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STUDY OF THE EMBRYONIC AND POST-EMBRYONIC STAGES OF DANIO RERIO (DANIO RERIO HAMILTON 1822) IN AQUARIUM CONDITIONS

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Abstract

The research was conducted from May to September 2025 in the aquarium complex of Baku State University. *Danio rerio* Hamilton, 1822 breeders, was used as the model research object. Their fertilized eggs were obtained under aquarium conditions, and their embryonic and post-embryonic developmental stages were studied. The duration of individual stages in the embryonic development of *Danio rerio* was determined: zygote (0-0.75 hours), cleavage (0.75-3.5 hours), blastula, characterized by the formation of the blastodisc (3.5-7.5 hours), gastrula, accompanied by the migration of cells of the southern pole and the formation of the three primary germ layers (ectoderm, mesoderm, and endoderm) (7.5-14 hours), somite formation (14-32 hours), organogenesis and twitching stages beginning with the heartbeat, as well as the hatching of free embryos from the eggs (50-75 hours). The effects of live and mixed feeds on the development of *Danio rerio* fry were studied comparatively. The creation of a complete chronological and morphological map of *Danio rerio* development can provide a reliable database for future research. The results obtained can be used in the educational process (as visual aids and information sources in the teaching of biology, embryology, and ichthyology), in scientific research, and in aquarium complexes (for the effective breeding of the species and obtaining healthy offspring).

Keywords: *Danio rerio*; embryonic and post-embryonic stages; live feed; mixed feed

1. Introduction

Danio rerio Hamilton, 1822 is a freshwater fish belonging to the family Cyprinidae. It is native to South Asian countries. It is a model organism in developmental biology and is known as the zebrafish in English-language literature. In scientific and popular science literature, there is no established successful term for this species, but the names zebrafish, *Danio rerio*, and striped danio are used. The term zebra danio is also used in neurobiological literature.

Danio rerio was proposed by George Streisinger [9] as a model object for studying vertebrate embryonic development and gene functions. The importance of this model organism has been confirmed in many genetic studies. *Danio rerio* is one of the few fish species that have been on orbital space stations. In 1976, fertilized eggs of *Danio rerio* were taken aboard the Soviet scientific station "Salyut-5" in a "biocat" thermostat capsule, and at the end of the first week of the flight, larvae hatched from them in a laboratory aquarium (they were later brought back to Earth for the continuation of research). For studying developmental biology, *Danio rerio* has several advantages over other vertebrates. Its genome, which is

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~70% like that of humans, its transparent embryos, rapid developmental period, and ease of breeding in laboratory conditions make this species an ideal object for science [3]. Its embryos develop rapidly, progressing from egg to the larval stage in just three days. The embryos are large, durable, robust, transparent, and develop outside the mother's body, which makes them easy to manipulate and observe.

Monitoring the morphological and physiological changes in the early developmental stages of these fish, as well as applying them for toxicological studies, is one of the most pressing issues in this field. Research conducted in aquarium conditions made to approximate the natural environment — opens the way for new discoveries that may be significant for both the field of ecology and for humanity in the future.

The main objective of the research is to study and describe in detail the main morphological features of the embryonic and post-embryonic development of *Danio rerio* under controlled, specific aquarium conditions (with precise determination of temperature, light regime, and hydro-chemical parameters of the water), as well as their chronological sequence and duration. It also aims to study the effect of feeding breeders with different—mixed and live—feeds on their developmental dynamics, reproduction, egg productivity, and embryonic and post-embryonic developmental stages.

2. Materials and Methods

The research was conducted from May to September 2025 in the aquarium complex of Baku State University. For this purpose, four aquariums with a water capacity of 45 liters were used. During the experiments, maintaining an optimal and stable aquatic environment in the aquarium complex was a priority issue. To this end, key hydro-chemical indicators (average temperature: kept constant in the range of 25-26°C, pH: a near-neutral environment of 7.0-7.8, general hardness GH: 8-16, dissolved oxygen O₂: 8.6-9.2, salinity, and total dissolved solids) were accurately measured and recorded daily using calibrated portable multi-parameter measuring devices (pH meter, EC/TDS meter, thermometer, oximeter). This monitoring is of particular importance for minimizing potential variability arising from environmental factors in future experiments and for making comparisons between control and experimental groups.

