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STRUCTURE, SIZE AND SPATIAL DISTRIBUTION OF PERCH (*PERCA FLUVIATILIS L.*) EGG COMPONENTS DURING INCUBATION

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ABSTRACT

Observations on the embryonic development of perch (*Perca fluviatilis L.*), carried out in horizontal and vertical light beams, revealed that volume of the entire complex (egg cell with membrane and jelly envelope) increased by the end of embryogenesis by some 50%.
The blastodisc, and thus also the embryo, occupied a lateral position within the egg, while structural fat droplets of considerable size, not integrated with the blastodisc, gathered at the egg top.

Key words: *Perca fluviatilis L.*, embryogenesis, egg, embryonic morphometry

INTRODUCTION

According to the systematics, *Perciformes* represent an evolutionary masterpiece. The order of perciforms comprises about 8 thousand species of fish, being the most numerous from among all vertebrate orders. High adaptability and plasticity of these fish enabled them to occupy not only marine, but also brackish and fresh waters.

Perch (*Perca fluviatilis L.*) belongs to the most popular fish of our ichthyofauna. It occurs in lakes and flowing waters, well oxygenated and with a not too rapid current. It is a predator; juveniles feed on invertebrates, while older specimens consume also fish, in this of its own species [31].

Sexual maturity is attained by perch males at the age of 2-3 years, and by females - at the age of 3-4 years [32], and according to Baruš and Oliva [3] males mature at the age of 1-3 years, and females at 2-4. Fecundity differs considerably depending on female size, and ranges from 12 to 300 thousand eggs [1, 5, 17,].

Depending on the geographical latitude, perch spawns in natural conditions in early spring, commencing from March in south regions and from June in the north [17, 21].

Perch spawning grounds are water areas having the bottom overgrown with a variety of submerged plants, and with patches of emergent vegetation. Underwater sandy and gravel bottom elevations are also preferred by this fish [14]. Eggs are laid in form of jelly bands 1-5.5 m long [2, 15, 21, 30], and about 6 cm wide according to Winterbert [30], while other authors [7] stated that they were 1-2 cm wide, and that about 80-90% of the egg bands were not longer than 0.5 m [22]. A lot of attention was given to female gonads of perch, this being so also because sex products ejected to water may be of an unusual shape [6, 9, 16, 19, 25].

Although perch is an ubiquitous fish commonly occurring in rivers and lakes, is characterised by extremely tasty meat, and is exceptionally attractive for anglers, we know much less about its embryonic development than of other fish inhabiting our waters. This may be due to the fact that we are still unable to culture perch, and that majority of the fishermen have for a long time considered it to be a weed fish.

Only Wintrebert [30] followed - to some an extent - perch development and described its jelly reproductive band, and Thomopoulos, in his book "Sur l'oeuf de *Perca fluviatilis L*" ("On perch egg") [24], gave a relatively accurate description of unfertilised perch egg, the process of perivitelline space formation, the effect of temperature and of I-, II- and III-valent ions on this process.

Other authors mentioned only perch egg diameter, even without bothering to say whether the measurements comprised the jelly envelope, although the numbers given suggest that the entire complex must have been measured (egg cell, swollen egg, and jelly envelope, see Table 1). Berg [4], who is also cited in Table 1, mentioned that the developing egg may increase its diameter even up to 3.5 mm.

Table 1. Diameter of perch eggs according to different authors

No	Egg diameter (mm)	Author
1	1.9	Dragin 1939
2	2.0–2.5	Berg 1949a
3	1.8–2.0	Kryžanovskij 1953
4	2.0–2.5	Bertin 1958
5	1.7–2.0	Bastl 1969
6	2.0–2.5	Nikolski 1970
7	2.0–2.5	Anisimova and Lavrovskij 1983

Duration of egg incubation depends in perch, as well as in other fish, on temperature. However, in contradistinction to other fish species, the number of thermal units (D°) from egg fertilisation to hatching varies within a surprisingly wide range (if one is to believe different authors), from 90 D° [1], through 175 D° at 12-14°C [15], to as many as 223 D° and 195 D° [12], so that the product of days and mean temperature is almost 2.5 times higher in the third instance compared to the first.

