brahmaputra River, Mymensingh, Bangladesh, of which, 60 fish stocked in each tank having an initial weight and length of 16.35 ± 0.57 g and 15.25 ± 0.38 cm, respectively, in the cisterns of 1.22 × 2.44 × 1.25 m (total 6 cisterns) maintained at 90 cm water depth. During the experimental period, a constant physico-chemical conditions of water such as temperature, pH, and dissolved oxygen (DO) were 26.5 ± 2 °C, 7.4 ± 0.2, and 6.7 ± 0.5 ppm, respectively, were maintained in each cistern. As the source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), dietary 1 % squid oil (SQO) was supplemented in the diet of the treated group (SQO diet) to compare while the control group offered with basal diet without SQO supplementation (CON diet). The fecundity, spawning, fertilization, hatching rate, and survival rate of fry in SQO group were significantly ($P < 0.01$) higher than in the CON group. Moreover, better early embryonic and larval development of fish was observed in the SQO group i.e. size of fertilized egg diameter, growth and early developmental stages of larvae but not significantly different from the control group. Collectively, the results of the present study showed that dietary SQO supplementation improved the breeding and reproductive performances of butter catfish. The findings of this study could assist to develop a nutrient-rich diet for the better broodstock development of butter catfish at the farm level which may ultimately reduce the mortality and poor survival of offspring of this commercially important catfish species.

* Corresponding author.
** Corresponding author.
E-mail addresses: tsmin@jejunu.ac.kr (T. Min), zakir.fbg@bau.edu.bd (Z. Hossain).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e24995
Received 31 March 2023; Received in revised form 13 December 2023; Accepted 18 January 2024
Available online 19 January 2024

1. Introduction

Proper domestication of fish, fry feeding, rearing, and species-oriented culture technique are all required for the sustainable aquaculture of any fish species [1]. For this, special nutrients are required for broodstock development of fish through the seedlings release and are also useful for the prevention of fish starvation, malnutrition, and the improvement of their growth after the release [2]. Despite species differences, it is commonly recognized that maternal nutritional diet has a direct impact on larval development that depend on endogenous energy reserves [3]. Early embryonic and larval development of fish mostly depends on the internal complement of vital nutrients present in the egg. Before and during oogenesis, the maternal diet influences these nutrients [4]. In the context of essential nutrients, dietary fatty acids are one of the strongest determinants of reproductive performance with enhanced reproductive fitness that can be obtained by increasing the dietary lipid levels [5].

Lipid is an important element of fish feed which serves as a basis of fatty acids, phospholipids, sterols, and fat-soluble vitamins necessary for the appropriate functioning of physiological processes and to some extent maintenance of the biological structure and the function of cell membranes [6]. Lipids are not only involved in regulating physiological activities and constructing tissues and organs but also the main sources of energy and fatty acids in terms of endogenous nutrients especially during the embryonic and larval development in fish [7–9]. Therefore, lipids and fatty acids especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) as the n-3 series of highly unsaturated essential fatty acids are play the key role in growth, embryonic development and survival of fish larva and their development [10]. Marine fish oils contain high concentration of EPA, DHA and a considerable levels of arachidonic acid (ARA), whereas none of these polyunsaturated fatty acids (PUFAs) are present in vegetable oils [11]. In case of freshwater fish, they can bioconvert the EPA, DHA and ARA from the fatty acids precursors, linoleic acid (LA) and alpha-linolenic acid (ALA) such as ARA from LA, and EPA and DHA from ALA through the desaturation and elongation process [12]. However, due to the competition of fatty acids, LA and ALA for the enzymes like desaturase and elongase can affect the ratio of PUFA contents in fish during the biosynthesis of EPA, DHA and ARA [13]. For this, freshwater fish required special nutrients such as EPA and DHA for the broodstock and larval development.

Squids contain a healthful omega-3 (or n-3) fatty acids such as EPA and DHA. From squid meal phospholipid, it was found that DHA (22:6n-3) consists of the highest content and the second-maximum amount is EPA (22:5n-3) among the n-3 fatty acids [14]. In addition, among the saturated fats, palmitic acid (16:0) consists of the highest at 33.4 % in squid oil [14]. As a source of essential fatty acids (EFA) such as EPA, DHA and arachidonic acid (ARA), dietary lipids showed a significant role in growth and survival rate of striped gourami fish [5,14]. The lipid content and composition of fish eggs vary with species and this state may change during different developmental stages according to the physiological events and energy demands of the eggs [15]. The (n-3) series of highly unsaturated fatty acids (HUFA) like EPA or 20:5(n - 3) and DHA or 22:6(n - 3) are believed to require for the production of a great number of fish larvae from brood fishes. Different studies have mentioned that DHA is superior to EPA for fish larvae as EFA implies a different physiological function [16]. The PUFAs especially EPA and DHA are considered to be present in large concentrations in fish eggs. During the periods of larval growth, fish larva has a limited ability for lipid biosynthesis due to the insufficient development of liver and intestinal cells [17]. So, it is essential to supply dietary PUFAs to fish larvae for optimum growth and functional activities. The PUFAs are incorporated into cellular and subcellular membranes and are required for optimal fish development as well as helping in the maintenance of membrane fluidity. While maintaining fluidity is crucial as PUFAs structure makes them more vulnerable to attack from reactive oxygen species (ROS) [18]. Furthermore, PUFAs have a significant impact on developing cell membranes during organogenesis in the fish body. It may also play key functions in regulating certain processes involved with membrane transportation, enzymatic activities, and receptor functioning, and serve as precursors for molecules with high activity, such as eicosanoids [19,20]. Squid is caught as a trash fish in Bangladesh. Its commercial sale has not started yet, as there are no commercial buyers in Bangladesh. With this research we aim use it commercially at low cost as there is no market value, which can be used by the farmers who can get squid oil at low cost.

Catfishes are an important part of the fish fauna in the wetlands of Bangladesh and many of them are economically important with high nutritive value, especially butter catfish, *Ompok pabda* belonging to the family, Siluridae and the order, Siluriformes, is an important candidate species for commercial aquaculture [21–23]. The butter catfish has high market demand and delicacy to the consumers in terms of nutritive value [23]. The fish is low in calories and packed with lean protein, healthy fats, vitamins, and minerals. It's particularly rich in heart-healthy omega-3 fatty acids and vitamin B12. Only 150 mt productions of this fish are recorded from different water bodies of Bangladesh [24]. In addition to Bangladesh, butter catfish is also distributed in *beels*, ponds, rivers and inundated fields of South-East Asian countries such as India, Pakistan, Afghanistan, Bhutan and Myanmar [25]. However, in recent years, due to indiscriminate fishing, habitat degradation, widespread use of pesticides and insecticides in agricultural fields, depletion of spawning grounds for this fish species and various ecological changes, its catches have significantly reduced from open waters that making it an endangered species in Bangladesh [25,26]. The reasons behind the alternation of this fish species is also resulting from the lack of proper knowledge on its domestication in culture system, breeding and farming technique and proper management [21,27]. Despite its greater ecological and economic value, this species did not receive sufficient attention in aquaculture. Proper feeding and best management practices can enhance the production of butter catfish at farm levels [23]. The scanty number of fish in natural water bodies and the high mortality rate of the larvae are the major constraints to the successful aquaculture of this important fish species [22]. This study aimed to evaluate the effects of dietary squid oil on the breeding performance, embryonic and larval development of endangered butter catfish.

2. Methods

2.1. Experimental setup and collection of fish

The present study was conducted from March to July 2021 at the Bangladesh Agricultural University, Bangladesh; located between 24°15′ and 25°12′ north latitudes and in between 90°04′ and 90°49′ east longitudes, respectively. Butter catfish, *Ompok pabda* collected from a wild source, the Brahmaputra River, Mymensingh, Bangladesh. The study was conducted in the cistern (1.22 m × 2.44 m × 1.25 m) according to the dietary treatments in triplicates (total 6 cisterns) based on completely randomized design (CRD). Prior to the execution of the experiment for 150 days, each cistern was stocked with 60 healthy fish (a total of 360 fish in 6 cisterns, maintaining a ratio of male: female to 1:1) with an average initial length of 15.25 ± 0.38 cm and an average initial weight of 16.35 ± 0.57 g. Fish were conditioned in the cisterns for two weeks. Water depth was maintained at 90 cm in each cistern throughout the study period. At the beginning and end of the experiment, fish were individually weighed using an electronic balance (AND-GULF, Model-EK600, UAE).

2.2. Experimental diets

In the present study, two diets were maintained with 30 % of protein levels. The experimental diet formulations include without (control diet, CON) or with EPA and DHA containing squid oil (SQO) supplemented diets shown in Table 1. In the treated diet, 1 % SQO was used as the source of EPA and DHA while soybean oil (locally purchased) was used in the control diet as the soybean oil is the world's second most commonly produced oil, low cost and good alternatives to fish oil [13,28]. Here, the collected SQO was extracted from squid using the method of Bligh and Dyer [29] in the laboratory of Genetics and Biotechnology, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. All the dry ingredients (fish meal, rice bran, wheat bran, maize meal, vitamin and mineral complexes) were mixed using the Pearson square method for feed formulation. The proximate composition of the main ingredients of the experimental diets such as fish meal, maize meal, rice bran, wheat bran, wheat flour, and the fatty acids composition of SQO is presented in Tables 2 and 3, respectively. The experimental diets were prepared by mixing the ingredients and distilled water (about 10 %) in a Hobart mixer and the resulting moist dough was pelleted using a meat mincer with a 1-mm die. Then the pellet feeds were kept in the tray and dried in the sun. After that, the pellet feeds as the control diet and the treatment diet were stored with tagging in the zipper bags in air-tight condition and kept at −20 °C for later use in the experiment.

