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Influence of Different Dissolved Oxygen Regimes on Embryonic Developmental Timing and Egg Morphometry in Striped Catfish *Pangasianodon hypophthalmus*

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Simple Summary

It has been demonstrated that dissolved oxygen levels can exert a significant influence on the development of embryos and the process of hatching. Consequently, ensuring adequate oxygenation during the incubation of eggs is imperative for the successful rearing of fish. The present study set out to compare four incubation systems providing varying water oxygen concentrations. The present study evaluated the embryonic development of striped catfish (*Pangasianodon hypophthalmus*), hatching time, egg size, and the incidence of developmental abnormalities. An increase in the oxygen concentration of the medium resulted in a delay in the process of hatching, as well as a greater increase in egg diameter and volume. Developmental abnormalities were identified in all of the systems that were examined. The findings of the study suggest that the conditions of oxygenation and the selection of the incubation system exert a substantial influence on the development of pre-hatching fish. These findings have the potential to contribute to the refinement of egg incubation methods and inform the development of practical guidelines for hatcheries.

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Abstract

Dissolved oxygen (DO) has been identified as one of the most significant and poorly controlled incubation variables in fish hatcheries. The objective of this study was to compare the effects of four incubation systems with varying DO concentrations on embryonic development of *Pangasianodon hypophthalmus*. Eggs from hormonally induced spawns were subjected to incubation under four different dissolved oxygen (DO) regimes: T1 (4.51 ± 0.46 mg L^{-1}), T2 (5.46 ± 0.08 mg L^{-1}), T3 (7.39 ± 0.07 mg L^{-1}), and T4 (8.12 ± 0.10 mg L^{-1}). No significant differences were observed between the treatments in terms of temperature,

pH, or conductivity. However, it is possible that differences in incubation system configuration may have contributed to the developmental responses observed. The development of the subjects was significantly delayed by higher DO levels, as evidenced by the T1 group hatching at 1841 ± 24 min, while the T4 group hatched at 2186 ± 5 min. In contrast, elevated DO levels resulted in significant increases in egg diameter, volume, and surface area, with increases of up to 46%, 215%, and 114%, respectively. These findings are consistent with increased perivitelline hydration, although water uptake and gas-exchange efficiency were not measured in this study. All treatments exhibited developmental abnormalities, indicating that oxygen is not the sole morphogenetic stressor. The findings revealed discrepancies in embryonic development and egg morphometry between the various incubation systems with differing dissolved oxygen levels. This will facilitate evidence-based hatchery management. The aerated hatching jar system (T4; 7.99–8.27 mg L^{-1}) yielded the largest eggs and the slowest development in the four systems that were tested. The findings of this study provide a valuable foundation for the enhancement of hatchery incubation operations. However, further validation is required to ensure the robustness of the conclusions.

Keywords: aquaculture; developmental acceleration; embryogenesis; hatching chronology; hatchery management; hypoxia; larvae; oxygen conditions

1. Introduction

Catfishes represent a highly diverse group of fish species that are of significant economic importance to freshwater fisheries. These species are of significance in the context of food security and rural livelihoods on a global scale, with contributions arising from both wild capture and aquaculture [1]. The striped catfish (*Pangasianodon hypophthalmus*) is a species of particular significance in the context of aquaculture. The advantages of this species include its capacity for air-breathing, rapid growth rates, its ability to accept formulated feeds, and its tolerance to low oxygen levels [2,3]. The production of this species has gradually expanded beyond its native geographical distribution, and it now accounts for a significant proportion of global freshwater aquaculture production [4].

It is important to note that this species does not spawn naturally in captivity. Consequently, the entire seed supply is contingent on hormonally induced breeding and meticulously controlled egg incubation [5,6]. In such circumstances, the quality of water is among the most critical factors determining the success of spawning and subsequent egg and larval development [7]. It is therefore recommended that a programme of continuous monitoring of water quality parameters be implemented, in order to ensure optimal growth and production. Aeration is widely regarded as a pivotal management strategy within the context of hatchery and nursery operations [8]. This is of particular importance in intensive culture systems, where high stocking densities reduce dissolved oxygen levels and compromise fish health [9].

Embryos of fish have been observed to demonstrate a high degree of sensitivity to oxygen conditions. The organisms under consideration are sedentary, and gases are exchanged by passive diffusion through the egg envelope. It therefore follows that the organisms are unable to escape from conditions that are not conducive to their survival [10]. A decline in ambient oxygen levels has been demonstrated to result in a decrease in the hatching rate, which has been shown to consistently cause developmental defects and mortality in the embryo [11]. This sensitivity is well documented across the taxa, with evidence of its impact observable in the developmental rate and survival in relation to the temperature-oxygen interaction [10]. It has been demonstrated that adverse incubation

conditions result in a reduction in emergence success at the population level. This reduction has been evidenced by multi-stressor analyses [12]. It has been demonstrated that developmental plasticity can offer partial compensation for the effects of hypoxia in ray-finned fishes. These effects are coordinated at the molecular level through the hypoxia-inducible factor pathway [13,14].

The production of high-quality seeds from striped catfish (*P. hypophthalmus*) is of paramount importance in the context of aquaculture. However, the extant literature on this subject is limited in scope and does not consider a wide range of factors. It is well documented that an increase in salinity has a detrimental effect on the development of embryos and larvae [15]. The temperature at which the eggs are stored has been demonstrated to impact the time to hatching and hatchability [16]. A number of hatchery-level experiments have also investigated the effects of light intensity and brood-stock characteristics on hatching [17,18]. It is evident that there is an absence of documented research that has systematically examined the levels of dissolved oxygen in common hatchery incubation systems for *P. hypophthalmus*. Dissolved oxygen levels are frequently observed to demonstrate a concomitant variation with temperature, pH, and electrical conductivity in hatchery water supplies. This covariation obscures the contribution of oxygen to development. Consequently, no evidence-based oxygen standards for the incubation phase of this species are available to hatchery operators.

The present study focused on the dynamics of dissolved oxygen in four incubation systems following the induction of spawning of *P. hypophthalmus.* The investigation sought to ascertain the effect of dissolved oxygen concentration on chronology of development, incubation time and egg morphometry. The present study establishes a correlation between the availability of oxygen and developmental outcomes, thereby revealing the confounding effect of oxygen and proposing oxygen standards for commercial hatcheries.

2. Materials and Methods

2.1. Experimental Site

The study was conducted at two research locations. The Fish Research Facility is part of the Department of Zoology at GC University, Lahore, Pakistan. The Sindhuan Fish Hatchery is part of the Department of Fisheries at the Government of Punjab, Pakistan. The health status of the broodstock, as well as the evaluation of egg diameter and quality, was conducted at the Fish Research Facility, which is affiliated with the Department of Zoology at GC University in Lahore, Pakistan. The process of induced spawning and the subsequent incubation of all treatments (T1–T4), across all replicate jars, was conducted at the Sindhuan Fish Hatchery, which is under the jurisdiction of the Department of Fisheries, Government of Punjab, Pakistan. This process was overseen by the relevant authorities, who also monitored the water quality and recorded developmental observations. The present study was conducted at a single hatchery site, which served as the location for all the treatments and replicates. These were subjected to the same ambient conditions during the incubation process. Consequently, the two facilities were not designated as separate trial sites, but rather functioned in a complementary manner within a single experiment.

2.2. Broodstock Choice and Artificial Spawning

Pangasianodon hypophthalmus specimens of both sexes were obtained from the broodstock pond of related production facilities and fish hatcheries. The total number of subjects in this study was 32, comprising 16 male and 16 female brooders. The fish were randomly

distributed to four dissolved oxygen (DO) treatments (T1–T4), with each treatment comprising four males and four females (eight brooders per treatment). The sample size was determined in accordance with standard hatchery breeding practices for the species in question, as well as to ensure sufficient replications between the various oxygen regimes. The disease-free brooders were selected and subsequently placed in circular tanks for the purpose of acclimatization. The continuous provision of aerated water was guaranteed in circular tanks. In the course of the research trials, the fish were not provided with sustenance.

Throughout the course of the manipulations, the brooders were anaesthetized using MS-222. The anesthetic was prepared from a 100 g L^{-1} buffered stock and subsequently diluted to a final concentration of 0.3 mL L^{-1} (30 mg L^{-1}). The extraction of milt was conducted from males that had been deeply anaesthetized, while the extraction of eggs was conducted from females. Both sexes received an identical anesthetic dose and handling procedure.