Range of temperatures in which the hatchlings are able to survive is from 6 to 22°C. The best developed and the longest larvae originate from eggs incubated in 12-16°C [3, 23]. According to Berg [5], newly hatched perch larvae are from 4 to 5.3 mm long, according to Rolik and Rembiszewski [20] - 6.5 mm, while Čihař [7] defined their length at 3-6 mm. The larvae carry a large, oval yolk sac containing (similarly as in course of the entire embryonic devel-opment) a large lipid drop; it seems that its role is hydrostatic.

The aim of this paper was to observed the embryonic development of perch with special attention given to morpho-mechanical peculiarities resulting - among others - from lateral positioning of the blastodisc (observed for the first time by Thomopoulos [24]), and from the presence in the ovum of a large lipid sphere not integrated with the blastodics. The latter fact may suggest "marine past" of this fish, in a similar way as has been earlier suggested in the case of stickleback [29].

MATERIALS AND METHODS

Studies were carried out in April and May 1998. Ready to spawn perch (*Perca fluviatilis L.*), both females and males, were caught in the Odra River estuary in the Szczecin Lagoon and transported to the laboratory, where artificial fertilisation was performed with the "dry" method. Delicate bands of eggs were then carefully distributed over some twigs placed in Weiss apparatuses and incubated in 14°C. Egg development was observed carefully collecting the samples of alive eggs in form of band fragments. Ovum structures were measured and embryo development observed using two equipment sets, each consisting of a microscope connected with a digital CCD camera, a monitor, and a video. The microscopes possessed 2× objectives produced by Nikon, enabling observation of large-sized objects (eggs) in the optical system. The digital camera was characterised by high reso-lution and ensured high quality of the image. Each set was also connected to a computer, so that the images could have been stored in computer's memory, and it was also possible to take pictures or perform a computerised analysis of the images whenever needed.. The results of the observations were registered on video cassettes, so that it was possible to perform a detailed and repeated

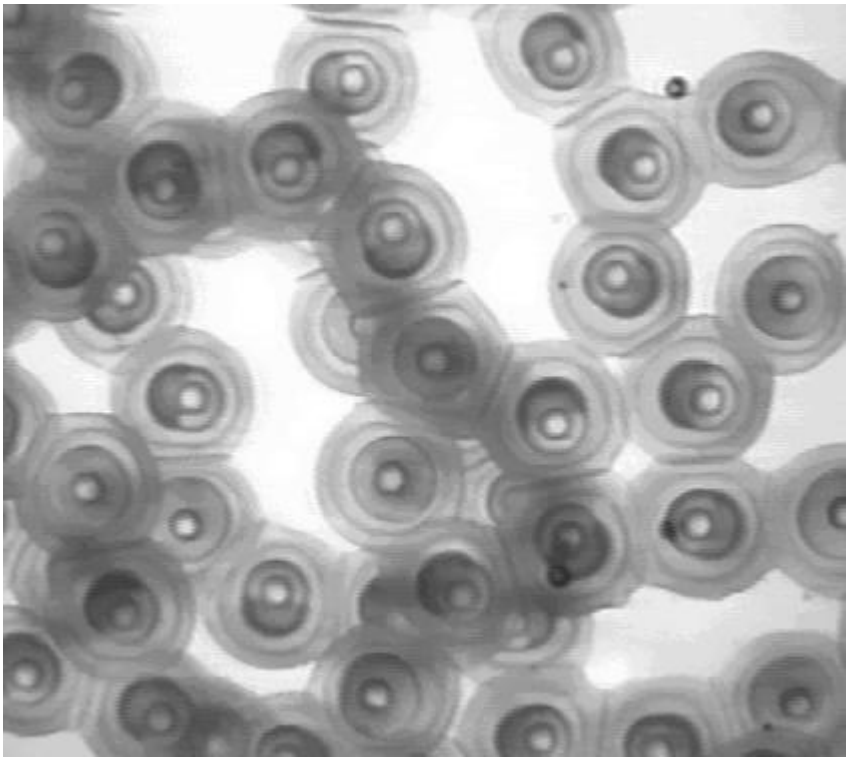
analysis, as also to determine position of the blastodisc and changes in spatial location of the developing structures.

