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PECULIARITIES OF EMBRYOGENESIS IN *SCARDINIUS ERYTHROPHTHALMUS* L.

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ABSTRACT

Morphomechanical changes appearing during embryonic development of *Scardinius erythrophthalmus* L. were followed. Upon complete egg hydration, the yolk together with the embryo was found to occupy about 30% of egg volume, the perivitelline space making up the remaining 70%. The embryonic disc, and the embryo later on, were always located laterally in the egg.
As in many other cyprinids, the developing eggs of *S. erythrophthalmus* contain no strucutral lipids in the form of droplets.
At the mid-point of embryogenesis, the yolk begins to divide; as a result, a vitellar diverticulum – elongating as the development continues – appears under the caudal part of the fast growing embryo. After hatching, the diverticulum is transformed into the posterior part of the body cavity. Until hatching, the *S. erythrophtalmus* embryos lack melanophores both in the skin and in the eyes.

Key words: *Scardinius erythrophthalmus*, egg structure, embryonic development, morphogenesis.

INTRODUCTION

Scardinius erythrophthalmus is a common, albeit not abundant, fish species occurring throughout Europe to the Urals. The species has not been recorded only in the Pyrenean Peninsula, Greece, on the Crimea, and in areas adjacent to Arctic seas [3, 13].

S. erythrophthalmus inhabits the littoral of standing and lentic waters, old river beds [21], and clear lakes with rich submerged vegetation [7]. Depending on the place of occurrence, the species begins to spawn when the water temperature reaches 14°C [15] in April, the spawning going on until June [2]. In colder areas, the spawning takes place from June until mid-July [14]; the eggs are then laid in two portions, the first portion consisting of 80% of all the eggs. Female fecundity, according to various authors, ranges from 20–60 thou. [15] to 100 thou. [9] to 232 thou. eggs [11], the discrepancies resulting most probably from differences in age and size of the females examined. Similarly [2], female age and size may affect egg size, the egg diameter varying from 1 [12] to 1.5 mm [11].

Incubation time is temperature-dependent and takes 5 days at 19°C [10] and 3 days at 24°C [6]. Similarly, Nikolski [11] observed incubation taking 3 days at 20–22°C. The newly hatched larva glues its head to the substrate and remains restive until the yolk sac is completely resorbed.

Although biology of *S. erythrophthalmus* is relatively well known, the minor economic importance of the species resulted in the fact that, as evidenced by the literature, the species' embryonic development and morphomechanics of the developing egg have not been paid too much attention to. It has been assumed that the embryonic development of *S. erythrophthalmus* proceeds as in other, commercially important teleost fish. Thus a view has been adopted that, in a freshwater fish egg, the blastodisc and the embryonic disc as well as the embryo itself place themselves in the apical part of the egg where the perivitelline space is at its largest. It turned out, however, that such a position is far from being a rule. As shown in our earlier studies, the stickleback blastodisc, and thus the embryonic disc, are always to be found laterally in the egg [18]. This is facilitated by the fact that, as opposed to salmonids and other fish [4, 16], lipid droplets in the stickleback eggs are not integrated with the embryonic disc; consequently, the disc remains lateral to the vitellar sphere and lipids are accumulated around the apical egg pole.

The embryonic disc in the bleak egg is situated laterally as well, although the underlying mechanism here is different and lipid droplets do not play any role as they are altogether absent [17].

It seems that the lateral position of embryonic disc in the egg is more common than it has been hitherto accepted; besides, morphomechanic changes involved in embryogenesis differ, sometimes considerably so, between species.

The above considerations necessitate that morphogenesis be followed in different fish species. This paper deals with the process in *S. erythropthalmus*, a representative of cyprinids.