In the course of the experiment, a dose of Ovaprim was injected into the muscles of both male and female fish, with the injection being administered beneath the dorsal fin. Ovaprim (Syndel Laboratories, Nanaimo, BC, Canada) was injected into both male and female subjects (weights 3.725–4.15 kg/specimen) on a single occasion. The injection was administered at a rate of 0.2 mL and 0.6 mL/kg live body weight, respectively. The brooders were once more maintained in disinfected circular spawning tanks. Subsequently, the male and female subjects were subjected to regular examinations at 30-min intervals until the occurrence of ovulation [19]. Subsequent to the confirmation of ovulation, the eggs were collected dry from the females and transferred into individual plastic bowls. The eggs were then placed in a dry location and tied with a dry cloth for several minutes. The male subjects were euthanized using MS-222 solution. Following this, the abdominal integuments were dissected and the testes removed. The testicles were then macerated in accordance with the methodology outlined by Kucharczyk et al. [20] to obtain sperm. Subsequent to the collection of sperm, the eggs were fertilized in accordance with the mechanics expounded by Kucharczyk et al. [20]. The temporal point at which water is added to the mixture of egg and sperm was hypothesized to correspond to the moment of egg activation and fertilization.

2.3. Experimental Design

Four females and four males were assigned to each of the four DO Treatments (T1–T4). The female subjects were individually stripped in a clean plastic bowl. The milt was then sprayed onto the eggs, which had been released into the plastic bowl, from the same male. Subsequent to the processes of fertilization, washing and degumming, the eggs from different females were maintained in separate hatching jars. Each female was then removed from the jars one by one, and eggs were fertilized using milt from a single male of the same treatment group (one female per jar and one male per jar). It is important to note that no egg batch was split between treatments. The four replicate jars per treatment constitute four independent parental crosses. The experimental design entailed the implementation of an incubation system as the primary unit of experimentation, with each system being replicated independently to ensure the robustness of the findings. Each system contained four replicates, with identical water volumes. The water exchange and aeration parameters were constant throughout the incubation period. The four incubation regimes compared in this study differed in terms of design with regard to the following parameters: container type, water flow and aeration.

The volume of eggs in each of the jars was estimated volumetrically immediately after the eggs were degummed. Each egg suspension was subsampled on three occasions, with 1 mL of solution being extracted from each sample, and the number of eggs was

counted using a stereomicroscope. Subsequently, the mean count per milliliter was recalibrated to correspond with the volume of the suspension that would be introduced to each jar. This ensured that each jar contained an approximate total of 3000 eggs (2800–3200).

The target density was consistent across all jars to eliminate the possibility of confounding effects arising from variations in oxygen treatment and egg density. In the course of the study, 50 eggs were obtained from each jar at each developmental stage and subjected to microscopic examination. For each of the 19 sampling stages, the mean number of eggs per jar was approximately 950, representing approximately 30% of the initial number of eggs. It was observed that egg densities and oxygen demands remained comparable across different treatments throughout the incubation period. This consistency was attributed to the uniform removal of eggs at predetermined intervals during the sampling process.

2.4. Water Quality Monitoring

Water quality parameters were measured over a period of eight hours till hatching. Throughout the duration of the experiment, the values of temperature, dissolved oxygen and electrical conductivity were meticulously recorded.

2.5. Examination of Embryonic Development

2.5.1. Embryo Care

After fertilization and several rounds of washing and degumming, the eggs were then released in egg incubation jars. It was observed that all jars had a continuous water supply, with supplementary aeration provided in T3 and T4 only, and not in T1 and T2.

2.5.2. Morphometry of Fish Ova

Eggs were harvested at each developmental stage and preserved in 10% neutral buffered formalin (approximately 4% formaldehyde, pH 7.2–7.4) for a minimum of 24 h prior to examination. The same protocol was used for all treatments, and measurements were made within two days of chemical fixation. The shrinkage correction factor was not applied because the dimensions of the fresh eggs were not measured prior to fixation. The morphometric values reported were those of the fixed-egg dimensions, which were then compared relatively among the various treatment groups. Given that fixation was observed for all treatments, any fixation-induced change remains constant from T1 to T4, and the relative differences between the oxygen regimes remain valid. Subsequently, the eggs were subjected to microscopic analysis using a light microscope (Smart Series System Microscope, Chongqing, China, RoHS, Serial Number: 2108545) that was equipped with an industrial digital camera (Industrial Digital Camera 14MP 1/2.3 Color USB 2.0 APTINA CMOS Sensor). The diameters, volumes and surface areas of the whole eggs were measured using an eyepiece micrometer. However, the dimensions of the yolks were not the subject of measurement.

2.5.3. Morphological Identification of Early Life Stages

The eggs were transferred into separate Petri dishes and examined using the time-lapse imaging technique. Continuous time-lapse imaging was performed from fertilization to hatching. A trinocular compound light microscope (Smart Series System Microscope, RoHS, Serial Number: 2108545) equipped with an industrial digital camera (Industrial Digital Camera 14MP 1/2.3'' Color USB 2.0 APTINA CMOS Sensor) was used to take the images at regular intervals. The time of first appearance of each developmental stage was determined from the image sequence for each incubation system. The images captured were utilized for the purpose of determining the egg stage. Embryos were observed

at different stages of development and subjected to a thorough morphological examination.

2.5.4. Morphological Identification of Deformed Eggs

The eggs were then visualized using a trinocular compound light microscope (RoHS Serial No. 2108545) and an industrial digital camera with an APTINA CMOS Sensor. Descriptive malformations were identified during the course of the experiment at a number of developmental stages in all of the treatments. However, the frequency of deformity was not quantified. The assessment of egg deformity was conducted by means of the following parameters.

Shape**:** An analysis of the egg's morphology revealed the presence of atypical shapes, including ellipsoidal, spherical, and other irregular forms.

Dimensions: The entire diameter of the egg was measured.

Chorion: The presence of any structural deformities, including thinning, thickening, or a ruptured surface in the external layer of the egg, is to be noted.

Vitellus: The yolk was examined for irregularities in appearance, such as homogeneity and granularity, as criteria for assessing yolk condition.

2.6. Statistical Analysis

The data underwent analysis using one-way ANOVA with the dissolved oxygen regime (T1–T4) as the only fixed factor, and Duncan's multiple range test was applied at $p \leq 0.05$ (SAS v9.1). The site was not included as a factor because all the incubation treatments and replicates were carried out at a single hatchery facility.

A total of 50 eggs were sampled from each of four replicate incubation systems (T1–T4) and examined microscopically at each developmental stage, and the time of first appearance of each stage was recorded. The data from four values derived from a single incubation system were averaged for each system and used in the analysis of variance (ANOVA) since the incubation system constituted the experimental unit. The number of independent replicates of systems per oxygen regime was found to limit statistical power at the system level.

3. Results

3.1. Water Quality Parameters During Egg Incubation and Free Embryo Development

The data for temperature, pH and electrical conductivity demonstrated no significant difference among treatments ($p > 0.05$; Table 1), while the data for dissolved oxygen indicated a significant difference among treatments ($p < 0.05$; Table 1). Electrical conductivity exhibited negligible variation across the incubation period, with only substantial disparities being evident in the subsequent stages. The differences in temperature, pH and conductivity were not significant, suggesting that the water quality conditions were fairly similar under the different treatments. Nevertheless, the developmental responses that were observed may be a consequence of the interaction between the availability of dissolved oxygen and the configuration of the incubation system.

Table 1. Environmental conditions recorded during egg incubation and free embryo development of striped catfish (*P. hypophthalmus*).

		T1	T2	T3	T4	Df Between Groups	Df Within Groups	F	*p Value*
Temperature) (°C)	0.00	28.2 ± 0.34	27.62 ± 0.57	27.9 ± 0.53	28.22 ± 0.53	3	16	0.32	0.811
	24 h	27.6 ± 0.46	28.76 ± 0.62	28.28 ± 0.42	27.74 ± 0.59	3	16	0.99	0.421
	48 h	29.3 ± 0.82	27.82 ± 0.53	28.72 ± 0.65	28.24 ± 0.68	3	16	0.89	0.467
pH	0.00	7.3 ± 0.07	7.46 ± 0.12	7.32 ± 0.07	7.50 ± 0.08	3	16	1.33	0.300
	24 h	7.42 ± 0.07	7.526 ± 0.12	7.41 ± 0.09	7.37 ± 0.11	3	16	0.47	0.709
	48 h	7.41 ± 0.09	7.37 ± 0.11	7.36 ± 0.13	7.6 ± 0.14	3	16	0.90	0.461
EC (mS/cm)	0.00	2.07 ± 0.03	2.078 ± 0.02	2.05 ± 0.03	1.98 ± 0.03	3	16	2.74	0.077
	24 h	2.078 ± 0.07	2.098 ± 0.06	1.93 ± 0.06	1.92 ± 0.04	3	16	2.75	0.077
	48 h	1.928 ± 0.06	1.92 ± 0.04	2.08 ± 0.03	2.09 ± 0.02	3	16	5.81	0.007
DO (mg L^{-1})	0.00	5.40 ± 0.10 ^{c}	5.55 ± 0.15 ^{c}	7.44 ± 0.14 ^{b}	8.27 ± 0.29 ^{a}	3	16	85.32	0.000
	24 h	4.46 ± 0.23 ^{d}	5.40 ± 0.12 ^{c}	7.26 ± 0.12 ^{b}	7.99 ± 0.29 ^{a}	3	16	133.07	0.000
	48 h	4.06 ± 0.06 ^{d}	5.41 ± 0.11 ^{c}	7.32 ± 0.13 ^{b}	8.07 ± 0.27 ^{a}	3	16	264.23	0.000

Data are expressed as Mean ± SE: Superscripts on different means within row differ significantly at $p \leq 0.05$.