2.3. Analyses of the fatty acids composition of squid oil and proximate composition ofFeed ingredients, and diets

The fatty acids analysis of squid oil and proximate composition of the dietary ingredients, and experimental diets were determined in the Nutrition Laboratory of the Faculty of Fisheries, BAU, Mymensingh following the standard methods given by the Association of Official Analytical Chemists [30]. Here, the fatty acids composition of squid oil, and proximate composition of ingredients and diets were analyzed before the execution of the feeding trial. For this, the fatty acids composition of squid oil was determined by gas chromatography mass spectrometry (GC-MS) (Trace GC, Thermo Finnigan, San Jose, CA, USA). Briefly, the sample was converted to fatty acid methyl ester (FAME) by transesterification method. The composition of FAMEs was determined by GC-MS with flame ionization detector, equipped with a Carbowax 007 capillary column (30 m × 0.25 mm inside diameter, film thickness 0.25 mm, QUADREX, Bethany, CT, USA). Injector and detector temperatures were 250 °C. The column temperature was programmed from 100 to 220 °C at a rate of 5 °C/min and 220 to 240 °C at a rate of 3 °C/min. Helium was used as the carrier gas. Fatty acid methyl esters were identified by comparison with known standards of FAME mix-37 (Supelco, Bellefonte, PA, USA). Protein was determined by following the standard Micro-Kjeldahl method (N × 6.25) after the determination of nitrogen (N) content through acid digestion, distillation, and

Table 1
Percentage of formulated diet ingredients (% on dry matter basis)[a].

Ingredients	CON diet (%)	SQO diet (%)
Fish meal	26.5	26.5
Maize meal	20.5	20.5
Rice bran	21.5	21.5
Wheat bran	19.5	19.5
Wheat flour	10.0	10.0
Vitamin premix	0.5	0.5
Mineral premix	0.5	0.5
Soybean oil	1.0	0.0
Squid oil	0.0	1.0
Proximate composition (% on dry matter basis)		
Moisture	11.1	11.2
Crude protein	30.0	30.1
Crude lipid	5.5	5.6
Crude ash	14.3	14.5
Crude fiber	8.6	8.7
Nitrogen free extract	30.3	30.5

[a] used Pearson square method for feed formulations and maintained 30 % protein level.

Table 2
Proximate composition of feed ingredients (% on dry matter basis).

Feed ingredients	Ash (%)	Moisture (%)	Lipid (%)	Protein (%)	Carbohydrate (%)
Fish meal	15.61	13.44	8.42	62.40	0.10
Maize meal	3.74	9.78	5.73	15.78	54.80
Rice bran	9.70	13.44	24.99	17.76	19.73
Wheat bran	4.93	10.64	4.43	14.57	55.72
Wheat flour	0.32	11.92	3.90	10.92	72.94

Table 3
Fatty acids composition of squid oil.

Fatty acids	Percentage (%)
16:0, palmitic acid	32.60
18:0, stearic acid	1.30
18:1 (n-9), oleic acid	2.40
18:2 (n-6), linoleic acid (LA)	1.00
20:2 (n-6), eicosadienoic acid	0.50
20:4 (n-6), arachidonic acid (ARA)	1.00
20:5 (n-3), eicosapentaenoic acid (EPA)	11.00
22:6 (n-3), docosahexanoic acid (DHA)	39.70
Others	10.50
Total	100.00

titration of the samples of feed ingredients and diets. Moisture content was determined by drying of the samples (0.1 g for each sample) in an electric oven at 105 °C for 24 h. Ash contents in the samples (1 g for each sample) were determined by incineration at 450 °C in a muffle furnace (Carbolite EML 11/6, Cadmus, UK) overnight. Lipid content was determined by extracting fats from the samples (1 g for each sample) with analytical grade acetone using a Soxhlet apparatus (Soxtec system 1046, Tecator AB, Foss, Hoganas, Sweden). Accurately weighed sample (0.5 g for each sample) was taken in a pre-weighed filter crucible and crude fiber was determined following standard procedure and finally calculated in percentage. Nitrogen free extracts (NFE) were estimated as a soluble carbohydrates by subtracting the sum of moisture, crude protein, crude lipid, ash and crude fiber from 100. Carbohydrate and NFE were calculated using the following formula:

$$\text{Carbohydrate (\%)} = 100 - (\text{moisture} + \text{crude protein} + \text{crude lipid} + \text{crude ash})$$

$$\text{NFE (\%)} = 100 - (\text{moisture} + \text{crude protein} + \text{crude lipid} + \text{crude ash} + \text{crude fiber})$$

2.4. Feeding of fish

Fish were fed with the formulated diets at 4 % body weight of fish twice a day for 150 days. Debris was siphoned out from the cistern twice in a week to keep cleanliness. Water was exchanged at least 50 % every day through the inlet and outlet system of the cisterns.

2.5. Physico-chemical parameters of water

Temperature, dissolved oxygen (DO), pH, ammonia, total alkalinity, transparency, and salinity of water in each cistern were recorded at weekly intervals. Temperature, dissolved oxygen (DO), pH, ammonia, total alkalinity, transparency, and salinity were measured by using a celsius thermometer, a portable digital oxygen meter (MI 605, Milwaukee instruments, Martini, MI), a portable digital pH meter (MICRO-TEMP, pH 500, Romania), the API® ammonia test kit, a digital total alkalinity meter (HANNA Instruments HI775, Romania), secchi disk reading and refractometer (HANNA Instruments 96801, Romania), respectively. During the experimental period, the physicochemical conditions of water did not vary significantly in the experimental cisterns such as the temperature: 26.5 ± 2.0 °C, pH: 7.4 ± 0.2, dissolved oxygen (DO): 6.7 ± 0.5 ppm, ammonia: 0.02 ± 0.0 mg/L, total alkalinity: 120.30 ± 6.4 mg/L, transparency: 25.20 ± 1.6 cm and salinity: 0.001 ± 0.0 ppt which were in the optimum levels.

2.6. Injection of pituitary gland (PG) hormone

In induced breeding of butter catfish, pituitary gland (PG) (United Agro Fisheries, Jashore, Bangladesh) solution was used as an inducing agent for the ovulation of the eggs according to Tumpa et al. [22] with little modification. Briefly, at the end of the experiment, the female fish were given an injection of 3 mg PG/kg body weight of fish and the male fish were given an injection of 6 mg PG/kg body weight as first dose. After 6 h, the second dose of PG was given in 16 mg PG/kg body weight of females and 6–8 mg

PG/kg body weight of males. For this, 3 male and 3 female gravid fish (1:1) were selected randomly from each cistern and injected accordingly based on the treatment groups.

2.7. Fecundity, ovulation, fertilization and collection of fish eggs

Fecundity is determined by the number of eggs produced by brood fish during a breeding season. In this study, the fecundity of the female fish was measured from March to July 2021. For this, two female fish from each cistern based on SQO diet group and CON diet group were sacrificed to collect ovaries during the months of March, April, May, June, and July and preserved the gonads in formalin for further calculation number of eggs in each ovary from the sacrificed fish. For the estimation of fecundity, the gravimetric method was used for studying fecundity, which is based on the relation between ovary weight and a number of eggs in the ovary [31,32]. For this, three subsamples of ovary were taken from the front, mid and rear sections of each ovary and weighed. The total number of eggs in each ovary subsample was then proportionally estimated using the equation:

Fecundity (F) = (ovary weight × number of eggs in the subsample)/sub-sample weight.

Brood fish selection is one of the most important aspects for successful induced breeding. In this study, good looking, healthy and sexually mature broods were selected for breeding [22]. Mature male and female broods were identified on the basis of secondary sexual characteristics. The mature males were identified by their flat abdomens and long protruded genital papillae. On the other hand, the females could be easily recognized by their soft and swollen abdomen as well as round and swollen urogenital papillae. After injection of PG, the males and females under each treatment were kept in the different cistern for stripping. The PG injected females were kept under observation to monitor if they exhibit any change in behavior. During this period close observation was done to see whether they make pairing or courtship behavior. The females were checked every hour after 8 h of first injection by gently pressing their abdomen to ascertain the ovulation. A fish was considered ovulated when there were extrusions of a few eggs upon gentle pressure on the abdomen from the anterior to posterior direction. The females upon ovulation were immediately stripped and eggs from each fish were collected in separate fertilization trays or Petri dish. The male could not be stripped for collection of milt. The male fish was anesthetized (150 mg/L solution of tricaine methanesulfonate or MS-222) and in the meantime, the testes from the respective male were dissected out from its body cavity and were macerated in 0.85 % sodium chloride (NaCl) solution. To initiate fertilization, the sperm suspension was mixed with eggs by gently stirring with a feather and water was added to the egg-sperm mixture to activate the sperms for fertilizing the eggs. Fertilized eggs were washed several times with clean water to remove the excess milt, blood, etc.

2.8. Determination of fertilization, hatching and survival rates of butter catfish

A batch of approximately 100 fertilized eggs was placed in each sieve (six replicates) to determine the fertilization and hatching rates according to the experimental diets. Soon after fertilization embryonic development started and the fertilized eggs assumed a watery appearance or were slightly transparent in color, while the unfertilized ones turned whitish and opaque as time passes. Within 6 h of incubation, the numbers of fertilized and unfertilized eggs from each sieve were counted based on the color and appearance of the eggs. The fertilized eggs began to change their size and color from yellowish to watery and transparent while unfertilized eggs turned opaque and whitish in color. The eggs of both treatments have been incubated at room temperature (25 °C). After the completion of hatching, the number of newly hatched larvae of every sieve becomes counted. Percent fertilization and hatching rates had been recorded as indices of the effectiveness of diet by using the following formula:

Fertilization rate (%) = ((number of fertilized eggs) / total number of eggs (fertilized + unfertilized)) × 100

Hatching rate (%) = (number of hatched eggs / total number of fertilized eggs) × 100

Survival rate (%) = (number of live hatchlings at the end / total number of live hatchlings at the beginning) × 100

2.9. Observation of embryonic and larval development stages of butter catfish

The fertilized eggs were collected randomly from the hatching trays with the help of a dropper and taken in a Petri dish containing water for observing the embryonic developmental stages of butter catfish at each 15 min, 30 min, and 1 h interval until the end of the hatching stages. The microscopic observation was continued until the first feeding of fry. At least 8–10 eggs were observed in each stage up to 5 days after hatching to achieve records.