To obtaining fertilized eggs, 4 breeder individuals (2 gravid females and 2 males) were used. Prior to the selection of broodstock for breeding, their morphophysiological condition was evaluated, including pronounced sexual dimorphism, the presence of nuptial coloration, and courtship behaviors such as males pursuing females. In most fish species, the emergence of these characteristics is typically triggered by sex pheromones during the spawning period [4].

The process of obtaining eggs from the breeder fish was continued under optimal conditions and was significantly optimized. To increase the spawning percentage and synchronize the process, a special technological environment was created. This environment included a complex of measures such as intensively feeding the breeders with high-protein live feeds for a period beforehand, sharp manipulation of the photoperiod (creating complete darkness for 24 hours), and increasing the water flow rate through filtration at the moment of spawning. The application of this improved methodology led to a noticeable increase in the quantity and quality of eggs obtained during the spawning process. After the eggs were collected, their fertilization rate was determined, and the viable fertilized eggs were transferred to Petri dishes and special incubation systems. After the incubation process was successfully completed, the resulting free embryos and larvae, as well as the fry from their subsequent developmental stage, are currently being raised in special grow-out aquariums under optimal hydro-chemical and temperature regimes.

To ensure the scientific accuracy of the experiments and the reliability of future results, the embryonic (blastula, gastrula, neurula, organogenesis) and post-embryonic (larval, fry, juvenile) developmental stages of the eggs obtained from *Danio rerio* were studied in detail under a high-precision microscope equipped with a digital camera.

To ensure a high survival rate and intensive growth of *Danio rerio* larvae and fry during their initial and most sensitive developmental stage, an optimal feeding strategy was applied. For this purpose, the mass incubation of *Artemia salina* cysts (eggs) was organized under laboratory conditions in accordance with high-tech standards. As a result of the incubation, which was carried out with precise control over salinity, temperature, aeration, and light regime in an incubation apparatus, live feed – *Artemia salina* nauplii - with high bioactivity and nutritional value were obtained.

To comparatively study the effect of live and mixed feeds on the development of *Danio rerio* fry, 2 additional aquariums with a water capacity of 10 liters were used. Initially, 23 forty-day-old *Danio rerio* fryers were placed in each of them (Table 1).

Table 1. Morpho-biological indicators of *Danio rerio* fry at the beginning of the research, before feeding

Variant I – Control				Variant II - Experimental		
No	P, mg	L, mm	F	P, mg	L, mm	F
1	9	12	0,5	9	11	0,7
2	8	11	0,6	9	10	0,9
3	11	13	0,5	11	12	0,6
4	14	13	0,6	8	9	1,1
5	8	10	0,8	9	10	0,9
6	7	9	1,0	9	10	0,9
7	8	10	0,8	11	12	0,6
8	9	11	0,7	11	12	0,6
9	8	10	0,8	8	9	1,1
10	11	13	0,5	8	8	1,6
11	10	12	0,6	11	12	0,6
12	9	10	0,9	10	11	0,7
13	9	11	0,7	9	10	0,9
14	8	9	1,1	10	11	0,7
15	10	11	0,7	9	10	0,9
16	10	11	0,6	9	10	0,9
17	11	12	0,8	10	11	0,7
18	10	11	1,9	8	9	1,1
19	8	8	1,6	8	8	1,6
20	7	8	1,0	8	10	0,8
21	7	9	0,5	8	10	0,8
22	10	11	1,0	8	9	1,1
23	9,2	10,1	0,9	9,1	10,1	0,9
M _{mean}	9,1	10,7	0,8	9,1	10,2	1,0

In the control (Variant I) and experimental (Variant II) aquariums, their feeding was conducted for 10 days using live (*Artemia salina* nauplii - Variant I) and starter compound feeds produced by the company Aller Aqua. The daily feed ration, given twice a day, constituted 2% of the fry's average mass. After the experiments were concluded, a series of morpho-biological indicators of the fry (length – L, mass – P, condition factor – F, specific growth rate – SGR) were determined and comparatively analyzed [5, 6, 7, 8, 11]. Student's t-test was used for the statistical analysis of the obtained results and to determine the mathematical significance of the differences between the variants [2].