MATERIALS AND METHODS

The study was carried out in June and July 1997 at the Department of Fish Anatomy and Embryology's field laboratory at Izdebno (District of Poznań). The observations concerned developing eggs and larvae of *S. erythrophthalmus*. Mature, ready to spawn individuals were removed from spawning concentrations forming near lake shores and transferred to tanks in a nearby hatchery, fed the same lake's water. In this way, water temperature and quality of the

natural spawning ground were maintained both during egg harvesting, dry fertilisation, activation, and incubation. The water temperature during the study was 21 ±2°C.

The fertilised and activated eggs were placed in spacious containers with running water. Whenever necessary, eggs were picked out from the containers for observations.

The observations were made using a system consisting of two microscopes, fitted with Nikon 2× objectives, coupled with a high resolution CCD digital camera (ensuring high quality images), a screen for on-line observations, a video recorder for recording and subsequent viewing and analysis of images, and a computer equipped with an appropriate image analysis software (Multiscan v. 6.08).

The microscopes allowed to view the eggs both vertically and horizontally (laterally). In the latter case, the eggs were placed in elongated, 2–4 mm wide, chambers mounted on a special shelf. The chambers were attached to the microscope stage, which facilitated handling. Morphodynamic changes in the egg were followed, from those involving the size of an egg and the yolk sphere to those in embryonic disc situation and spatial distribution of the developing embryonic structures.

RESULTS

Similarly to other cyprinids, embryonic development in *S. erythrophthalmus* is relatively short; in our work (mean temperature of 21 ±2°C) took 74 D°, i.e., 1776 h°.

The eggs differed in size. Egg diameter varied from 1.2 to 1.5 mm (1.38 on the average), which – as converted to volume – yielded the smallest and largest egg volumes of 0.90 and 1.77 mm^3 (1.38 mm^3 mean volume). Similar proportions apply to the sizes (volumes) of vitellar spheres in the egg, from the smallest diameter of 0.8 mm to the largest diameter of 1.05 mm, the respective volumes being 0.27 and 0.61 mm^3. Thus the volumes of both the eggs and vitellar spheres varied widely, the highest values being almost twice the lowest ones.

Water absorption by an activated egg was very fast and terminated after 15 min. The process was accompanied by smoothing out and stretching of the egg membrane, clearly wrinkled in the laid egg (Fig. 1).

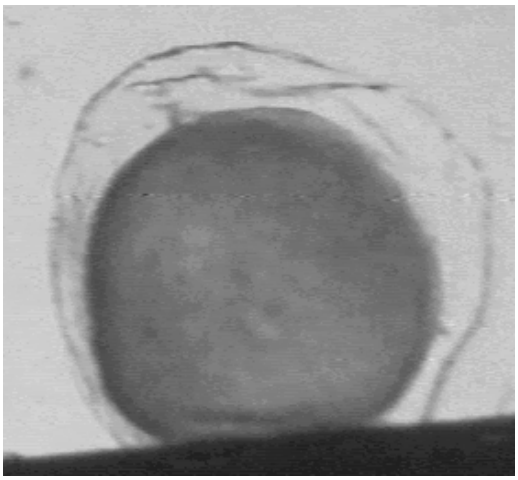

Fig. 1. Formation of perivitelline space; egg membrane wrinkled (lateral view)

The space between the egg membrane and the vitellar sphere was 0.38 mm on the average, i.e., 28.8% of the egg height (Fig. 2).

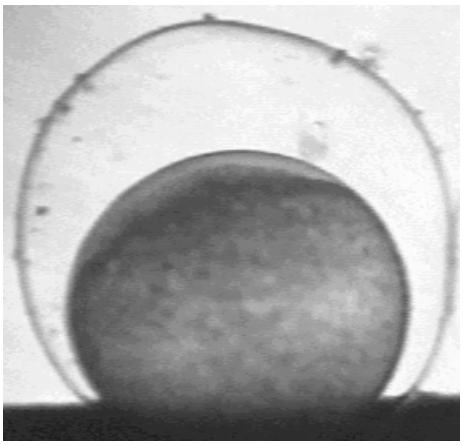

Fig. 2. Lateral view of egg 6 min after activation

The reception mound began to emerge immediately after fertilisation (2–5 min after an egg had been placed in water); its formation, as a result of ectoplasm movement towards the embryonic pole, proceeded throughout the period of egg swelling (Figs 3 a, b). During formation of the first cleavage (30 min) and after two blastomeres had been formed, the embryonic disc produced a sizeable mound above the vitellar sphere, the mound making up 1/3 of the sphere diameter (Fig. 4).