2.10. First feeding of the hatchlings of butter catfish

Although the hatchlings of butter catfish get nutrients from the yolk sac up to 3 days after hatching, the larvae began out first feeding on 36–72 h post-hatching at the ambient temperature of 27–29 °C. Hard-boiled chicken egg yolk supplied as a first feed for the hatchlings up to satiation level. The boiled egg yolk was mixed with water through a fine sieve. After 3–4 d, live tubificid worms (finely chopped) were provided to larvae.

2.11. Statistical analysis and images capture

The results were expressed as mean ± SD in triplicates by using a Microsoft Excel sheet on Windows 2010. The differences between control and treatment groups were analyzed using by paired-samples T-test using the statistical software package SPSS 22. The statistical significance was set at $P < 0.05$ and $P < 0.01$, as compared to the control group. Embryonic and larval developmental stages of *O. pabda* were photographed (40x) under a binocular microscope (OLYMPUS CX41RF, Tokyo, Japan) with a digital camera (Pixel 5.0) (OLYMPUS DP22, Tokyo, Japan).

3. Results

3.1. Effects of squid oil on the fecundity of butter catfish

In the present study, the fecundity of butter catfish was found significantly higher ($P < 0.01$) in SQO treated brood compared to the control during the spawning season (April to July) and despite the values started to decrease after June, the SQO group maintained the significantly higher fecundity until the month of July. The fecundity of fish in the present study was ranged between 3421 ± 210 to 15521 ± 342 eggs/fish during the experimental period. However, the fecundity of the treated fish was the peak in the month of June as 15521 ± 342, whereas 13143 ± 321 eggs/fish was found in the control (Table 4).

3.2. Effects of squid oil on the fertilization, hatching and survival rates of butter catfishlarvae

Fertilization of the experimental fish happened immediately after ovulation. The fertilization rate in the SQO-treated group was significantly greater ($P < 0.05$) than in the control group. The fertilization rates were recorded in June as 92.23 ± 0.69 % and 81.21 ± 0.74 % with SQO-treated fish and the control group, respectively. The number of hatchlings was counted by visual observations. The number of spawn/larvae in each bowl was counted in June. The hatching rate in the SQO-treated group was significantly higher ($P < 0.05$) than in the control. The hatching rates were 87.43 ± 0.71 % and 78.29 ± 0.47 % with SQO-treated fish and the control group, respectively. The survival of fish fry is a crucial indicator of good reproductive performance. The survival rate of larvae in the SQO-treated group was significantly greater ($P < 0.05$) than in the control group. However, in the present study, the survival of fish larvae were 76.62 ± 0.82 % and 62.31 ± 0.65 % with SQO-treated fish and the control group, respectively (Fig. 1).

3.3. Effects of squid oil on the embryonic development of butter catfish

Embryonic developmental stages of the control and SQO treated groups were measured after the successful breeding of butter catfish. Each of the stage was captured by using a camera with a digital microscope. The diameters of eggs were measured in the 'mm' scale of the live sample by using the software "ImageJ". In this study, the diameters of SQO-treated fish eggs were somewhat larger than the control. The embryonic developmental stages took place within the chorion and ended up with a hatching phage. The stages and time intervals of butter catfish fertilized eggs were thoroughly showed in Table 5 as well as Figs. 2 and 3.

The fertilized egg started with a thin perivitelline surface that separated the ovum's membrane from the rest of the ovum cell. The color of fertilized eggs of butter catfish was brownish and it appeared as adhesive, spherical, and transparent in both of the experimental groups. The size of fertilized egg diameter was measured 96.72 ± 1.04 μm in control fish (Fig. 2b), whereas for the SQO-treated fish (Fig. 3b) it was 98.82 ± 1.14 μm in diameter. Then the blastodisc of the eggs were formed (Figs. 2c and 3c). The initial cleavage (2-cell stages) stage was recognized in fertilized eggs which appeared within 36 min of the post-fertilization. The average diameter of this stage was 105.58 ± 0.52 μm in control fish (Fig. 2d) and 107.48 ± 0.79 μm was in SQO-treated fish (Fig. 3d). The second cleavage (4-cell stage) appeared approximately within 46–48 min after the fertilization and the average diameter of the control (Fig. 2e) and treated (Fig. 3e) groups were 113.45 ± 1.13 μm and 114.57 ± 1.03 μm, respectively. To form the multicellular blastodisc, the construction of blastomere cells increased as time progressed and attained 8 cells, 16 cells, 32 cells, 64 cells, 128 cells and 512 cells within 60 min, 1:08 h, 1:12–1:13 h, and 1:22 h, respectively after the fertilization. The mean diameter was observed 116.33 ± 0.76, 117.65 ± 0.62, 116.38 ± 0.73, 116.21 ± 1.87 μm, 116.11 ± 1.12 μm, and 115.97 ± 0.53 μm for the control diet group (Fig. 2f–k), and 118.37 ± 0.86, 118.65 ± 0.68, 117.58 ± 0.94, 117.71 ± 2.17, 116.93 ± 0.63 μm, 116.37 ± 0.72 μm for the SQO diet group (Fig. 3f–k).

At the morula stage, blastulation progressed to form a multicellular blastodisc within 2:06 h after fertilization and the mean

Table 4
Fecundity (eggs/fish) variation in different month of female butter catfish[a].

Month	CON diet	SQO diet
March	3421 ± 210	3472 ± 231
April	5432 ± 243	7228 ± 221**
May	9314 ± 231	12054 ± 237**
June	13143 ± 321	15521 ± 342**
July	8653 ± 265	10928 ± 245**

[a] Values of each parameter in the same row with asterisk are significantly different (**$P < 0.01$). Values are presented as mean ± SD of samples in triplicates (n = 3).

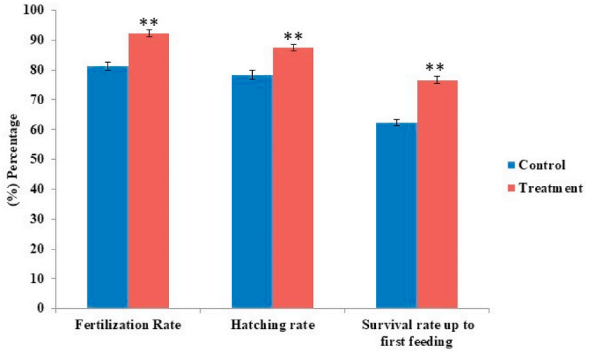

Fig. 1. Percentage (%) of fertilization rate, hatching rate and survival rate of butter catfish up to first feeding. Data represented as mean ± SD in triplicates (n = 3) (*t*-test, $^{**}P < 0.01$).

Table 5
Embryonic development of control and SQO treated diets for *O. pabda*.

Time after spawning	Development stage	Key description	Control diet		SQO diet	
			Egg size (μm) (mean ± sd)	Fig. 2	Egg size (μm) (mean ± sd)	Fig. 3
0 min	Fertilized egg	Eggs were adhesive, spherical, transparent and brownish in color.	96.72 ± 1.04	b	98.82 ± 1.14	b
36 min	2 cell stage	First cleavage	105.58 ± 0.52	d	107.48 ± 0.79	d
46–48 min	4 cell stage	Second cleavage	113.45 ± 1.13	e	114.57 ± 1.03	e
60 min	8 cell stage	Third cleavage	116.33 ± 0.76	f	118.37 ± 0.86	f
1:08 h	16 cell stage	Fourth cleavage	117.65 ± 0.62	g	118.65 ± 0.68	g
1:12–1:13 h	32 cell stage	Fifth cleavage	116.38 ± 0.73	h	117.58 ± 0.94	h
1:22 h	64 cell stage	Sixth cleavage	116.21 ± 1.87	i	117.71 ± 2.17	i
2:06 h	Morula stage	Blastulation progresses to form a multicellular blastodisc	111.29 ± 1.10	l	112.24 ± 1.02	l
3:30 h	Blastula stage	A third of egg space covered by the blastoderm cells	113.26 ± 0.37	n	115.59 ± 0.83	n
5:00 h	Yolk plug stage	Yolk invasion complete and cephalic region gets thicker in size	120.28 ± 2.13	o	121.23 ± 2.02	o
7:00–8:00 h	Kidney shaped embryo	Elongated embryo with rudimentary notochord	119.73 ± 0.54	p	120.36 ± 0.67	p
9:00–10:00 h	Enlarged embryo	The cephalic and caudal end becomes almost differentiated	120.55 ± 0.25	q	121.95 ± 0.17	q
11:00–12:00 h	Kupffer's vesicle formed	An oval area is observed at the base of caudal region to form kupffer's vesicle	117.73 ± 1.32	r	118.30 ± 1.43	r
13:0–14:00 h	Optic vesicle developed	The tail rudiment gets separated and optic vesicle fully developed	113.81 ± 1.12	s	114.67 ± 1.03	s
15:00–16:00 h	Rapid twisting movement	Yolk mass differentiated in to yolk bulb. The caudal region at this stage found more active.	113.32 ± 1.78	t	114.99 ± 1.92	t
17:00–18:00 h	Fully active embryo	The egg membrane becomes decomposed and lost its shape	119.62 ± 1.01	u	120.77 ± 0.99	u
20:00–21:00 h	Just before hatching	Embryo with prominent eye with rudiments of maxillary barbells	121.14 ± 0.43	v	122.04 ± 0.31	v
22:00 h	Hatchling	Hatching of embryos start	126.78 ± 0.73	w	127.80 ± 0.80	w

diameters were 111.29 ± 1.10 μm and 111.97 ± 1.04 μm for the control (Fig. 2l and m) and 112.24 ± 1.02 μm, 112.92 ± 1.42 μm for SQO-treated (Fig. 3l and m) fish. The blastula stage characterized with a third of egg space covered by the blastoderm cells within 3:30 h after fertilization where the average diameter was 113.26 ± 0.37 μm for the control (Fig. 2n) and 115.59 ± 0.83 μm for the SQO-treated group (Fig. 3n). The yolk plug stage was attained within 5:00 h of fertilization, as yolk invasion completed and the cephalic region got thicker in size, and the average diameter of the control group was 120.28 ± 2.13 μm (Fig. 2o) and the SQO group was 121.23