3. Results and discussion

The spawning process of the *Danio rerio* breeders occurred on June 17, 2025, at 6 AM. The total number of fertilized eggs obtained from 2 gravid breeders was 620. The fertilization rate of the eggs was 80%, and the number of free embryos hatched from the eggs was 496. In general, the duration of the individual stages in the embryonic development of *Danio rerio* was determined as follows: zygote (0-0.75 hours), cleavage (0.75 – 3.5 hours), blastula, characterized by the formation of the blastodisc (3.5-7.5 hours), gastrula, accompanied by the migration of cells of the southern pole and the formation of the 3 primary germ layers (ectoderm, mesoderm, endoderm) (7.5-14 hours), somite formation (14-32 hours), organogenesis and twitching stages beginning with the heartbeat, as well as the hatching of free embryos from the eggs (50-75 hours).

The first systematic classification of *Danio rerio* embryonic development was prepared by C.B.Kimmel and colleagues [1].

Continuous observations and initial biometric measurements have confirmed that, because of using live feeds for 10 days, the specific growth rate (SGR) and the condition factor (F), which expresses the mass gain resulting from feeding, were significantly higher in fry compared to those fed with the commercial compound feed. The results of the experiments are presented in the second table (Table 2).

Table 2. The effect of live and mixed diets on the morpho-biological parameters of *Danio rerio* fryers

Variant I – Control					Variant II - Experimental			
No	P, mg	L, mm	F	SGR, %	P, mg	L, mm	F	SGR, %
1	56	20	0,7	13	42	17	0,8	11
2	121	26	0,7	19,9	65	19	0,9	14.1
3	14	10	1,4	1,6	22	15	0,6	4.7
4	21	15	0,6	2,8	15	11	1,1	1,8
5	21	15	0,6	6,7	24	15	0,7	2,8
6	15	11	1,1	5,2	55	18	0,9	5,2
7	21	15	0,6	2,8	55	18	0,9	4,7
8	25	16	0,6	3,0	15	11	1,1	0,9
9	54	18	0,9	5,5	25	16	0,6	3,3
10	25	17	0,5	2,4	50	17	1,0	5,3
11	93	23	0,8	6,5	25	16	0,6	2,4
12	40	17	0,8	4,3	17	12	0,7	1,5
13	68	20	0,8	5,9	28	15	0,8	3,3
14	20	14	0,7	2,6	30	15	0,9	3,2
15	75	21	0,8	5,8	21	13	0,9	2,4
16	67	20	0,7	5,5	50	17	1,0	5,0
17	50	17	1,0	4,4	51	17	1,0	4,7
18	23	13	1,0	2,4	20	14	0,7	2,6
19	20	14	0,7	2,6	15	11	1,1	1,8
M _{mean}	43,6	16,9	0,8	5,4	33	15,1	0,8	4,1

Post-embryonic development covers the stage of the *Danio rerio* fish from hatching until maturity. M.Westerfield [9] classifies these stages as follows:

- **Larval stage:** 3-30 days. During this period, the fish feeds on the remains of its yolk sac and then transitions to active feeding. Fins begin to develop in the larvae, vision strengthens, and reflexes form.
- **Juvenile stage (1–3 months):** This is the transition period to sexual maturity. The body shape resembles that of adults, but the reproductive organs are not yet fully formed.
- **Adult stage (starting from approximately the 3rd month):** Sexual maturity is attained, and the fish become ready to reproduce.

Studies show that while an increase in temperature accelerates embryonic development, conditions above 32°C led to an increase in deformities and mortality. At lower temperatures, development slows down, and morphological defects occur [1].

Creating a complete chronological and morphological map of the development of *Danio rerio* can provide a reliable database for future research.

The obtained results can be used in the educational process (as a visual aid and information source in the teaching of biology, embryology, and ichthyology), in scientific research, and in aquarium complexes (for the effective breeding of the species and obtaining healthy offspring).

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