The dissolved oxygen levels were measured at four different time points during the incubation period, with mean values of 4.51 ± 0.46, 5.46 ± 0.08, 7.39 ± 0.07 and 8.12 ± 0.10 mg L^{-1} for T1–T4, respectively (see Table 1 for details). The time-effectiveness of DO separation serves to both confirm the experimental design and reinforce the sound interpretation of the oxygen-dependent developmental effects.

3.2. Effect of Dissolved Oxygen on Embryonic Development and Hatching Time

Embryonic development systematically followed all typical developmental stages of teleost development, such as cleavage, morula, gastrula, organogenesis, and hatching (Figures 1–7). The morula stages (see Figure 2C–E) follow conventional descriptive terminology for meroblastic fish embryos and are used to indicate the accumulation of blastomeres into the blastoderm cap, rather than a solid morula. However, the findings of the present study demonstrate that dissolved oxygen levels have a significant impact on developmental timing (see Table 2). The cleavage stages, including the blastodisc, the initial cell division, and lower DO treatments (T1 and T2), occurred at a considerably earlier stage than higher DO treatments (T3 and T4). This trend was maintained in all subsequent stages, resulting in a statistically significant delay in hatching time as the concentration of dissolved oxygen (DO) was elevated ($p < 0.001$). The incubation period of the highest oxygen treatment (T4) was the longest, with an average of 345 min of incubation before hatching, in comparison to the incubation period of the lowest DO treatment (T1). It was observed that lower oxygen treatments resulted in the premature completion of all treatments when compared with higher oxygen treatments. This discrepancy was evident during the formation of the blastodisc and the initial cleavage divisions. Consequently, the impact is observed to be on the rate of development rather than on the hatching date. It is not possible to provide an explanation for this accelerated development in this context, since no measurements of metabolic rate, oxygen consumption and stress-related variables have been conducted.

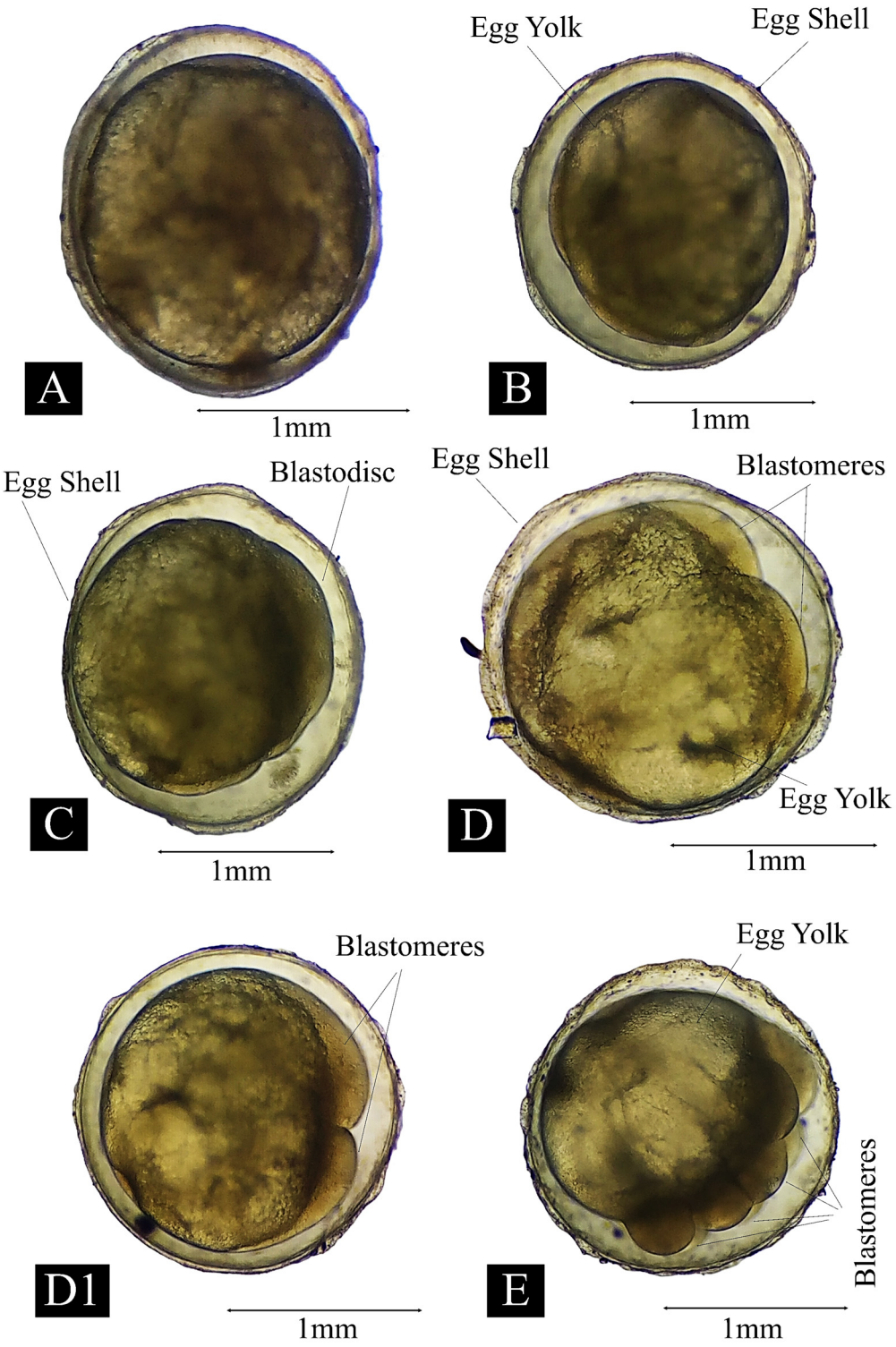


Figure 1. Photomicrograph of ova of striped catfish (*Pangasianodon hypophthalmus*): (**A**) = Unfertilized egg, (**B**) = fertilized egg, (**C**) = blastodisc, (**D**) = 2-cell stage, (**D1**) = 2-cell stage, (**E**) = 8-cell stage.