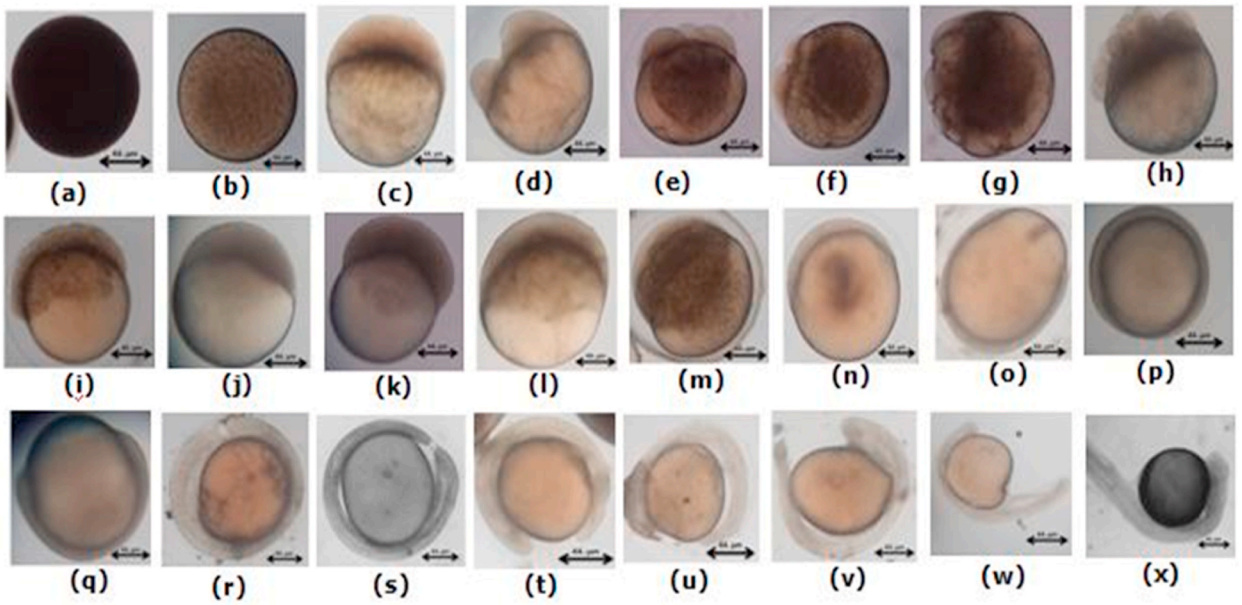

Fig. 2. Early embryonic developmental stages of control group of butter catfish, (a) Unfertilized egg, (b) Fertilized egg, (c) Blastodisc formation, (d) 2- cell stage, (e) 4-cell stage, (f) 8-cell stage, (g) 16-cell stage, (h) 32- cell stage, (i) 64-cell stage, (j) 128-cell stage, (k) 512-cell stage, (l) Oblong stage, (m) Sohere stage, (n) 50 % epiboly, (o) 90 % epiboly, (p) Epiboly complete, (q) Bud stage, (r) Somite stage, (s) Segmentation-I, (t) Segmentation-II, (u) Segmentation-III, (v) Segmentation-IV, (w) Newly hatched larvae, (x) 24 h old larvae. Scale bar: 44 μm.

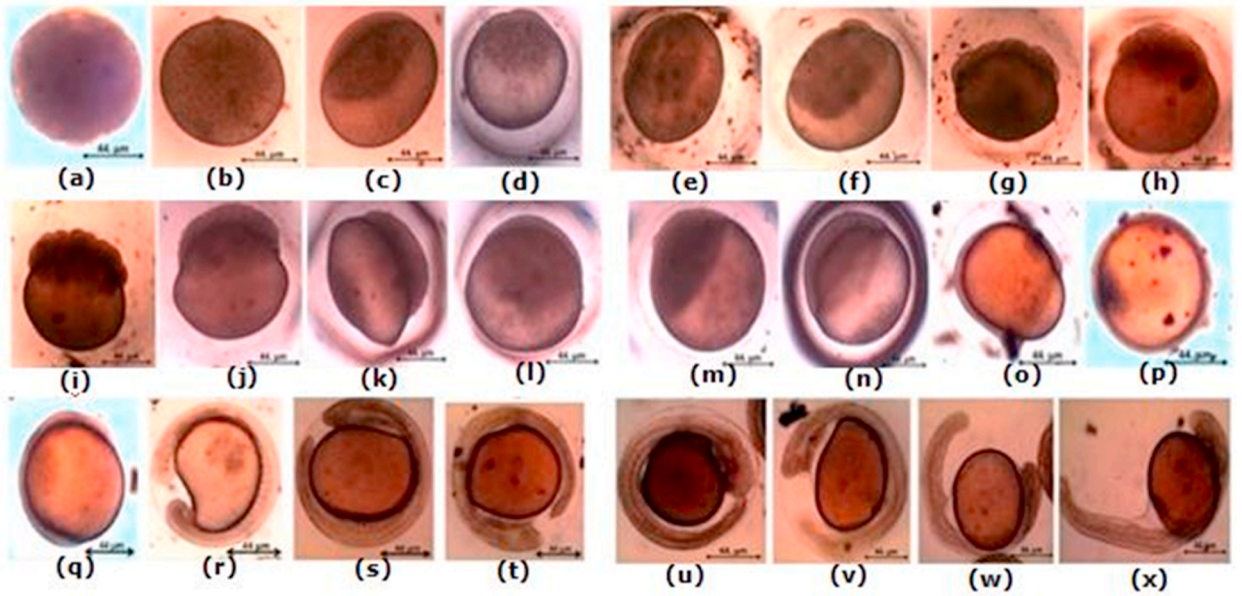

Fig. 3. Embryonic development of butter catfish for squid oil (SQO) treated group (a) Unfertilized egg, (b) Fertilized egg, (c) Blastodisc formation, (d) 2- cell stage, (e) 4-cell stage, (f) 8-cell stage, (g) 16-cell stage, (h) 32- cell stage, (i) 64-cell stage, (j) 128-cell stage, (k) 512-cell stage, (l) Oblong stage, (m) Sohere stage, (n) 50 % epiboly, (o) 90 % epiboly, (p) Epiboly complete, (q) Bud stage, (r) Somite stage, (s) Segmentation-I, (t) Segmentation-II, (u) Segmentation-III, (v) Segmentation-IV, (w) Newly hatched larvae, (x) 24 h old larvae. Scale bar: 44 μm.

± 2.02 μm (Fig. 3o). The earliest sign of the embryo was visible and showed as the kidney-shaped embryo after 7:00–8:00 h of post-fertilization and characterized with elongated embryo and rudimentary notochord. The average diameter of the control group was 119.73 ± 0.54 μm (Fig. 2p) and the SQO group was 120.36 ± 0.67 μm (Fig. 3p). At the 9:00–10:00 h after fertilization, an enlarged embryo appeared where the cephalic and caudal end became almost differentiated. At that moment, the average diameter of control

was 120.55 ± 0.25 μm (Fig. 2q) and the SQO group was 121.95 ± 0.17 μm (Fig. 3q). At the 11:00–12:00 h from fertilization, an oval area was observed at the base of the caudal region to form kupffer's vesicle where the average diameter of control was 117.73 ± 1.32 μm (Fig. 2r) and the SQO group was 118.30 ± 1.43 μm (Fig. 3r).

After 13:0–14:00 h of post-fertilization, the optic vesicle fully developed where the tail rudiment got separated. The average diameter of control was 113.81 ± 1.12 μm (Fig. 2s) and the SQO group was 114.67 ± 1.03 μm (Fig. 3s). At the 15:00–16:00 h after fertilization, rapid twisting movement stages appeared where yolk mass differentiated into yolk bulb, and the caudal region at this stage was more active. The average diameter of the control group was 113.32 ± 1.78 μm (Fig. 2t) and the SQO-treated group was 114.99 ± 1.92 μm (Fig. 3t). A fully active embryo was noticed after 17:00–18:00 h of post-fertilization where the egg membrane became decomposed and lost its shape. The average diameter of the control (Fig. 3u) and SQO-treated (Fig. 3u) groups were 119.62 ± 1.01 and 120.77 ± 0.99 μm, respectively. The embryo's development continued within 20:00–21:00 h after post-fertilization, and its movement became stronger with time until the embryo was able to break away from the encompassing membrane. Just before the hatching, the embryo pounded speedily by its tail and it became free with a prominent eye with rudiments of maxillary barbells. The average diameter of the control (Fig. 2v) and treated (Fig. 3v) groups were 121.14 ± 0.43 and 122.04 ± 0.31 μm, respectively. The larvae started hatching at 22:00 h of fertilization. After hatching the larvae were straight and can be differentiated by a head, trunk as well as tail, and its length was 126.78 ± 0.73 and 127.80 ± 0.80 μm, respectively for the control (Fig. 2w) and treated (Fig. 3w) group of butter catfish.