RESULTS

As mentioned above, perch lays the eggs in form of characteristic bands. In our experiment and in conditions of artificial spawning, length of the egg bands was from 1 to 2.5 m and their width - up to 4 cm.

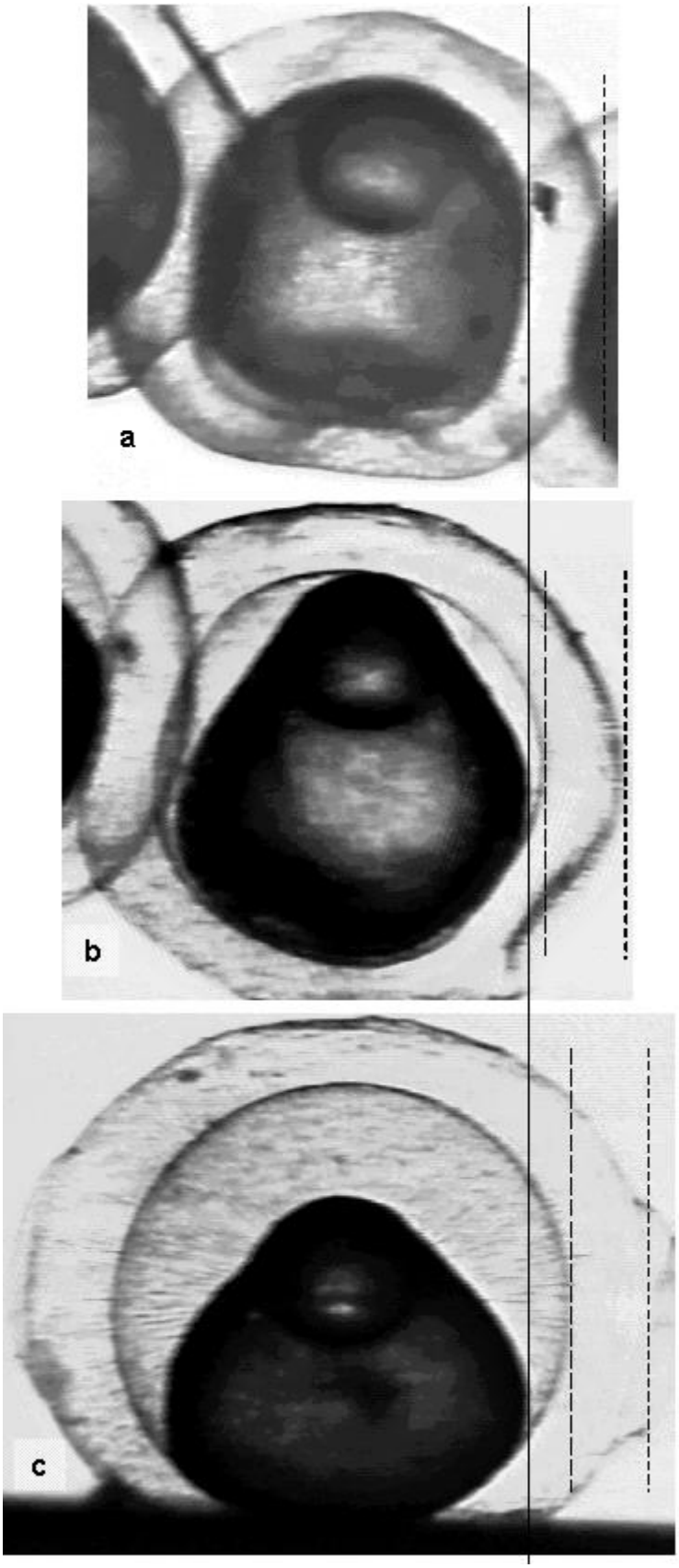
The jelly substance making up the bands and enveloping the eggs was not uniform. Its particular fragments enveloped the eggs with a layer of the same thickness, forming separate structural and spatial complexes, which nevertheless adhered strongly to each other, so that it was very difficult to separate them (Fig. 1). Dimensions of the jelly layer enveloping particular eggs changed during egg swelling (water absorption). Layer thickness increased from 0.14 mm immediately post the fertilisation to 0.20 mm at the end of egg swelling, and then remained unchanged throughout egg incubation period. In the last stage of embryogenesis, when the embryo prepares itself to leave the egg membranes, the jelly layer become a little thinner, of about 0.17 mm.