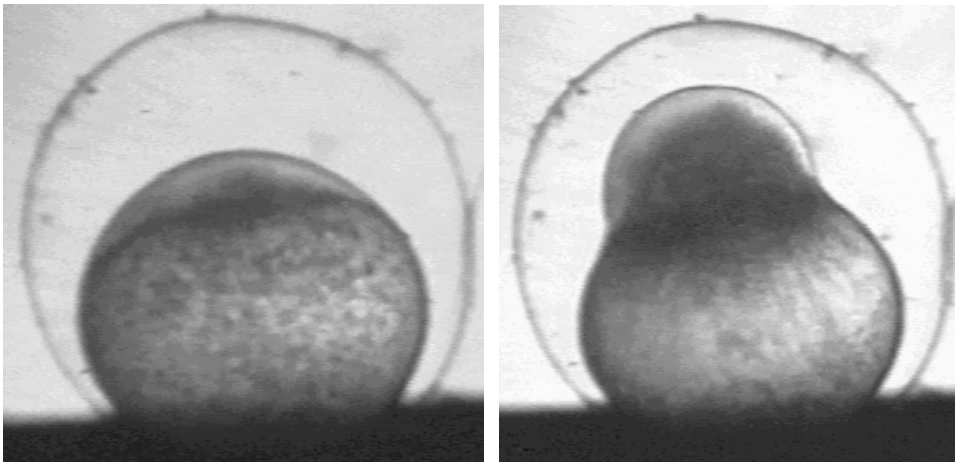

Fig. 3. Formation of reception mound after activation
a—13 min; b—27 min (lateral view)

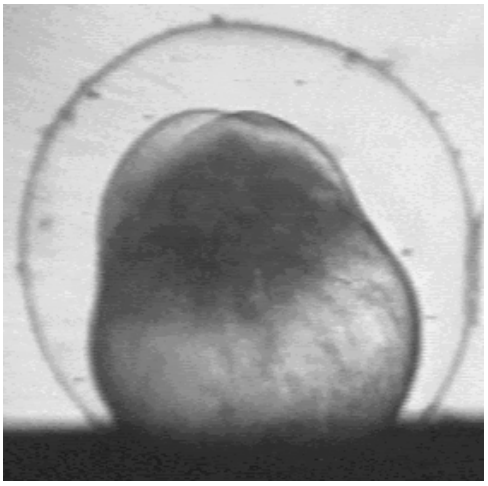

Fig. 4. Formation of first cleavage on raised disc (34'30")

The embryonic disc was always placed laterally, although the angle between the disc and the horizontal plane differed in each case. The lateral position was maintained throughout the developmental stages from the morula until the complete embryo was formed (Figs 5 a-e).