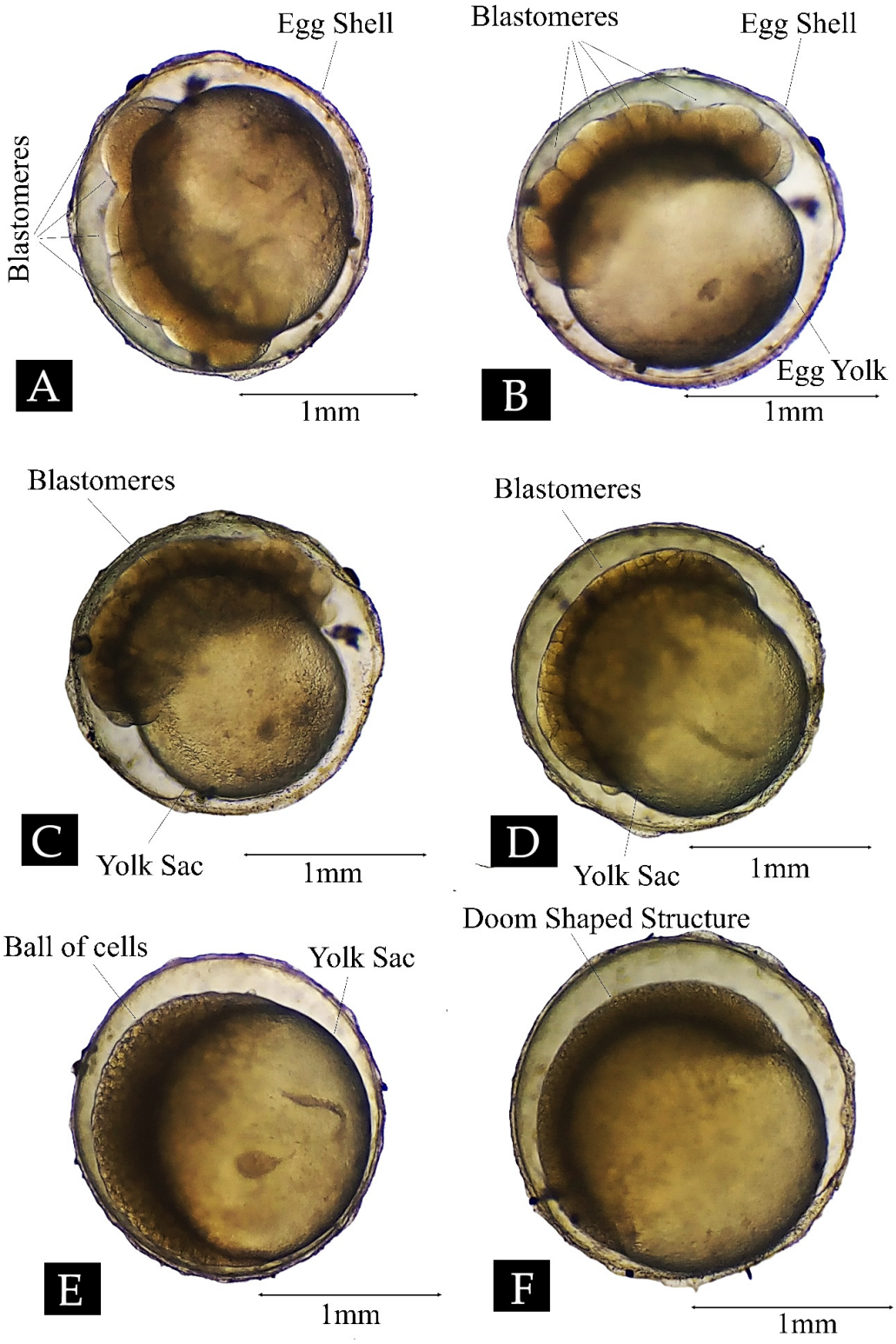


Figure 2. Photomicrograph of ova of striped catfish (*Pangasianodon hypophthalmus*): (**A**) = 16-cell stage, (**B**) = 32-cell stage, (**C**) = early morula, (**D**) = mid-morula, (**E**) = late morula, (**F**) = early gastrula.

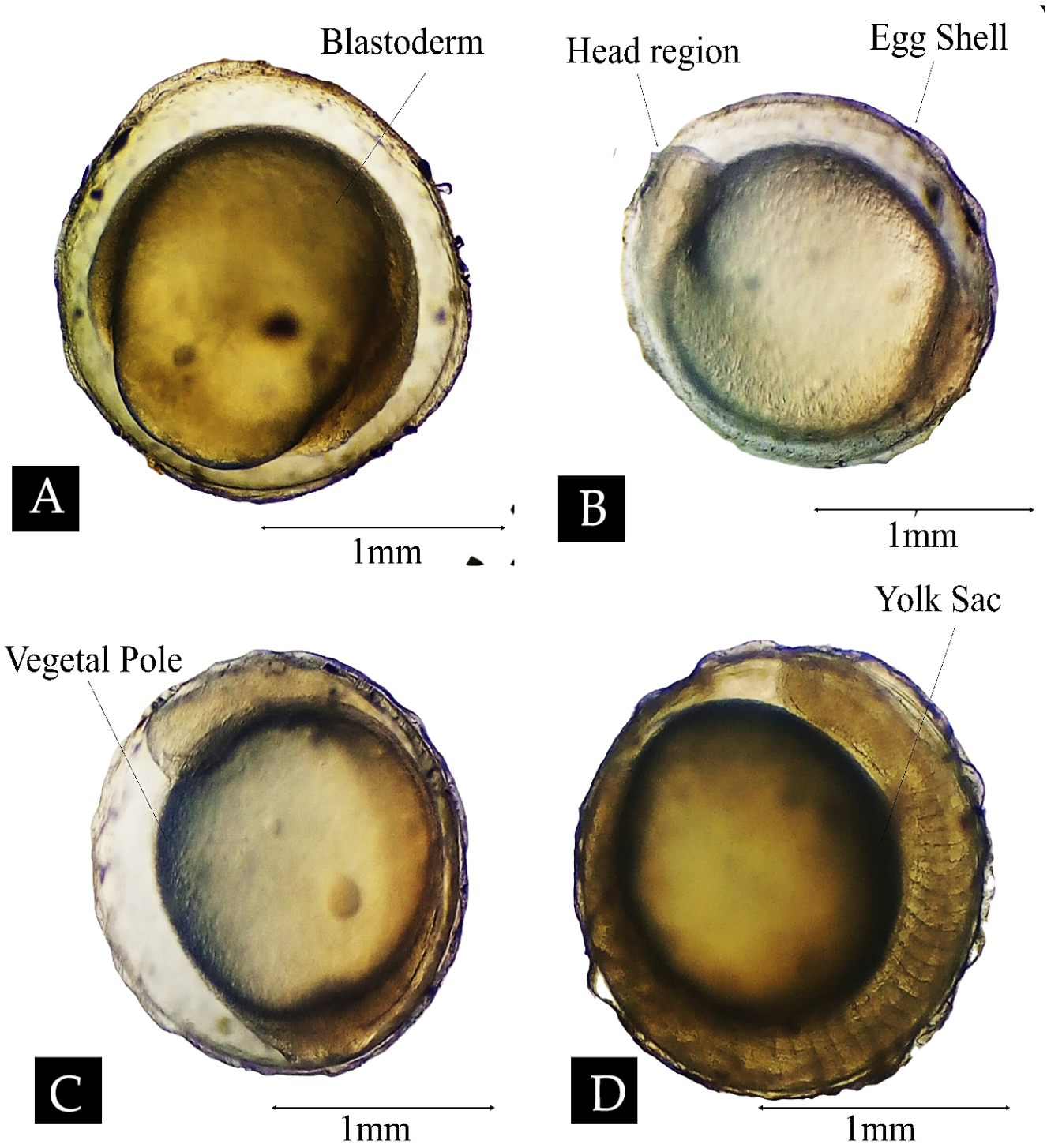


Figure 3. Photomicrograph of ova (*Pangasius hypothalamus*): (**A**) = mid-gastrula, (**B**,**C**) = yolk plug stage, (**D**) = somite formation.