Fig. 1. Fragment of a lace-like jelly band with eggs.



Water absorption by the eggs (egg swelling) lasted for about 50 min. in 14°C, and for 25 min. in 26°C (Fig. 2a, b, c). Due to this, the eggs and their envelopes considerably increased in volume, from 1.55 mm^3 to 2.35 mm^3 (i.e. one egg absorbed 0.90 mm^3 of water), out of which 0.5 mm^3 of water was absorbed by the perivitelline space.

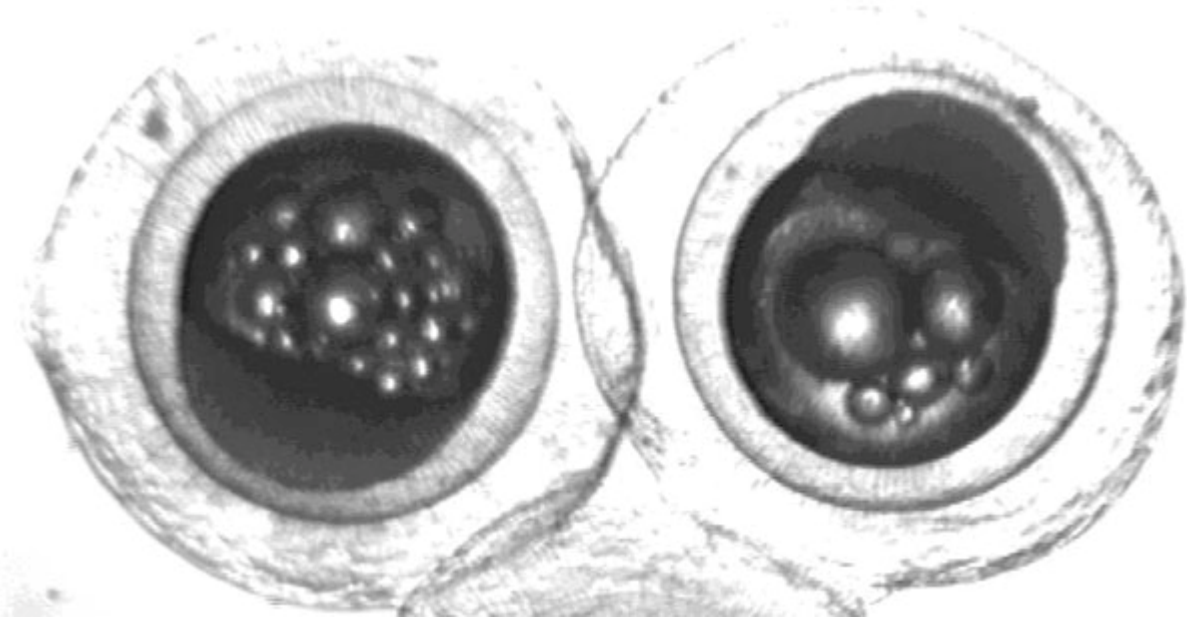
Fig. 2. Increase of the size of perch eggs during activation in water, at 14°C, after:
a – 1 min.
b – 15 min.
c – 50 min. (egg swelling process completed, side view).



The egg cell (yolk sphere) measured ∅ = 1.03 mm, viz. its volume was 0.57 mm^3 (10 minutes after the fertilisation), whereas swollen egg (without jelly envelope, 50 min. after the fertilisation) measured ∅ = 1.25 mm on the average (volume 1.02 mm^3).