3.4. *Effects of squid oil on the larval development of butter catfish*

The growth and early developmental stages of larvae of the SQO treated group were found different from those of the control group by estimation. The larval development stages are given in Table 6 and Figs. 4 and 5 over time. Just before hatching, the egg membrane became decomposed and lost its shape. It appeared with vigorous thrashing movements and frequent whipping motion of the embryo. The diameter size were 121.14 ± 0.43 and 122.04 ± 0.31 μm for the control (Fig. 4A) and SQO-treated (Fig. 5A) groups of the experiment, respectively. Newly hatched larvae appeared with lacking mouth and pectoral fin, the head region was attached to the yolk sphere and the body was observed without any pigmentation. The diameter size of the newly hatched larvae for the control (Fig. 4B) and SQO-treated (Fig. 5B) groups were 126.78 ± 0.73 and 127.80 ± 0.80 μm, respectively. After 6 h, some star or branch-shaped melanophores were distributed on both sides of the body and back of the head and somite. The body of the larvae was straight, floating on the surface of the water. The brain was slightly visible. A prominent notochord was found and barbells partially appeared. The diameter size of the larvae were 139.27 ± 0.83 and 148.68 ± 0.35 μm for the control (Fig. 4C) and SQO-treated (Fig. 5C) groups, respectively. After 12 h, three pairs of barbels appeared, and among them, the maxillary pair was visible. The heart became more distinct and blood circulation was continued. The diameter size of the 12 h old hatched larvae for the control (Fig. 4D) and treated (Fig. 5D) groups were 164.29 ± 0.71 and 179.64 ± 0.53 μm, respectively. About 24 h old larvae, the lateral line was visible as well as swim bladder and nostril were formed. Larvae remained motionless in the surface layer of water. Pigmentation extended to the yolk sac both dorsally and ventrally. The diameter size of the 1-day old larvae were 253.61 ± 0.21 and 274.38 ± 0.43 μm for the control (Fig. 4E) and SQO-treated (Fig. 5E) groups, respectively. After 2 days, typical star-shaped melanophores clusters appear around the

Table 6
Larval development of control and SQO treated diets for *O. pabda*.

Phase	Progress in larval development	Control diet		SQO diet	
		Size of larvae (μm) (mean ± sd)	Fig. 4	Size of larvae (μm) (mean ± sd)	Fig. 5
Just before hatching	Egg membrane became decomposed and lost its shape. Vigorous thrashing movements and frequent whipping motion of the embryo.	121.14 ± 0.43	A	122.04 ± 0.31	A
Newly hatched larvae	Lack of mouth and pectoral fin, head region was attached to the yolk sphere. Body was observed without any pigmentation.	126.78 ± 0.73	B	127.80 ± 0.80	B
6 h old larvae	Some star or branch shaped melanophores distributed on both side of the body and back of the head and somites. The body of the larva was straight, floating on the surface of the water. The brain was slightly visible. Prominent notochord was found and barbell partially appeared.	139.27 ± 0.83	C	148.68 ± 0.35	C
12 h old larvae	Three pair of barbell appeared and among them the maxillary pair was clearly visible. Heart became more distinct and blood circulation was continued.	164.29 ± 0.71	D	179.64 ± 0.53	D
24 h old larvae	Lateral line was visible. Swim bladder and nostril also formed. Larvae still remained motionless in the surface layer of water Pigmentation extended to the yolk sac both dorsally and ventrally	253.61 ± 0.21	E	274.38 ± 0.43	E
2 d old larvae	Typical star-shaped melanophores clusters appear around the final tract of the intestine.	436.76 ± 0.28	F	496.27 ± 0.33	F
3 d old larvae	Yolk sac was gradually disappeared and was absorbed to half size. Mouth was gradually developed and feeding commenced at this stage	692.48 ± 0.27	G	746.28 ± 0.37	G
4 d old larvae	Tail and caudal fin formation were in progress and both maxillary and mandibular barbells were formed	834.78 ± 0.26	H	893.67 ± 0.42	H
5 d old larvae	Mouth was fully developed and functional Yolk substances were completely absorbed The larvae were free swimming and fully capable to capture food	1186.78 ± 0.73	I	1226.78 ± 0.73	I

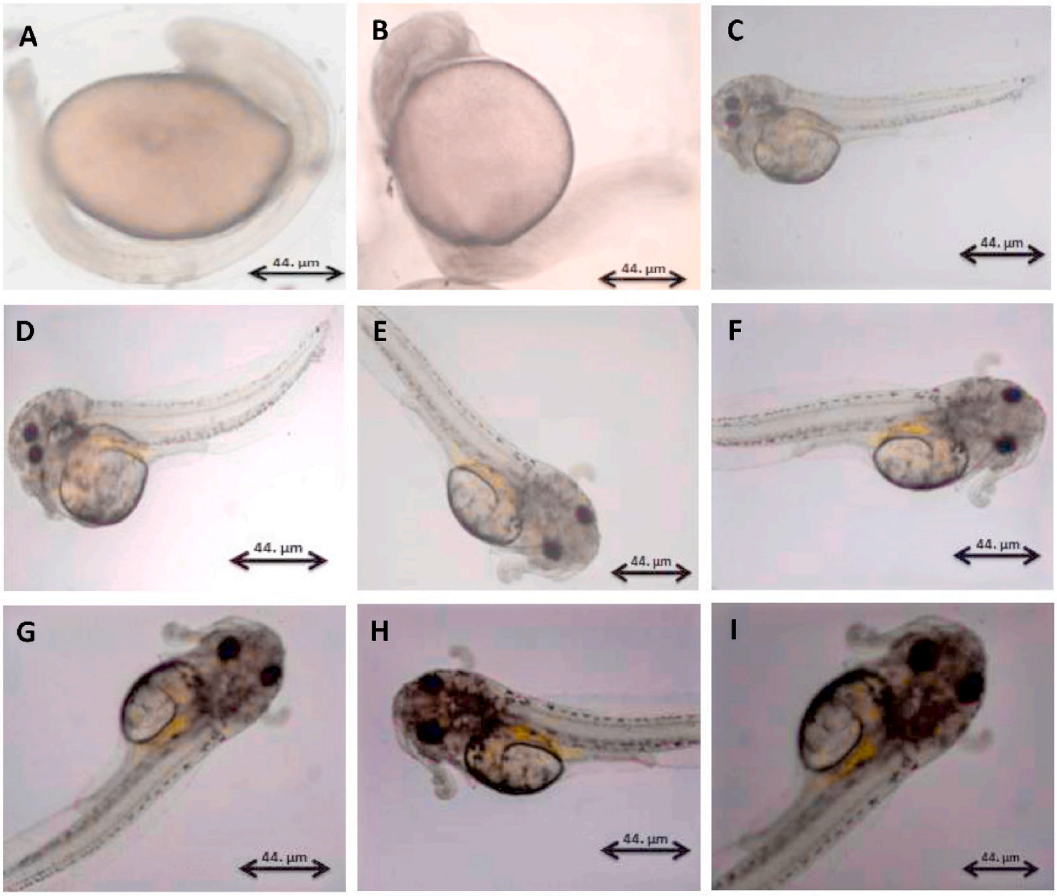

Fig. 4. Larval developmental stages of butter fish for control group; A. Just before hatching (23.00 h), B. Newly hatched larvae, C. 6 h old larvae, D. 12 h old larvae, E. 24 h old larvae, F. 48 h (2 d) old larvae, G. 72 h (3 d) old larvae, H. 96 h (4 d) old larvae and I. 120 h (5 d) old larvae. Scale bar: 44 µm.

final tract of the intestine. The diameter size of the 2 days old hatched larvae for the control (Fig. 4F) and treated (Fig. 5F) groups were 436.76 ± 0.28 and 496.27 ± 0.33 µm, respectively. After 3 days, the yolk sac was gradually disappeared and absorbed in the body into half size. The mouth of the larvae was gradually developed and feeding commenced at this stage. The diameter size of 3 days' larvae were 692.48 ± 0.27, 834.78 ± 0.26 and 1186.78 ± 0.73 µm for the control (Fig. 4G–I), and 746.28 ± 0.37, 893.67 ± 0.42 and 1226.78 ± 0.73 µm for the SQO-treated (Fig. 5G–I) groups of the study.

4. Discussion

4.1. Fecundity of butter catfish

Fecundity is one of the most significant biological features of fish which must be understood in order to explain the variations in the level of reproduction as well as to make efforts to increase the amount of harvest. Numerous factors influence the fecundity value, including age, size, ambient temperature, dietary constituents, and stocking ratio [33,34]. The number of ripe or mature eggs that a hatching fish produces in a certain spawning time can easily be determined at full maturity by artificially striping the eggs to estimate fecundity [35]. In the present study, the fecundity of butter catfish was found to be significantly higher ($P < 0.01$) in SQO-treated brood compared to the control during the spawning season and despite the values starting to decrease after June, the treatment group maintained the significantly higher fecundity until the month of July which might be attributed to the direct positive effect of SQO diet on the fecundity of butter catfish. Purkayastha et al. [36] found a strong correlation between female size and fecundity of *O. pabda*, with the average relative fecundity of 18,000–22,000 eggs/100 g fish which showed little higher fecundity than the present study. According to Chakrabarti et al. [37], *O. pabda* has a relative fecundity of 2,00,000–2,50,000/kg body weight.