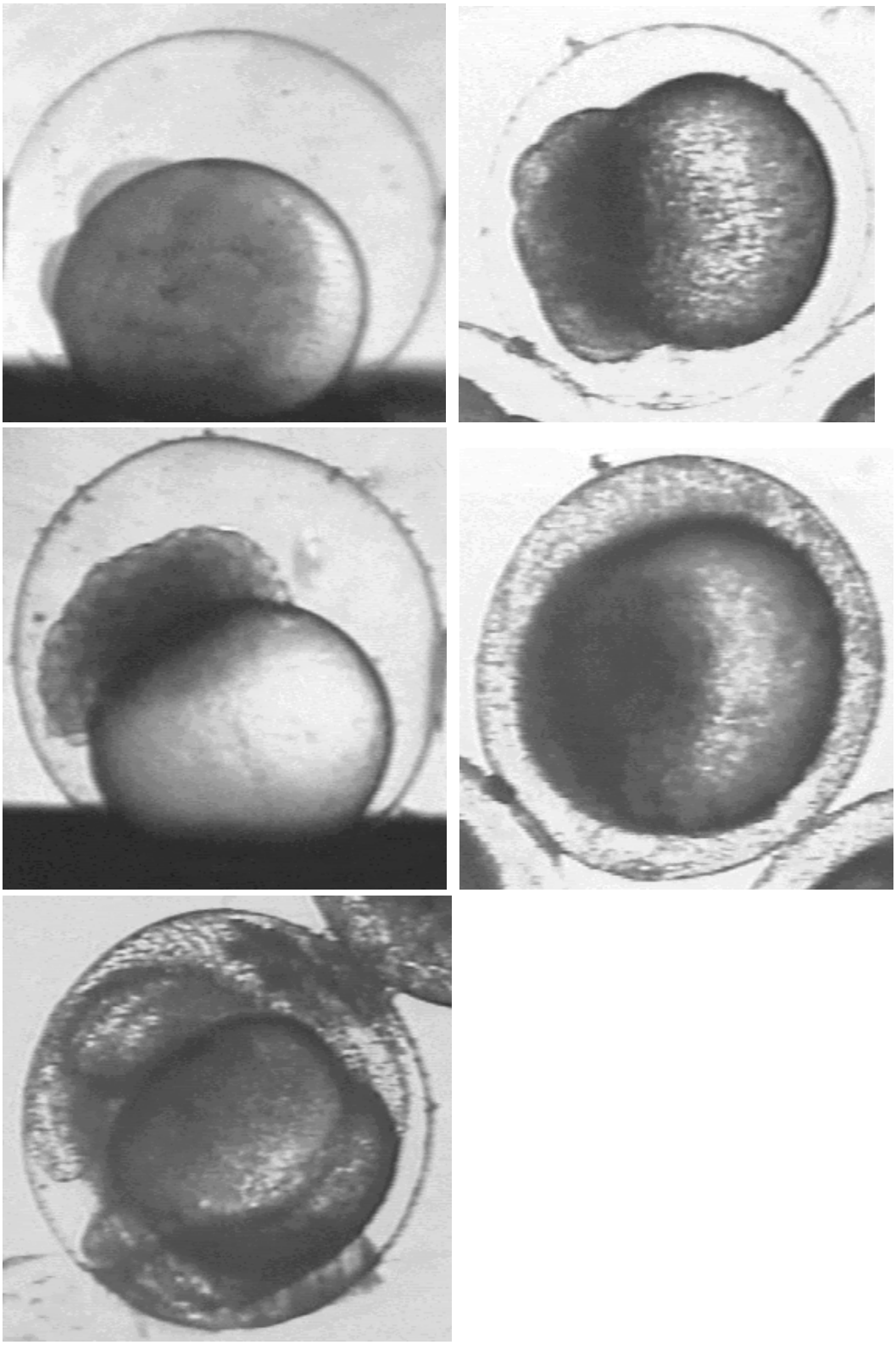

Fig. 5. Lateral position of embryonic structures at different stages of embryogenesis a—2-blastomere stage (1h 03'); b—8-blastomere stage (1h 12'); c—morula (2h 43'); d—gastrula (4h 50'); e—embryo after 500 h°

It is noteworthy to observe that neither near the embryonic disc nor anywhere else in the egg cell and in the vitellar sphere was the so-called structural lipid visible in the form of droplets. The vitellar sphere which, on activation, was an integral part of the egg cell, retained its original regularly spherical shape throughout all the processes related to embryo formation on its surface. However, just after gastrulation was completed (180 h°), the rapidly elongating caudal part of the embryo was observed to rise above the yolk surface and to pull out part of the yolk in the form of an elongated diverticulum (Fig. 6 a) connected with the embryo. The diverticulum grew longer and longer with time (Fig. 6 b). The elongation rate was, however, slower than growth of the embryo, as a result of which it was, just before hatching (Fig 7 a, b), equal in length to the tail and adhered tightly to it after hatching (Fig. 8). After some time, the diverticulum seemed to have transformed into the posterior part of the larval body cavity.