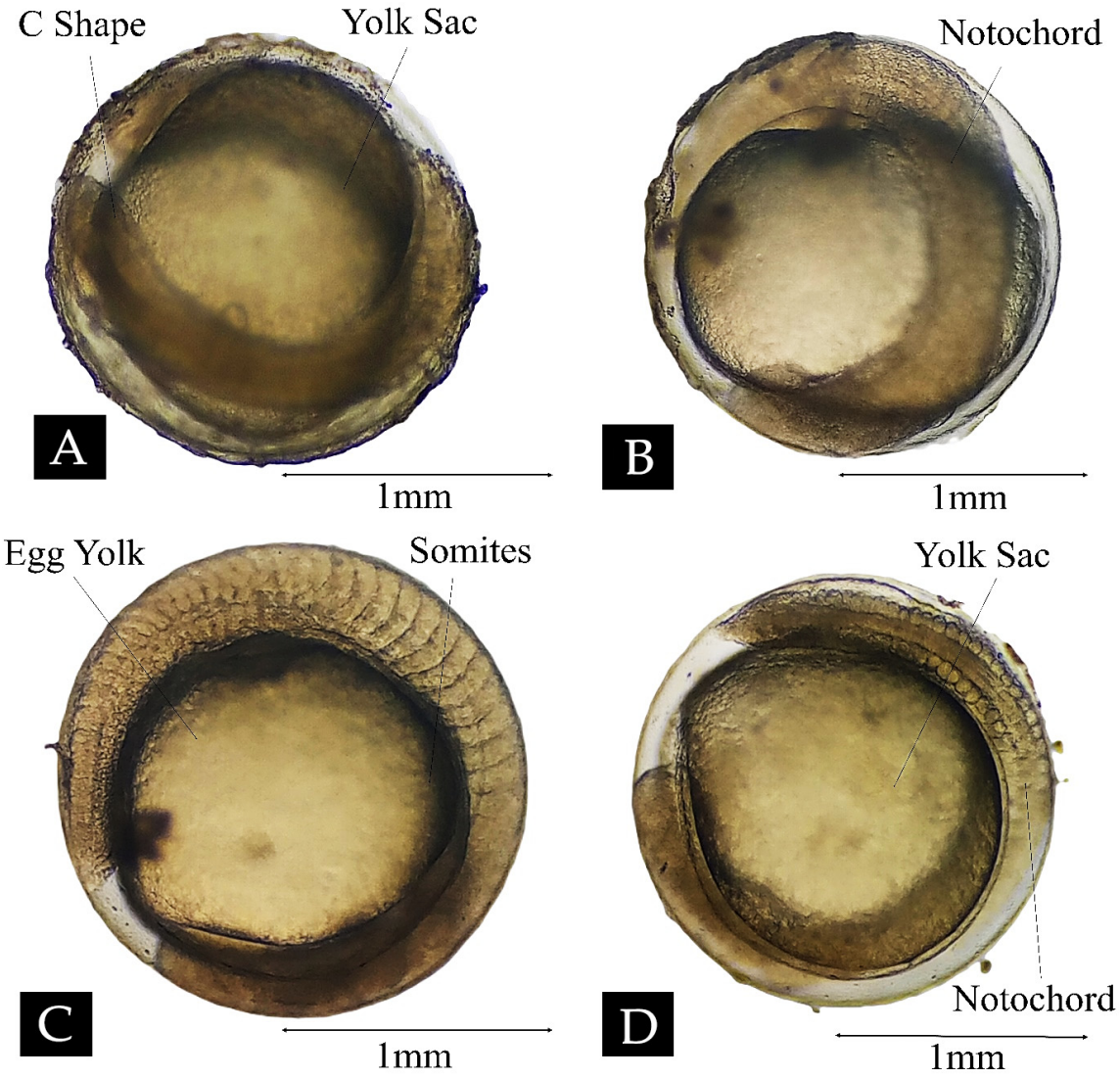


Figure 4. Photomicrograph of ova of striped catfish (*Pangasianodon hypophthalmus*): (**A**) = C-shape, (**B**) = notochord formation, (**C**) = 31-somite stage, (**D**) = notochord formation.

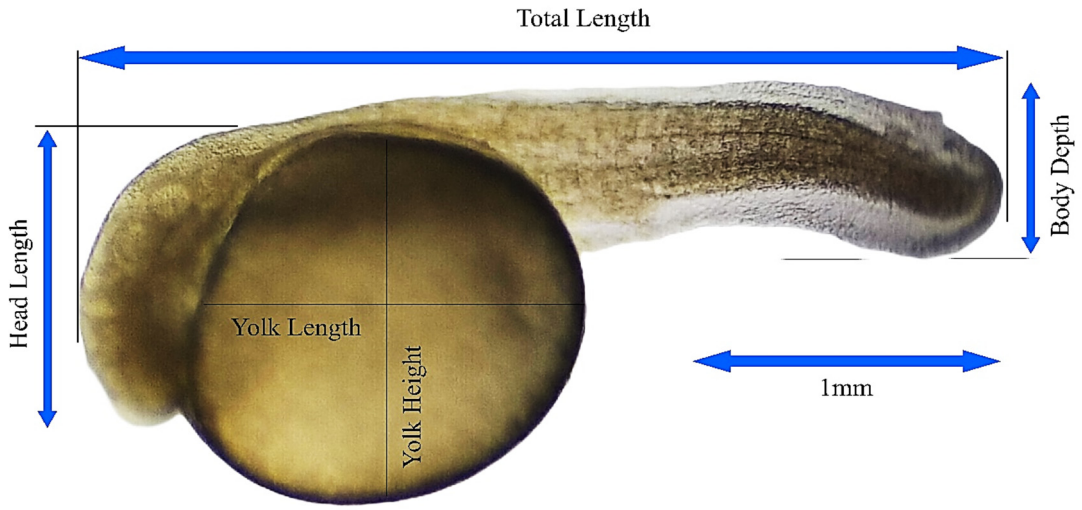


Figure 5. Photomicrograph of hatchling striped catfish (*Pangasianodon hypophthalmus*).

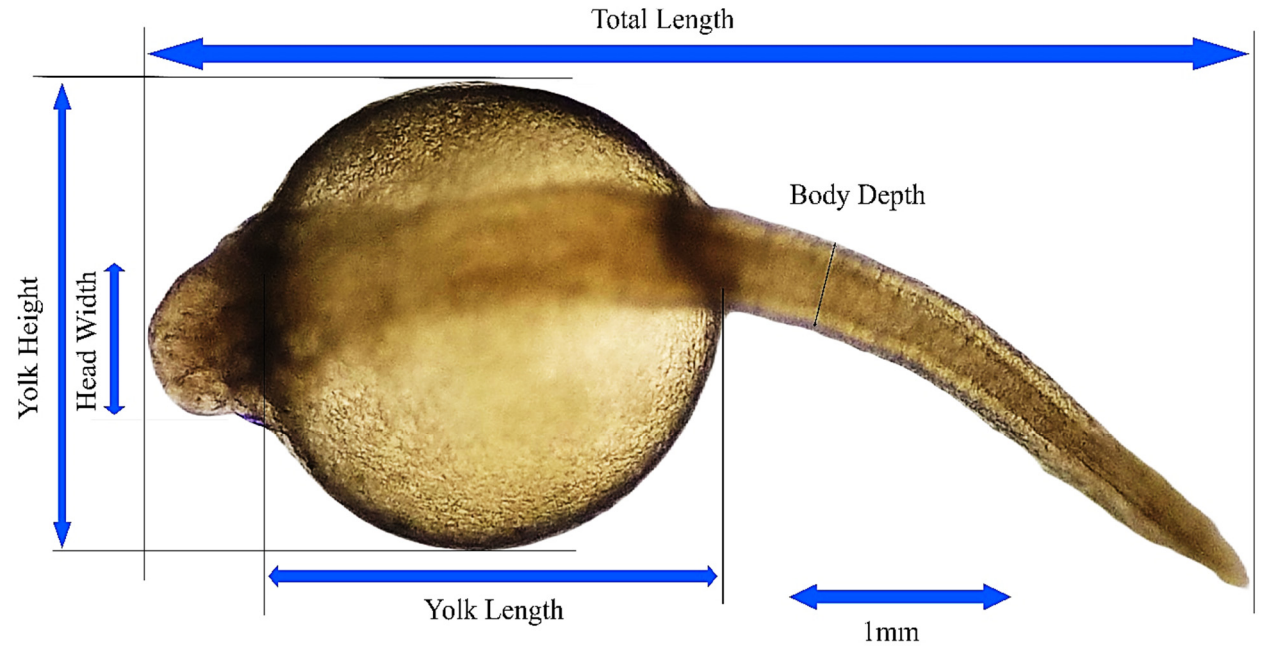


Figure 6. Dorsal view of hatchling striped catfish (*P. hypophthalmus*).

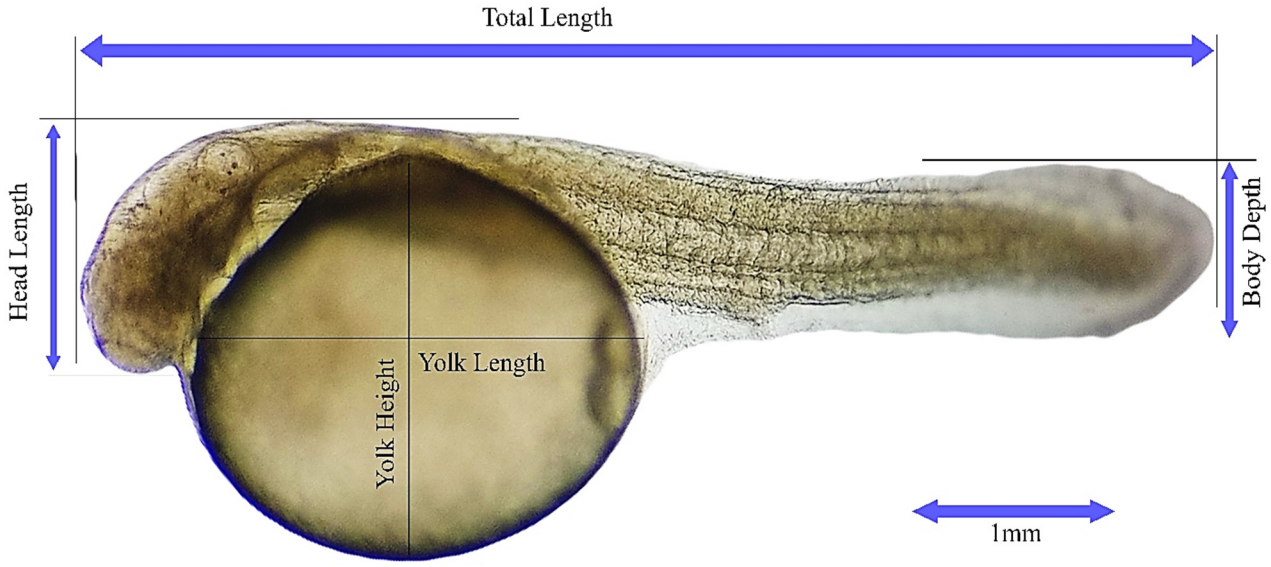


Figure 7. Larvae of striped catfish (*Pangasius hypothalamus*) after 4 h post-hatching.