Peak season (May to July) for butter catfish fecundity corresponds with the onset of rainfall and floodwaters [21]. Besides, in this study, both the treatment and control groups showed the highest fecundity in June. Banik et al. [38] also observed the fecundity of *O. pabda* ranged from 15560 ± 185 to 17369 ± 213 during June to August with the pick time in June. Tsadik and Bart [33] reported

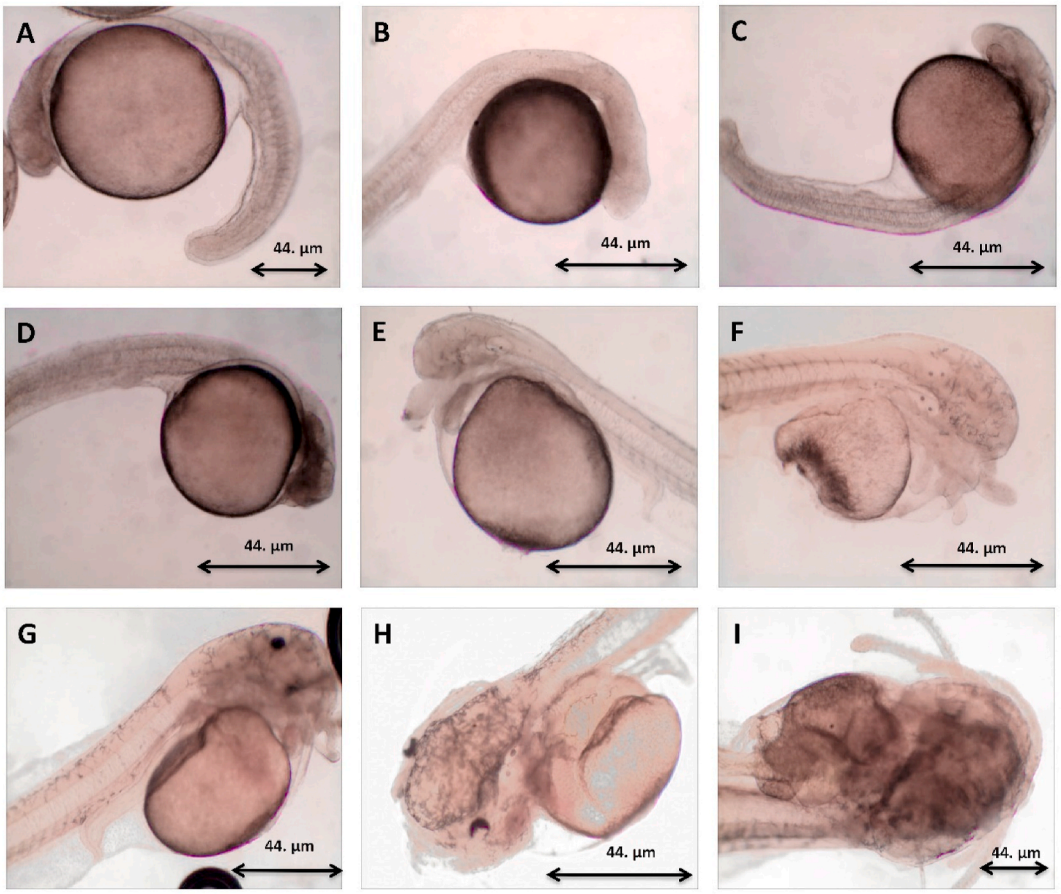

Fig. 5. Larval developmental stages of butter fish for squid oil (SQO) treated group; A. Just before hatching (23.00 h), B. Newly hatched larvae, C. 6 h old larvae, D. 12 h old larvae, E. 24 h old larvae, F. 48 h (2 d) old larvae, G. 72 h (3 d) old larvae, H. 96 h (4 d) old larvae and I. 120 h (5 d) old larvae. Scale bar: 44 µm.

nearly similar results in their study for *Oreochromis niloticus*. Parameswaran et al. [39] reported the *Ompok pabda* breeding season from May to August and, June was the peak month. However, Hossain et al. [21] observed the fecundity of the fish for three months like April, May, and June, where the values were 9165 ± 428, 11368 ± 719, and 10091 ± 515, respectively with the peak value in May. Therefore, it would be more reasonable to conclude that PUFA containing SQO contributes to attaining a greater body size that eventually makes more eggs to release.

4.2. Fertilization, hatching, and survival of butter catfish larva

Fertilization, hatching, and survival rate are the determinants of breeding performance for a fish species that ultimately decides whether the farming of that fish would be economical or not [36]. Therefore, the reproductive performance of broodstock is strongly affected by the nutritional composition of diet in many fish species [40]. In the current investigation, fertilization, hatching, and survival rates were significantly greater in the SQO-treated group than in the control which is in agreement with Banik et al. [38]. Therefore, a clear effect of feed ingredients is visible in the present study. Hossain et al. [21] agreed with the present studies and also found almost similar results of the treated group. Therefore, in our current findings, a greater rate of fertilization, survival, and hatching in treated brood fish might be attributed to the supplementary effect of dietary SQO diet containing EPA and DHA.

4.3. Embryonic development of butter catfish

The diameters and age are important determinants for the developmental stages of the embryo and larvae of butter catfish, which indicates better maturation of brood female is crucial for the better quality of eggs, larvae, and embryo [41]. Raizada et al. [42] and Chakrabarty et al. [43] reported the successful spawning of *O. pabda* in captive condition. Likewise, in the present study, the diameter of SQO-treated fish with mature egg was 98.82 ± 1.14 µm which was slightly greater than the control 96.72 ± 1.04 without significant differences. The color of the eggs were brownish and adhesive which is comparable to those of other catfish species, for example, *M. cavasius* [44], *O. malabaricus* [45] and *H. fossilis* [46]. Eggs evolved with a sticky coating to keep them from drifting in water

currents and to provide an adequate oxygen supply [46]. In this study, the initial cleavage-stage appeared within 36 min of the post-fertilization period. Sarma et al. [3] also found the same findings that the first cleavage started with the formation of blastodisc began 36 min after fertilization, as two blastomeres were created by dividing a crescentic light region over one end of the enormous yolk. In this study, the first cleavage, which occurred about 30 min after fertilization, was followed by 16 celled stages in 70 min, is an agreement with Chakrabarty et al. [43]. The second cleavage (four-cell stage) started after 10–12 min and the third cleavage (eight-cell stage) followed in another 12 min. In the current study, the formation of the multicellular blastodisc as well as construction of blastomere cells increased as time progressed and attained 8 cells, 16 cells, 32 cells, and 64 cells within 60 min, 1:08 h, 1:12–1:13 h, and 1:22 h, respectively after the fertilization. Likewise, *Pangasius pangasius* developed in 64-cell stages as well as morula stage at 1.27–1.30 h and 03.43 ± 00.33 h [47].

In the current investigation, the morula stage of *O. pabda* was reached at 02:05 h after fertilization. Sarma et al. [3] found the same duration for morula stage which attained at 02:06 h after fertilization of the egg. In addition, in this study, in the case of both of the control and the SQO-treated fish, the blastula stages appeared in 3.30 h after the fertilization of eggs making fully active embryo within 17–18 h, and the hatching occurred at 22 h after fertilization. Chattopadhyay [48] found that the blastodisc were formed within 30 min after fertilization of eggs. In this experiment, the blastula stage was characterized with one third of egg space covered by the blastoderm cells within 3:30 h after fertilization where the average diameter was 113.26 ± 0.37 μm for the control and 115.59 ± 0.83 μm for the SQO-treated group, respectively. Sarma et al. [3] also found a similar result in their experiment. In the present work, the gastrula attained within 5:00 h of fertilization where the yolk invasion completed and the cephalic region got thicker in size. The average diameter of gastrula for the control and treatment groups were 120.28 ± 2.13 μm and 121.23 ± 2.02 μm, respectively. Puvaneswari et al. [46] reported that *H. fossilis* required 7 h to reach the gastrula stage. Moreover, Tumpa et al. [22] observed the gastrula stage in *O. pabda* between 4.50 and 5.00 h after fertilization. In the case of *O. pabo,* it took 22 h or 23 h for the eggs to hatch after fertilization based on Sarma et al. [3] and Tumpa et al. [22], respectively. Conversely, *O. bimacuatus* hatched out 22 ± 11 h post-fertilization (hpf) with the yolk sac totally absorbed. After 5 h, 7–8 h, 9–10 h, 11–12 h, 17–18 h, and 22 h of post-fertilization, yolk plug stage, kidney-shaped embryo, expanded embryo, Kupffer's vesicle generation, optical vesicle, fully active embryo, and hatchling stage were detected, respectively. Similarly, *O. bimacuatus* hatched out in 21 ± 1 h post-fertilization (hpf) and the yolk-sac was totally absorbed in 48 hpf [42]. Additionally, *C. batrachus* [49], *H. fossilis* [46], *M. aculeatus* [50], and *M. cavasius* [44] were also reported to hatch in 26 h, 23 h, 31.45 h, and 17–21 h, respectively.

In this study, the larvae were straight after hatching and could be distinguished by a head, trunk, and tail. The length of freshly hatched *O. pabda* larvae was measured at 126.78 ± 0.73 μm in the control group and 127.80 ± 0.80 μm in the treated group, which was transparent and pale yellow in color.