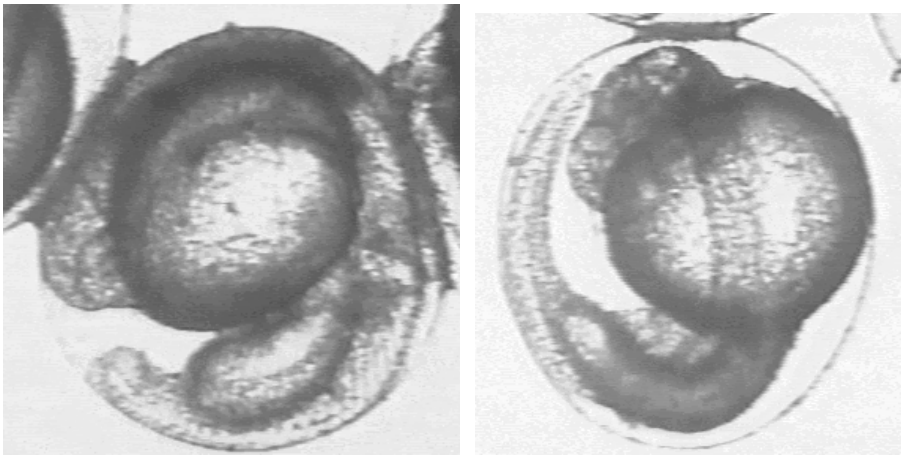

Fig. 6. Yolk sac diverticulum
a—initial phase (630 h°); b—more elongated and slim (714 h°)

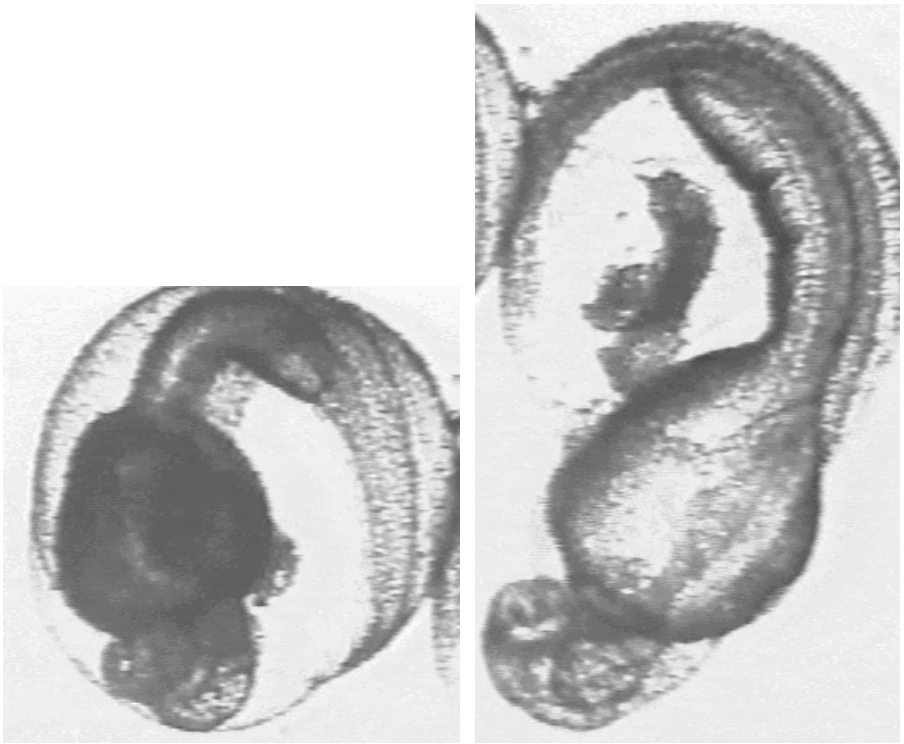

Fig. 7. Hatching of larvae
a—stretching of egg membrane where it comes in contact with the embryonic head;
b—larva breaking free of membrane