Table 2. Effect of DO level on hatching time (minutes post-fertilization) in striped catfish (*Pangasius hypothalamus*). The results are presented as mean ± SE.

Treatment	T1	T2	T3	T4	Df Between Groups	Df Within Groups	F	*p* Value
Unfertilized egg	0	0	0	0				
Fertilized egg/zygote	0	0	0	0				
Blastodisc Formation	22.0 ± 0.71 ^{c}	26.8 ± 2.33 ^{b}	28.8 ± 1.02 ^{b}	33.4 ± 1.21 ^{a}	3	16	10.583	*0.000*
2 cell	30.6 ± 0.81 ^{c}	34.0 ± 2.00 ^{bc}	37.0 ± 1.10 ^{b}	41.8 ± 1.28 ^{a}	3	16	12.037	*0.000*
8 cell	43.4 ± 1.86 ^{c}	53.8 ± 1.88 ^{b}	51.2 ± 2.76 ^{b}	61.2 ± 1.16 ^{a}	3	16	13.504	*0.000*
32 cell	104.6 ± 4.48 ^{c}	117.0 ± 4.54 ^{b}	123.4 ± 3.47 ^{ab}	131.6 ± 2.42 ^{a}	3	16	8.863	*0.001*
Early Morula	193.8 ± 3.84 ^{c}	209.6 ± 3.08 ^{b}	226.6 ± 4.03 ^{a}	229.0 ± 1.79 ^{a}	3	16	24.703	*0.000*

Mid Morula	228.2 ± 4.79 ^{c}	247.8 ± 3.02 ^{b}	255.8 ± 3.28 ^{ab}	263.0 ± 3.58 ^{a}	3	16	16.204	*0.000*
Late Morula	313.2 ± 5.25 ^{d}	333.8 ± 5.23 ^{c}	347.6 ± 2.68 ^{b}	361.8 ± 2.8 ^{a}	3	16	24.546	*0.000*
Early Gastrula	421 ± 2.51 ^{d}	452.6 ± 4.01 ^{c}	463.2 ± 3.31 ^{b}	486.6 ± 3.75 ^{a}	3	16	62.631	*0.000*
Late Gastrula	520.6 ± 5.12 ^{c}	550.6 ± 3.11 ^{b}	571.2 ± 3.99 ^{a}	575.4 ± 3.93 ^{a}	3	16	37.237	*0.000*
Embryonic body formation	625.2 ± 7.05 ^{c}	658.2 ± 5.12 ^{b}	669.6 ± 2.29 ^{b}	684.6 ± 4.34 ^{a}	3	16	25.443	*0.000*
Somite appearance	691.6 ± 8.94 ^{c}	708.4 ± 6.45 ^{bc}	723.6 ± 3.67 ^{ab}	737 ± 4.57 ^{a}	3	16	9.828	*0.001*
Early C-shaped Embryo	731.2 ± 7.00 ^{c}	759.4 ± 4.63 ^{b}	757.2 ± 2.33 ^{b}	778.2 ± 2.15 ^{a}	3	16	18.530	*0.000*
Yolk Plug	813.0 ± 5.43 ^{c}	855.6 ± 2.46 ^{b}	867.6 ± 3.72 ^{b}	884.4 ± 3.87 ^{a}	3	16	57.728	*0.000*
Late C-shaped Embryo	893.6 ± 7.84 ^{d}	917.2 ± 5.42 ^{c}	954.8 ± 5.62 ^{b}	976.6 ± 4.38 ^{a}	3	16	39.125	*0.000*
Start of Body movements	1299.2 ± 28.66 ^{b}	1409.6 ± 22.90 ^{b}	1475.2 ± 19.12 ^{b}	1549.6 ± 13.25 ^{a}	3	16	23.898	*0.000*
Hatching	1841.4 ± 23.56 ^{d}	1973.6 ± 40.65 ^{c}	2048.6 ± 15.92 ^{b}	2186.4 ± 4.82 ^{a}	3	16	33.463	*0.000*

Superscripts on different means within row differ significantly at $p \leq 0.05$.

3.3. Effect of Dissolved Oxygen on Egg Morphology

The findings concerning the effect of dissolved oxygen on egg morphometry demonstrated that egg diameter, volume, and surface area increased in proportion to the concentration of dissolved oxygen (see Table 3). The increase in egg volume was substantially greater in T4 than in T1, with percentage increases of 214.88 ± 18.70% and 69.91 ± 6.38%, respectively ($p < 0.001$; Table 3). The augmented egg swelling evident in DO conditions may be indicative of enhanced water absorption and chorionic expansion capacity, which could result in an elevated oxygen diffusion capacity during protracted incubation periods.

Table 3. Effect of DO level on change in egg morphology of striped catfish (*P. hypophthalmus*). The values are presented as mean ± SE.

Treatment	T1	T2	T3	T4	Df Between Groups	Df Within Groups	F	*p* Value
Change in egg diameter (%)	19.25 ± 1.49 ^{c}	31.65 ± 5.58 ^{b}	38.08 ± 3.66 ^{ab}	46.34 ± 2.87 ^{a}	3	16	9.493	*<0.001*
Change in egg volume (%)	69.91 ± 6.38 ^{c}	133.06 ± 29.01 ^{b}	165.52 ± 22.61 ^{ab}	214.88 ± 18.70 ^{a}	3	16	8.475	*<0.001*
Change in egg surface (%)	42.30 ± 3.57 ^{c}	74.55 ± 14.66 ^{b}	91.19 ± 10.50 ^{ab}	114.49 ± 8.45 ^{a}	3	16	8.475	*<0.001*

Superscripts on different means within row differ significantly at $p \leq 0.05$.

3.4. Embryonic Deformities

Embryonic deformities were prevalent and observed in all treatments, including unfertilized eggs up to an advanced embryonic state of notochord formation and C-shaped embryos (see Figures 8 and 9; Table 4). The abnormalities that were observed included irregular cleavage, abnormal gastrulation, malformed yolk plugs and disfigured axial structures. It was observed that malformations occurred during a number of developmental stages. Despite the absence of an attempt to quantify the frequency of occurrence, this section provides a description of the types of abnormalities observed, as opposed to their incidence.