4.4. Larval development of butter catfish

During the early stages of a fish's life, nutrition is especially vital for its healthy development [40]. Comprehension of larval fish's particular food requirements can increase the efficiency and quality of fish produced in a culture system [3]. In the present study, the newly hatched larvae appeared with the absence of a mouth and pectoral fin, the head region was linked to the yolk sphere and the body was observed without any coloration. The diameter size of the newly hatched larvae for the control and treated groups were 126.78 ± 0.73 and 127.80 ± 0.80 μm, respectively. Similarly, Sarma et al. [3] observed the larvae had a prominent eye, an oval yolk sac that was yellowish-green in appearance, and an unpigmented body. The newly hatched larvae of *M. montanus* was 3.0 ± 0.1 mm in lengths [51], whereas the length of *H. fossilis* was 2.5 ± 0.2 mm which were transparent and somewhat brown in color [46], while Rahman et al. [44] found *M. cavasius* has a length of 2.59–2.62 mm. In this study, the 6 h old larvae were identified by the presence of melanophores in the shape of stars or branches on both sides of the body, the rear of the head, as well as somites. The larval structure was straight and floated on the water surface. A small portion of the brain was seen. Barbels near the mouth were partially visible and a significant notochord was identified. The diameter size of the larvae was 139.27 ± 0.83 and 148.68 ± 0.35 μm for the control and treated groups of the experiment, respectively. Tumpa et al. [22] also found for the 6 h old larvae were transparent on both sides of the yolk sac with several star-shaped dark black-brown melanophores. In this study, 12 h old larvae with three pairs of barbels developed and the maxillary pair was identifiable among them. The heart began to distinguish itself as the blood circulation continued. The diameter size of the 12 h old hatched larvae for the control and SQO-treated groups were 164.29 ± 0.71 and 179.64 ± 0.53 μm, respectively. Tumpa et al. [22] found that the body of larvae was straight and floated on the water surface with a conspicuous notochord at 12 h old. The *Notopterus notopterus* showed similar results with a deep yellow to the dark pink color of their body [52]. A lateral line was seen in the current experiment for 24 h old larvae with the developed swim bladder and nostril. In the water surface layer, larvae stayed motionless. Pigmentation spread both dorsally and ventrally to the yolk sac. The diameter size of the 1-day old larvae were 253.61 ± 0.21 and 274.38 ± 0.43 μm for the control and treated groups of this study, respectively. Tumpa et al. [22] reported after 24 h of hatching, *O. pabda* larvae were immobile in the water surface with a lateral line. The study found that near the last tract of the intestine, typical star-shaped melanophores clusters appeared in 2 days old larvae. The diameter size of the 2 days old hatched larvae for the control and treated groups were 436.76 ± 0.28 and 496.27 ± 0.33 μm, respectively. Tumpa et al. [22] found the swim bladder and nostril after 48 h of hatching. In the present study, the yolk sac of a three-day-old larva gradually diminished and was absorbed to half of its size. At this time, the mouth was fully formed and feeding began. The diameter size of 3 days old larvae were 692.48 ± 0.27 and 746.28 ± 0.37 μm for the control and treated groups, respectively. In an experimental findings, Sarma et al. [3] reported that the mouth was developed gradually and feeding began three days of age and from the third day forward, the yolk sac began to dissolve gradually in butter catfish. Likewise, Chakrabarty et al. [43] detected three days for absorption of yolk sack in *O. pabda,* while the pectoral fin was clearly visible, caudal fins were underway of formation and barbels were developed on both the

maxillary and mandibular sides. However, *P. pangasius* yolk sac absorption observed on day four post-hatch period [47]. This time varies among the fish species such as *C. batrachus* for 72 h [53] and *N. nandus* for 64.0 ± 0.30 h [54]. Fish egg quality, early embryonic and larval development are affected by maternal diet [3]. The mobilization of fish brood nutrition determines egg and sperm quality [56]. It is possible to improve the quality of *O. pabda* broodstock by supplementing diets with squid oil, which leads to spontaneous spawning under captive circumstances [22]. As a result, it is critical to provide fish larvae with dietary squid oil as a source of EPA and DHA to ensure optimal development and function.

Nonetheless, in the present study, the use of squid oil has not available as well as the use of fish oil and soya oil in Bangladesh, as there has been less research on the DHA and EPA availability in squid. In this experiment we used soya oil in the control group and squid oil in treatment group and got better results in squid oil. We assumed that this research will make fish producers interested in the use of squid oil and they will use it commercially instead of discarding squid as trash fish.

5. Conclusion

Fish nutrition is one of the most important factors influencing the breeding performance, embryonic and larval development of cultured fish. The lack of nutrient-rich feeds causes mortality and poor survival rate in fish which impedes the need to produce enough fingerlings to meet the high demand for fish. In recent years, due to feeding management and various ecological changes, butter catfish catches have significantly reduced from open waters that made it an endangered species. In the present study, the best breeding performances, fecundity, embryonic and larval developments were achieved by the butter catfish fed the squid extracted oil containing EPA and DHA in the diet. The finding of the present study warranted further research on the utilization of squid oil at different levels for *O. pabda* based on nutrigenomic approaches in the diet of this important commercial fish species.

Ethics statement

This study was approved by the Institutional Animal Care and Use Committee, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh (AWEEC/BAU/2021).

Data availability

All data generated or analyzed during this study are included in this published article.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Md Saddam Hossain: Formal analysis, Investigation, Writing – original draft. **Mohammad Moniruzzaman:** Formal analysis, Methodology, Software. **Shahanaj Parvin Rumki:** Formal analysis, Investigation. **Tutul Kumar Saha:** Formal analysis, Investigation. **Mohammad Matiur Rahman:** Formal analysis, Investigation. **Sungchul C. Bai:** Resources, Validation, Writing – review & editing. **Taesun Min:** Resources, Validation, Writing – review & editing. **Zakir Hossain:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Krishi Gobeshona Foundation (KGF project ID: TF 40-F/17) under Bangladesh Agricultural Research Council (BARC) to Zakir Hossain. The research work was also supported by the Basic Science Research Program (2019R1A6A1A11052070) under the Ministry of Education and the Basic Science Research Program (2022R1A2B5B02001711) under the Ministry of Science and ICT through the National Research Foundation of Korea (NRF) to Taesun Min. Mohammad Moniruzzaman would like to acknowledge the National Research Foundation of Korea (NRF) for providing the postdoctoral research fellowship under the Brain Pool Program (Grant No. 2019H1D3A1A01101555) funded by the Ministry of Science, ICT, and Future Planning.

References

[1] M.N. Sarowar, M.Z.H. Jewel, M.A. Sayeed, M.F.A. Mollah, Impacts of different diets on growth and survival of *Channa striatus* fry, Int. J. BioRes. 1 (2010) 8–12.

[2] Y. Yamagashi, H. Mitamura, H.N. Arai, Y. Mitsunaga, Y. Kawabata, M. Khachapicha, T. Viputhanumas, Feeding habits of hatchery reared young Mekong giant catfish in a fish pond and in Mae Peum reservoir, in: N. Arai (Ed.), Proceeding of the 2nd International Symposium on SEASTAR 2000 and Asian Bio Logging Science (The 6th SEASTAR 2000 Workshop), 2005, pp. 17–22.

[3] D. Sarma, J. Das, A. Dutta, U.C. Goswami, Early embryonic and larval development of *Ompok pabo* with notes on its nursery rearing, Eur. J. Exp. Biol. 2 (2012) 253–260.

[4] P. Laevens, E. Lebegue, H. Jaunet, A. Brunel, P. Dhert, P. Sorgeloos, Effect of dietary essential fatty acids and vitamins on egg quality in turbot broodstocks, Aquacult. Int. 7 (1999) 225–240.

[5] M.S. Hossen, A.H.M.M. Reza, S.F. Rakhi, Z. Hossain, Performance of different dietary lipids on growth indices and survival of striped gourami, *Colisa fasciatus* (Perciformes: Osphronemidae) fry, Int. J. Biochem. Biophy. 2 (2014) 1–7.

[6] J. Ghanawei, L. Roy, D.A. Davis, I.P. Saoud, Effects of dietary lipid levels on growth performance of marbled spine foot rabbitfish *Siganus rivulatus*, Aquaculture 310 (2011) 395–400.

[7] J.W. Hong, S.H. Lee, M. Moniruzzaman, Y. Park, S.H. Won, H.Y. Jo, S.S.O. Hung, S.C. Bai, Dietary eicosapentaenoic acid requirement of juvenile rock bream, Oplegnathus fasciatus. Aquacult. Nutri. 24 (2018) 36–46.

[8] J.W. Hong, S.H. Lee, H. Yun, M. Moniruzzaman, Y. Park, E. Shahkar, M. Seong, S.C. Bai, The optimum dietary docosahexaenoic acid level based on growth and non-specific immune responses in juvenile rock bream, *Oplegnathus fasciatus*, Aquacult. Res. 48 (2017) 3401–3412.

[9] J.S. Huang, R.X. Li, R.T. Xie, Y.M. Chen, J.D. Zhang, E. Amenyogbe, G. Chen, Changes in amino acid and fatty acid composition during early development in cobia (*Rachycentron canadum*), Front. Mar. Sci. 9 (2022) 995616.

[10] Y.D. Jia, Z. Meng, X.F. Liu, J.L. Lei, Biochemical composition and quality of turbot (Scophthalmus maximus) eggs throughout the reproductive season, Fish Physiol. Biochem. 40 (2014) 1093–1104.

[11] E. Shahkar, H. Yun, S.H. Lee, D.J. Kim, S.K. Kim, B.I. Lee, S.C. Bai, Evaluation of the optimum dietary arachidonic acid level and its essentiality based on growth and non-specific immune responses in Japanese eel, *Anguilla japonica*, Aquaculture 452 (2016) 209–216.

[12] K. Scharnweber, F. Chaguaceda, P. Eklov, Fatty acid accumulation in feeding types of a natural freshwater fish population, Oecologia 196 (2021) 53–63.

[13] C.C. Hilbig, N.F. Nascimento, A.L. Heinen, A.T. Neto, J.P. Funghetto, R.A. Bombardelli, F. Meurer, L.S.O. Nakaghi, Effects of dietary fatty acids on the reproduction of South American female catfish *Rhamdia quelen* (Quoy & Gaimard, 1824), Latinoam. J. Aquat. Res 47 (3) (2019) 456–466.

[14] M.S. Hossen, A.H.M.M. Reza, S.F. Rakhi, K. Takahashi, Z. Hossain, Effects of polyunsaturated fatty acids (PUFAs) on gonadal maturation and spawning of striped gourami, *Colisa fasciatus*, Int. Aquat. Res. 6 (2014) 65.