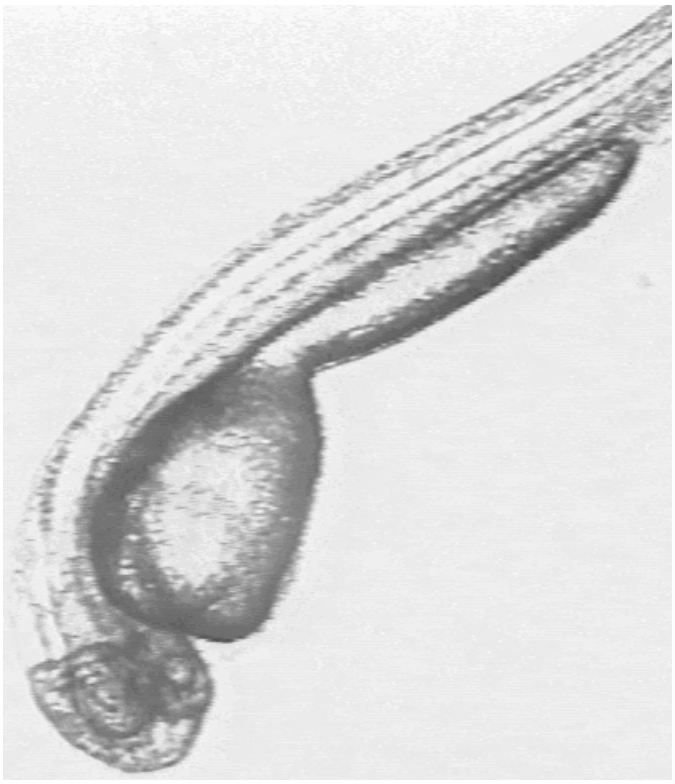

Fig. 8. Hatched larva; yolk sac oval in shape, diverticulum elongated

DISCUSSION

Many of the observations described in this paper have never so far been recorded in the literature on mechanics of the embryogenesis related to structural transformations occurring during that process. The reason is that, perhaps, only "static" images preserved in one way or the other have been observed only or some elements of the developing embryo's dynamics have been viewed, during short periods of time, with the classic vital microscopy allowing observations in the vertical light beam.

The methodology used in this work and involving continuous vital observations made it possible to thoroughly monitor, both in the vertical and horizontal light beams, changes occurring throughout the embryogenesis [17]. This, in turn, facilitated a very thorough and comprehensive recording of morphological changes in the developing embryo. The analysis of those changes allowed to better comprehend the biological meaning of the changes.

Results of the studies described in this paper, observations, and conclusions drawn from our earlier research on embryos of stickleback, bleak, carp, sun bass, and pike show that teleost morphogenesis is very diversified and much more complex and far-fetching than it was realised in previous studies described in the literature.

The results of observations on morphomechanical changes occurring during embryogenesis of *S. erythrophthalmus* show that the changes, although generally conforming to the general patterns known in ichthyology, involve details which deviate from those patterns and occasionally differ substantially from them.

The sizeable perivitelline space, three times as large as the egg cell itself, is one of such details. The large perivitelline space has its advantage, primarily because the rapidly developing embryo which, on termination of its development in the egg, will be almost 7 times the egg cell (vitellar sphere) diameter in length and almost 5 times longer than the egg diameter, finds enough space to easily fit between the egg membranes. Besides, the large perivitellar space ensures that the embryo can move without difficulty, thus enhancing respiration, a process exceptionally intensive in *S. erythrophthalmus* embryos due to a favourable area to volume ratio.