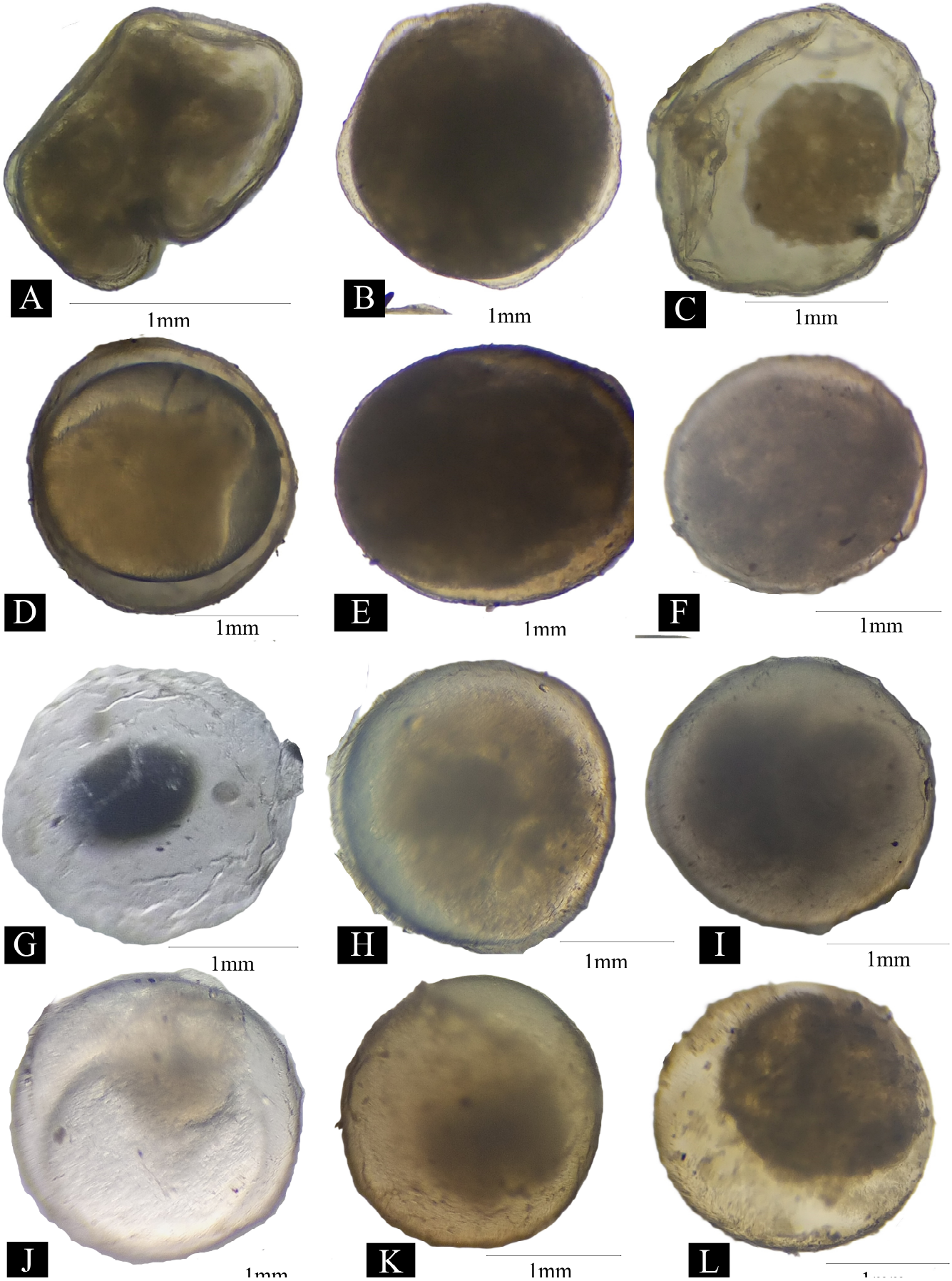


Figure 8. Photomicrograph of ova of striped catfish (*P. hypophthalmus*) (Table 4).

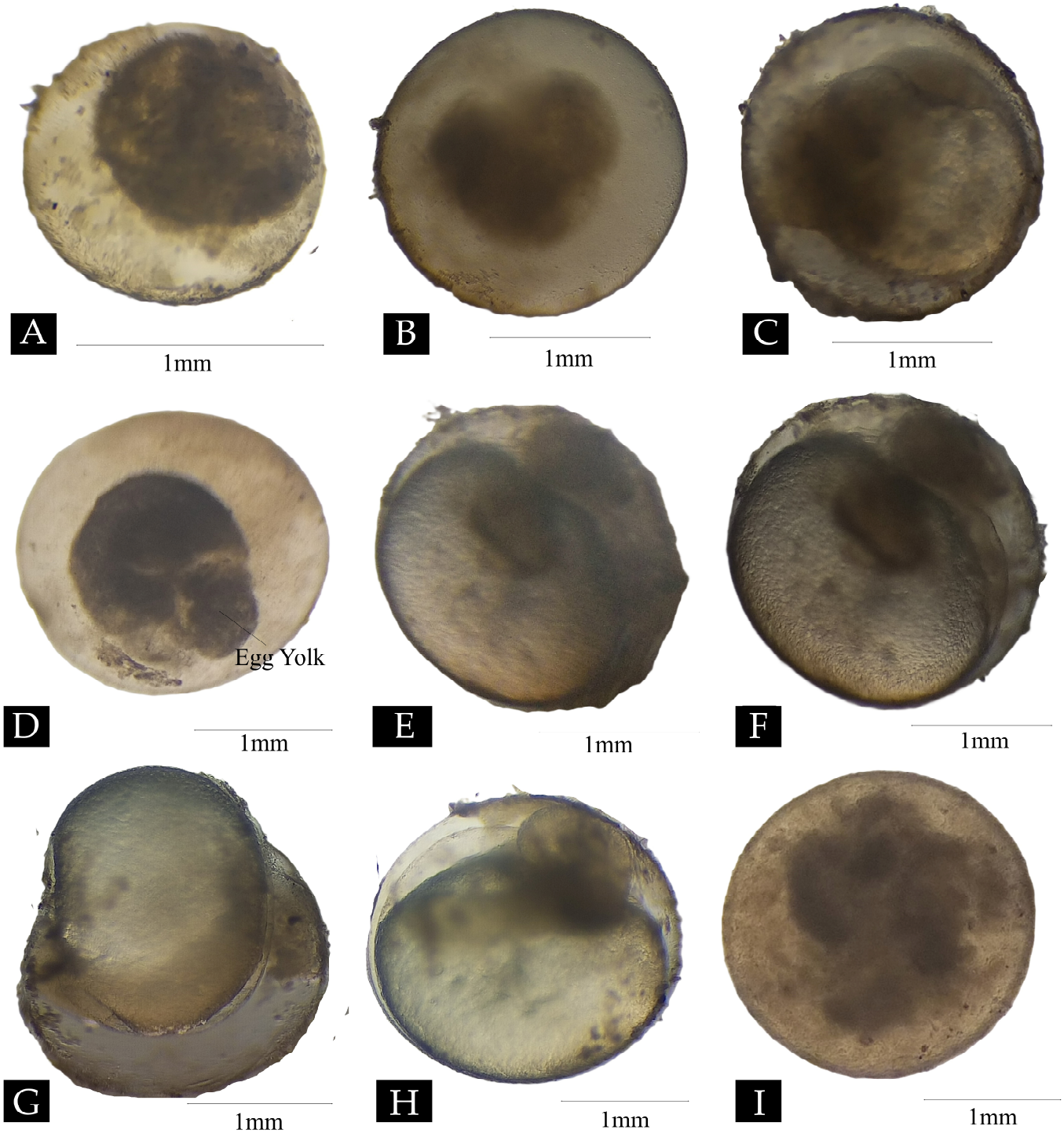


Figure 9. Photomicrograph of striped catfish (*P. hypophthalmus*) eggs.

Table 4. Description of deformed ova stage of striped catfish (*P. hypophthalmus*); mpf stands for minutes post-fertilization.

Figure	Deformed Eggs
Figure 8A	Deformed egg at unfertilized egg stage
Figure 8B	Deformed egg at fertilized egg stage
Figure 8C	Deformed egg at morula stage
Figure 8D	Deformed egg at gastrula stage
Figure 8E	Deformed egg at late gastrula stage
Figure 8F	Deformed egg at yolk plug stage

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Figure 8G	Deformed egg at embryonic body formation
Figure 8H	Deformed egg at embryonic body formation
Figure 8I	Deformed egg at C-shaped embryo (625 mpf)
Figure 8J	Deformed egg at notochord formation (644 mpf)
Figure 8K	Deformed egg at C-shape (663 mpf)
Figure 8L	Deformed egg at C-shape (813 mpf)
Figure 9A	Deformed egg at C- stage (846 mpf)
Figure 9B	Deformed egg at (893 mpf)
Figure 9C	Deformed egg at (956 mpf)
Figure 9D	Deformed egg at (997 mpf)
Figure 9E	Deformed egg at (1098 mpf)
Figure 9F	Deformed egg at (1299 mpf)
Figure 9G	Deformed egg at (1443 mpf)
Figure 9H	Deformed egg at (1549 mpf)
Figure 9I	Deformed egg at (1841 mpf)

4. Discussion

The present study's principal strength lies in the robust correlation between biological responses and the concentration of dissolved oxygen. The correlation remains unaltered by temperature ($p > 0.05$), pH, which remains within the optimal range ($p > 0.05$), and conductivity, which exhibits a change only after 40 and 48 h ($p = 0.028$ and 0.007). This isolation is of critical importance, given that water-quality variables in hatcheries tend to be co-varying, making it difficult to make inferences from a single factor. However, in *Heteropneustes fossilis*, the larval weight exhibited a correlation with both pH and DO, thereby precluding the drawing of conclusions regarding individual factors [21]. The two key factors that impact the breeding success are temperature and DO [22]. The four DO regimes were four incubation systems: circular tank (T1), flow-through (T2), aerated flow-through (T3), and aerated hatching jars (T4). A substantial variation in DO was evident among all treatments at all sampling times ($p < 0.001$). T1 ranged from 4.06 to 5.40 mg L^{-1}, near the hypoxia threshold of 5–6 mg L^{-1} [23], while T4 ranged from 7.99 to 8.27 mg L^{-1}, approaching full aeration. The design is evidently intended to convey a particular message regarding the function of the hatchery. The assertion is that the oxygen environment of the embryos is contingent upon the selection of the incubation system.