[15] T. Mukhopadhyay, S. Ghosh, Lipid profile and fatty acid composition of two silurid fish eggs, J. Oleo Sci. 56 (2007) 399–403.

[16] T. Watanabe, Importance of docosahexaenoic acid in marine larval fish, J. World Aquacul. Sot. 24 (1993) 152–161.

[17] S. Fontagné, I. Geurden, A.M. Escaffre, P. Bergot, Histological changes induced by dietary phospholipids in intestine and liver of common carp (*Cyprinus carpio L.*) larvae, Aquaculture 161 (1998) 213–223.

[18] P.P. Vince, J. Werner, in: P. Olivar, J.J. Govoni (Eds.), Vitamins A and E in the Maternal Diet Influence Egg Quality and Early Life Stage Development in Fish: a Review, Recent Advances in the Study of Fish Eggs and Larvae M, 2006, pp. 41–57. Barcelona (Spain).

[19] J.C. Navarro, J.R. Sargent, Behavioural differences in starving herring *Clupea harengus* L. Larvae correlate with body levels of essential fatty acids, J. Fish. Biol. 41 (1992) 509–513.

[20] D.R. Tocher, Metabolism and functions of lipids and fatty acids in teleost fish, Reviews in *Fish. Sci* 11 (2003) 107–184.

[21] M.S. Hossain, M.R. Ali, M.I. Rahman, A.K.M.M. Hasan, M.M. Iqbal, S.K. Barman, Induced breeding of *Ompok pabda* with S-GnRHa, Int. J. Nat. Sci. 6 (2017) 141–147.

[22] I.J. Tumpa, M.S. Hossain, M.N. Uddin, Z. Hossain, Spawning and developmental biology of endangered Pabdah catfish, *Ompok pabda* (Hamilton, 1822), Asian J. Med. Biolog. Res. 6 (2020) 449–459.

[23] P. Chakraborty, M.R. Islam, M.A. Hossain, U.K. Fatema, D.C. Shaha, M.S.A. Sarker, T. Akter, Earthworm meal (*Perionyx excavatus*) as an alternative protein source to fish meal in feed for juvenile butter catfish (*Ompok pabda*), Aquacult. Int. 29 (2021) 2119–2129.

[24] FRSS, Yearbook of Fisheries Statistics of Bangladesh, Fisheries Resources Survey System 25 (2020) 42. Department of Fisheries, Bangladesh.

[25] M.Y. Hossain, M.A. Hossen, K. Yahya, M.M. Islam, M.A. Islam, K.K.U. Ahmed, M. Begum, Threatened fishes of the world: *Ompok pabda* (Hamilton, 1822) (Siluriformes: Siluridae), Croatian J. Fish. 73 (2015) 183–185.

[26] IUCN Bangladesh, Red list of Bangladesh volume 5: freshwater fishes, IUCN, International Union for conservation of Nature, Bangladesh Country Office, Dhaka, Bangladesh 16 (2020) 360.

[27] B.K. Chakraborty, Z.A. Mirza, A. Chakraborty, Reproductive cycle of the endangered Pabda, *Ompok pabda* (Hamilton-Buchanan, 1822) in Bangladesh, Asian Fish Sci. 23 (2010) 301–320.

[28] C.L. Aysi, J.L. Zhao, Recent development in the use of palm oil in aquaculture feeds a review, Int. J. Sci. Technol. Res. 3 (2014) 259–264.

[29] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917.

[30] AOAC, Official Methods of Analysis of Association of Official Analytical Chemists, twenty-first ed., 2020 (Washington, DC).

[31] J.R. Hunter, S.R. Goldberg, Spawning incidence and batch fecundity in northern anchovy, Engraulis mordax, Fish. Bull. 77 (1980) 641–652.

[32] H. Murua, G. Kraus, F. Saborido-Rey, A. Thorsen, P. Witthames, S. Junquera, Procedures to estimate fecundity of wild collected marine fish in relation to fish reproductive strategy. J. Northwest Atlant, Fish. Sci. 33 (2003) 33–54.

[33] G.G. Tsadik, A.N. Bart, Effects of feeding, stocking density and water-flow rate on fecundity, spawning frequency and egg quality of nile tilapia, Oreochromis niloticus (L.). Aquaculture 272 (2007) 380–388.

[34] D.M.B. Helmizuryani, R.I.S. Aminah, K. Khotimah, The gonadal maturation of climbing perch, *Anabas testudineus* (Bloch, 1792) with dietary supplement add on feed, AACL Bioflux 13 (2020) 885–892.

[35] T.B. Bagenal, Aspects of fish fecundity, in: D.G. Shelby (Ed.), Ecology of Fresh Water Fish Production, second ed., Black Well., London, 1978, pp. 101–135.

[36] S. Purkayastha, S. Sarma, U.K. Sarkar, W.S. Lakra, S. Gupta, S.P. Biswas, Captive breeding of endangered *Ompok pabda* with Ovatide, J. Appl. Aqua. 24 (2012) 42–48.

[37] P.P. Chakrabarti, S.C. Mondal, D.N. Chattopadhaya, R.N. Mandal, B.N. Paul, Pabda-Seed Production & Culture, Central Institute of Freshwater Aquaculture, Bhubaneswar, India, 2012, p. 44.

[38] S. Banik, P. Goswami, S. Malla, *Ompok pabda* (Hamilton-Buchanan, 1822): an endangered Catfish of Tripura, India: reproductive physiology related to freshwater lotic environment, J. Env. 1 (2012) 45–55.

[39] S. Parameswaran, C. Selvaraj, S. Radhakrishnan, Notes on the life history and biology of the cat fish *Ompok pabda* (Hamilton), J. Zool. Soci. Ind. 23 (1971) 137–150.

[40] S. Gupta, A review on feeding and reproductive biology of *Ompok pabda* with an emphasis on its conservation, J. Aqua. Res. Dev. 9 (2018) 525.

[41] A. Rahman, S. Yeasmine, M.M. Khan, Growth Survival and Production of a threatened siluried catfish, *Ompok pabda* (Hamilton) Fingerlings in nursery ponds, Malays. Appl. Biol. 37 (2008) 47–56.

[42] S. Raizada, K.K. Lal, U. Sarkar, P.K. Varshney, V. Sahu, K.C. Yadav, P. Agnihotri, A. Awasthi, J.K. Jena, Captive breeding and embryonic development of butter catfish (*Ompok bimaculatus*, Bloch 1794), a threatened fish of Indian sub-continent in northern India, Proc. Nat. Acad. Sci., India Sec. B, Biol. Sci. 83 (2013) 333–339.

[43] N.M. Chakrabarty, P.P. Chakrabarty, S.C. Mondal, N. Sarangi, Artificial breeding seed production and rearing of butter fish *Ompok pabda* - a significant mile stone in technology advancement, Fishing Chimes 26 (2008) 134–136.

[44] M.R. Rahman, M.A. Rahman, M.N. Khan, M.G. Hussain, Observation on the embryonic and larval development of Silurid catfish, Gulsha (Mystus cavasius), Pak. J. Biol. Sci. 7 (2004) 1070–1075.

[45] C. Vijayakumar, Early embryonic and larval development of *Ompok malabaricus*, Int. J. Biol. Tech. 1 (2010) 131–133.

[46] S. Puvaneswari, K. Marimuthu, R. Karuppasamy, M.A. Haniffa, Early embryonic and larval development of Indian catfish, *Heteropneustes fossilis*, Eurasian J. Biosci. 3 (2009) 84–96.
[47] S. Ferosekhan, S.K. Sahoo, S.S. Giri, A. Saha, M. Paramanik, Embryonic and larval development of yellow tail catfish, Pangasius pangasius. J. Aqua. Res. Dev. 6 (2015) 6.
[48] N.R. Chattopadhyay, Comparative efficacy of carp pituitary extract and Ovaprim during captive breeding of Cat Fish, Pabda (*Ompok pabda*), J. Agri. Sci. Bot. 2 (2018) 3–12.
[49] D. Roy, A. Mitra, M. Biswas, S. Chakraborty, S. Pal, S. Homechaudhuri, Early ontogeny of the Asian catfish Magur, *Clarias batrachus* (Linnaeus, 1758), Int. J. Fish Aqu. Stud. 7 (2019) 287–292.
[50] S.K. Sahoo, S.S. Giri, A. Saha, S. Chandra, A.K. Sahu, N. Sarangi, Embryonic development of the spiny eel, *Mastacembelus aculeatus* (Bloch, 1786), Ind. J. Fish. 54 (2007) 333–337.
[51] A.J. Arockiaraj, A. Mohammed, S.S. Haniffa, S.P. Singh, A.Z. Taiwanica, Cyclic changes in Gonadal maturation and Histological observations of threatened freshwater catfish *Mystus montanus* (Jerdon, 1849), Acta Ichthyol. ET Piscat. 14 (2003) 23–32.
[52] S.M. Srivastava, A. Gopalakrishnan, S.P. Singh, A.K. Pandey, Embryonic and larval development of threatened bronze featherback, *Notopterus notopterus* (Pallas), J. Exp. Zool. 15 (2012) 425–430.
[53] S.K. Sahoo, S.S. Giri, A.K. Sahu, Effect of stocking density on growth and survival of *Clarias batrachus* (Linn.) larvae and fry during hatchery rearing, J. Appl. Icht. 20 (2004) 302–305.
[54] S.F. Rakhi, M.S. Hossen, A.H.M.M. Reza, Z. Hossain, Embryonic development and growth performances of an endangered fish species *Nandus nandus*: effects of dietary polyunsaturated fatty acids supplementation, Iran. J. Fish. Sci. 14 (2015) 1029–1052.