The lateral position of the embryonic disc and the embryo itself may be explained by the fact that, similarly to other cyprinids, the eggs lack structural lipids in the form of lipid droplets. In other teleosts whose eggs do possess lipid droplets integrated with the embryonic discs, the droplets raise the disc towards the egg apex. This biological mechanism proved unnecessary in *S. erythrophthalmus* and in some other species, mainly cyprinids, because such position of the disc and the anterior part of the embryo would restrict, or hamper, a fast increase of the caudal part of the embryo, and render it virtually impossible later on, because the embryo would be forced to squeeze in between the membrane and yolk in the bottom part of the egg.

The formation of a large vitellar diverticulum underneath the caudal part of the embryo is very interesting. The diverticulum elongates with time and becomes cylindrical after hatching. The elongated diverticulum, situated close to the embryo provides the latter with structural and energetic materials which the embryo needs in large amounts due to its high demand both for structural materials indispensable in intensive growth in size, formation of internal organs, and tail muscles and for energetic resources necessary at increasing motility when the circulatory system is not yet operative. The narrowing enables executing lateral movements of the caudal part of the embryo. The subsequent fate of the diverticulum shows that it transforms into the posterior part of the body cavity.

A similar process, although proceeding in a somewhat different way, was observed in the bleak, a species of a similar duration of embryonic development under similar or identical thermal and oxide conditions [17].

A few hours before hatching the embryo becomes very motile. It rotates 2–3 times around its axis, the frequency of rotations increasing with time. Between the rotations, the embryo pushes hard on the egg membranes from inside, which is one of the reasons why the egg changes in shape from spherical to a flattened sphere. Adhering tightly to the membrane, the embryo squeezes the hatching enzyme from special glands [1, 5, 8, 20] and the membrane, digested on its internal surface, becomes thinner and more elastic in certain places. This is particularly well visible, as a local mound, where the membrane comes in contact with the embryonic head (Fig. 7 a). This is the area where the membrane ultimately breaks up. The head and anterior part of the embryo, sprung by the elasticity of the tail, are pushed out of the membrane. After several strong movements of the tail the larva breaks free entirely.

The hatching larvae have resorbed more than 50% of the storage materials. In spite of that, the yolk sac of the hatched larvae restricts their motility and swimming speed to such a degree that the larvae seem to be forced to attach themselves to submerged plants to which the eggs were glued beforehand. Attaching prevents the larvae from falling down into the bottom mud where they would be in danger of being affected by hypoxia, bacteria, etc. Besides, weighted down by the yolk sac, they would hardly have a chance to escape predation of the larvae,

hatched somewhat earlier, of perch, pikeperch, or stickleback. Moreover, attachment to a substrate in the water column enhances respiration during a strong wave action.

The absence of pigment in the embryos and hatched larvae may be regarded as a special kind of predator evasion mechanism.

CONCLUSIONS

1. The perivitelline space in *S. erythrophthalmus* eggs is very large and amounts to 70% of the activated egg volume.
2. The embryonic disc and the embryo itself are always situated laterally in the egg.
3. The *S. erythrophthalmus* eggs lack structural lipids in the form of droplets mounted underneath the embryonic disc.
4. The spherical yolk sac begins to divide on embryo formation, as a result of which a sizeable diverticulum appears underneath the caudal part of the embryo; the diverticulum gradually elongates and becomes cylindrical after hatching.
5. The hatching larvae of *S. erythrophthalmus* lack melanophores both in the skin and in eye balls.