4.1. Oxygen-Driven Acceleration of Embryonic Development

The most significant finding is the systematic, oxygen-dependent deceleration of development that occurs in conjunction with increasing oxygen levels. It was observed that all of the recorded milestones were postponed sequentially from T1 to T4. Blastodisc formation occurred at 22 ± 0.71 min in T1 versus 33.4 ± 1.21 min in T4. The mean duration of the first appearance was found to be 691.6 min, with a standard error of ±8.94 min, as opposed to 737 min, with a standard error of ±4.57 min. The onset of body movements occurred at 1299.2 ± 28.66 min, as compared to 1549.6 ± 13.25 min. Hatching occurred at 1841.4 ± 23.56 min versus 2186.4 ± 4.82 min ($p < 0.001$). The mean hatch time for T4 embryos was found to be 345 min later than that of T1 embryos. In the present study, the presence of hatching was observed at 30.7, 32.9, 34.1 and 36.4 h in T1–T4, respectively. The values obtained in the present study exceed both the 18–24 h hatching time reported for *Pangasianodon hypophthalmus* [24,25] and the upper range of 23–34 h reported earlier for catfish species at 26–30 °C [26]. Therefore, it can be concluded that all four oxygen treatments resulted in hatching times that exceeded the upper limits of the published ranges. Furthermore, it was observed that the higher the oxygen treatment, the more delayed the hatching was. In this study, developmental rate and hatching time are considered to be

two distinct endpoints. The developmental rate is indicated by the time of first appearance of each stage, and the hatching time is represented by the time of hatching.

As has been exhaustively demonstrated in the extant literature, the hatching of *P. hypophthalmus* occurs within 18–24 h of fertilization [24,25]. The hatching process is initiated within 23–34 h at temperatures ranging from 26 to 30 °C, under constant flow of fresh water [26]. The following ranges for oxygen-driven acceleration have been documented: T4 treatment (lasting approximately 36.4 h) exceeds the established range, while T1-T3 exhibited significantly earlier hatching, indicating the potential for oxygen-driven acceleration.

In the absence of optimal conditions for development within the egg environment, the hatching of aquatic embryos may occur prematurely as an adaptive escape response. The occurrence of premature hatching in fish has been documented in the scientific literature [27,28]. This, however, does not provide an adequate explanation for the previously observed blastodisc and cleavage stages, as there is no pre-existing mechanism for hatching at these stages. The observed phenomenon was thus termed "developmental acceleration" under conditions of hypoxia, as opposed to the alternative appellation of "premature hatching". It is important to note that both the metabolic stress and adaptive escape explanations are merely hypotheses; as such, direct measurements of metabolic rate, oxygen consumption, and stress markers are required in order to provide a more robust evidence base. This trend is also evident in *P. hypophthalmus*, and the data presented herein suggest that oxygen effects commence at the earliest cleavage divisions.

The step-wise progression of developmental milestones with DO supports the role of dissolved oxygen as a critical limiting factor in fish embryogenesis. As demonstrated in [12], the process of development is retarded and the success of hatching is diminished under hypoxic conditions. The transition from the acceleration phase to the failure phase is characterised by a pronounced shift, with only 4.9% of zebrafish eggs successfully hatching at a DO level of 0.5–0.8 mg L^{-1} after 96–260 h, in contrast to the 48–60 h normoxic period. The hatching process was found to be entirely suppressed at a DO concentration of 3–4 mg L^{-1} in yellow perch [29]. The effect is species-specific, and the location of the lower boundary is more significant for hatchery practices than the presence of the effect itself.

4.2. Oxygen-Dependent Egg Hydration and Morphometry

The egg morphometric data reveal an additional dimension of oxygen dependence that is not frequently quantified. The percentage increases in egg diameter (19.25 ± 1.49% in T1 to 46.34 ± 2.87% in T4), volume (69.91 ± 6.38% to 214.88 ± 18.70%), and surface area (42.30 ± 3.57% to 114.49 ± 8.45%) all rose proportionally with DO ($p < 0.001$). This egg swelling is due to the formation of the perivitelline space between the chorion and the perivitelline membrane. The final volume is the result of a combination of two factors, namely the turgor pressure of the perivitelline fluid and the elasticity of the chorion [30]. The process of egg hydration is subject to active regulation, energetic support and mediation by aquaporin channels and vitelline membranes [31,32]. As demonstrated in the present study, the phenomenon of swelling was found to be associated with ambient oxygen levels. This finding is consistent with the extant research, which indicates that embryos developing under oxygen saturation levels below the standard parameters exhibit a reduced size in comparison to the standard [22]. It has been demonstrated that the larger surface area in T4 increases the diffusive interface for gas exchange. This is of particular significance given that the chorion functions as an additional barrier [33]. The morphometric changes observed are indicative of an increase in perivitelline water uptake and an increase in diffusive surface. However, it should be noted that the measurement of water uptake, membrane permeability, aquaporins and oxygen diffusion was not included in

the study. Moreover, it is important to note that the total surface area in itself does not necessarily indicate improved gas exchange, since the surface area-to-volume ratio, diffusion distance, and chorion thickness have not been examined. Consequently, the present association remains a hypothesis that is yet to be subjected to direct empirical testing.

4.3. Hypoxia-Induced Morphogenetic Abnormalities

Embryonic deformities were prevalent and observed in each treatment, including unfertilized eggs, notochord formation and C-shaped embryos. These encompassed abnormal gastrulation, irregular cleavage, disfigured yolk plugs, and disfigured axial structures. This breadth is analogous to the extant literature, which demonstrates hypoxia to be a morphogenetic stressor. In the context of hypoxia, fish embryos exhibit developmental asynchronization, a phenomenon that frequently gives rise to congenital defects, including those affecting the spinal and vascular systems [22]. It has been established that other fish species are susceptible to deformities induced by hypoxia. The presence of body and caudal-fin deformities in post-hatching Atlantic salmon fry was found to be associated with oxygen saturation levels of 60%, indicating that oxygen deprivation was responsible for these abnormalities [34]. A study on anemonefish revealed that exposure to 4.0 and 2.5 mg L^{-1} DO resulted in a reduction in notochord length and lower survival compared to the control group [35]. All treatments, including unfertilized eggs and embryos at the notochord and C-shape stages, exhibited malformations. However, no quantification of deformity was conducted. In all observed cases, deformities were identified, and it was determined that oxygen was not the sole cause. The potential causative factors include oocyte quality and the methodologies employed during washing and degumming. The observations are of a descriptive nature, and counts by stage are required in order to assess treatment effects.

4.4. Long-Term Consequences of Embryonic Oxygen Exposure and Implications for Hatchery Oxygen Management

There is evidence to suggest that oxygen levels during early development can affect later physiological function in aquatic organisms. As demonstrated in the relevant literature, alterations in physiological responses have been reported following developmental hypoxia exposure in red drum [36] and carry-over effects of early hypoxia exposure on tolerance and growth in Pacific abalone [37]. In a similar manner, research on *Fundulus heteroclitus* and other fish species has demonstrated the role of early environmental conditions in determining the environmental tolerance of later life [38,39]. The results of the study suggest that the incubation conditions of the embryo may have a broader impact than previously thought, extending beyond the process of hatching. However, it remains uncertain whether analogous outcomes can be observed in *P. hypophthalmus*, as the present study did not encompass extended evaluation of larval performance.

Furthermore, adequate oxygen supply is imperative for the effective management of hatcheries. Embryonic development of common carp has been the subject of study in conditions of oxygenated water [40], and ultrafine-bubble oxygenation technology has been the focus of investigation for the purpose of improving larviculture performance in freshwater fish [41]. As indicated by the relevant literature, including the works of [26], there have been documented cases of flow-through incubation systems for *P. hypophthalmus*. Furthermore, the necessity of continuous oxygen management has been emphasised in hatchery guidelines [42]. The extant literature suggests that the provision of oxygen is of significance during the process of fish embryogenesis. However, it is important to note that the optimal requirement for oxygen may vary among species and incubation parameters. The present study provides valuable comparative information regarding the embry-

onic development and egg morphometry of *P. hypophthalmus* under different DO conditions. This information could contribute to the enhancement of hatchery practices. The study incorporated both maternal and paternal effects, with four females and males allocated to each treatment. Notably, egg batches were not divided across treatments. Consequently, the variability observed among parents was compounded by that inherent to the jars, thereby precluding the ability to distinguish between the contributions of parents and the treatment. It is recommended that future research employ split-family designs, standardise incubation parameters, and assess hatching success and larval performance. This will assist in elucidating the effects and provide evidence-based oxygen management practices for commercial hatcheries.

5. Conclusions

The present study demonstrated that the embryonic developmental timing and egg morphometry of *P. hypophthalmus* exhibited significant disparities amongst four incubation regimes, under varying dissolved oxygen concentrations. The lowest dissolved oxygen treatment (T1) exhibited a significant decrease in egg diameter, egg volume and surface area, while the highest dissolved oxygen treatment (T4) exhibited a significant increase in egg diameter, egg volume and surface area, accompanied by an extended incubation period. In all treatment groups, developmental abnormalities were observed, indicating the potential involvement of additional factors. The findings of this study provide valuable insights into the early embryonic development and hatchery incubation management. However, further testing is required to ascertain hatching success and larval quality before optimal oxygen recommendations can be made. Further studies are required to account for parental effects, the nutritional status of broodstock, the quality of eggs, and subsequent larval performance. The objective of such studies would be to inform evidence-based hatchery management practices.

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