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REFERENCES

1. Baburina E.A., G.A. Buznikow, 1957: Zeliezy vyluplenija embrionov kubanskogo rybca kak istocnik gialuronidazy i fiermienta vyluplenija [Hatching glandsof the embryos of the zanthe as the source of hyaluronidase and the hatching enzyme]. Dokl. AN SSSR, 113, 1387–1391. (In Russian).
2. Baruš V., O. Oliva, 1995: Michulovci (Petromyzontes) a ryby (Osteichthyes) [Lampreys (Petromyzontes) and fishes (Osteichthyes)]. Vol. 2, Academia, Praha. (In Czech).
3. Berg L.S., 1949: Ryby presnych vod SSSR i sopredelnych stron [Freshwater fishes of USSR and neighbouring states]. Izd AN SSSR, Moskva. (In Russian).
4. Devillers Ch., 1961: Structural and dynamic aspects of the development of the teleostean egg. Advances in Morfogenesis, vol. 1, Academic Press, New York–London.
5. Hayes F.R., 1942: The hatching mechanism of salmon eggs. J. Exp. Zool., 83: 357–373.
6. Kryžanowskij S.G., 1949: Ekologomorfologičeskije zakonomernosti razwitija karpovych, v'junowych i somovych ryb (Cyprinoidei i Siluroidei) [Ecomorphology of development of cypinid and siluroid fishes]. Tr. Inst. Morf. Životn., 1, 5–332
7. Lusk S., V.Baruš, J. Vostradovsky,1983: Ryby v našich vodách [Fishes in our waters], Českoslov. Akad. VÉD, Praha. (In Polish).
8. Łuczyński M., 1985: Wykluwanie się ryb. W: Fizjologia ryb [Hatching of fish. In: Physiology of fish], AR-T Olsztyn.
9. Mihalik J., F. Reiser, 1990: Nasze ryby [Our fishes], PWRiL Warszawa. (In Polish).
10. Młyniec B., 1991: Wzdręga (*Scardinius erythrophthalmus* L.). W: Ryby słodkowodne Polski [M. Brylińska ed.] [(*Scardinius erythrophthalmus* L.). In: Freshwater fishes of Poland], PWN, Warszawa: 207–212.
11. Nikolski F., 1970: Ichtiologia szczegółowa [Detailed ichthyology]. PWRiL, Warszawa.
12. Rolik H., J.M. Rembiszewski, 1987: Ryby i krągłouste (Pisces et Cyclostomata) [Fishes and cyclostomes]. PWN, Warszawa: 168–171. (In Polish).
13. Staff F., 1950: Ryby słodkowodne Polski i krajów ościennych [Freshwater fishes of Poland and neighbouring states]. Warszawa.
14. Stehlik J., 1968: Plodnost perlina – ((*Scardinius erythrophthalmus* L.)..), z Klíčavske nádrže [Fecundity of (*Scardinius erythrophthalmus* L.)]. Acta musei Silesiae, A, 17: 81–88.

15. Szczerbowski J., 1993: Rybactwo śródlądowe [Inland fisheries], IRŚ, Olsztyn.
16. Winnicki A., 1967: Plasticity and hardening of the rainbow trout egg membranes. Bull. Acad. Pol. Sci., Cl. II, 15 (12): 779–783.
17. Winnicki A., A. Korzelecka, 1997: Morphomechanical aspects of the development of the bleak (*Alburnus alburnus* L.). Acta Ichth. Piscat., 27: 17–26.
18. Winnicki A., A. Korzelecka, A. Tański, 1997: New data on apply reproduction and early ontogenesis of stickleback (*Gasterosteus aculeatus* L.). Arch. Ryb. Pol., (in press).
19. Winnicki A., M. Stańkowska–Radziun, K. Radziun, 1970: Structural and mechanical changes in the egg membranes of *Salmo gairdneri* Rich. during the period of hatching of the larvas. Acta Ichth. Piscat., 1: 7-18.
20. Wintrebert P., 1912: Le mécanisme de l'eclosion chez la truite arc-en-ciel. C. R. Soc. Biol., 72: 724-727.
21. Załachowski W., 1997: Ryby [Fishes], PWN, Warszawa.

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