The so-called structural fat present in the yolk sphere was not integrated with the blasto-disc and occupied upper position in the egg (Fig. 2c). It could be present as one large ball with a few smaller ones, or as a number of small droplets - sometimes as many as 16 (Fig. 3), the overall volume of which amounted to 0.035 mm^3. Since egg volume (without jelly envelope) was 1.02 mm^3, fat droplets represented 3.4%. It is worth noting that volume of structural fat did not change in course of the whole embryogenesis. Lower specific weight of the fat droplets resulted in their gathering at the upper cell pole, causing a characteristic uplift and a change of egg cell shape from spherical to pear-like, this being well visible in the horizontal light beam (Fig. 2c).

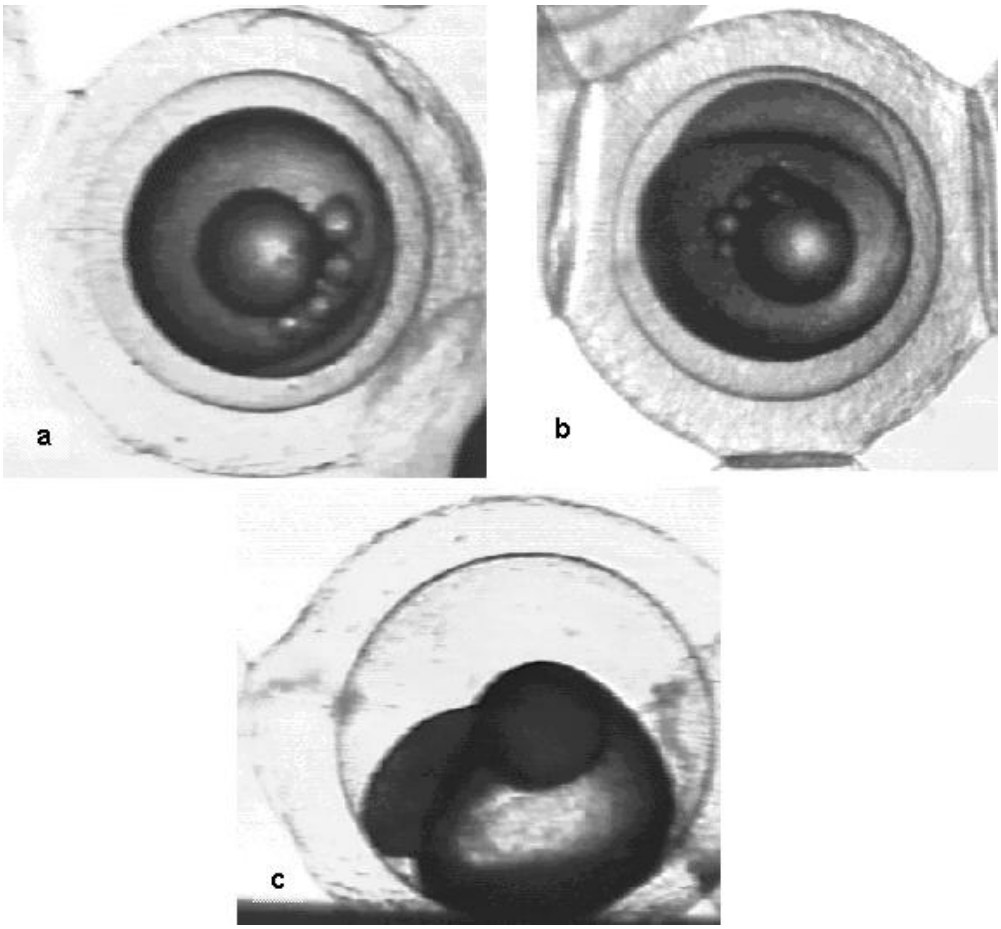
Fig. 3. Size of structural fat droplets not integrated with the blastodisc in perch eggs (a, b – view from above)



Mechanical pressure does not change the spherical shape of the egg as such; only the outer layer of the complex, i.e. the jelly envelope, will then become flattened. Duration of the embryonic development of perch in Weiss incubators was 126 D° in 14°C (3024 h°). Fertilisation rate was very high (over 90 %).

Location of the blastodisc. Immediately after activation, considerable ectoplasm uplifting took place at the upper pole, most probably due to quasi-peristaltic movements, giving the origin to the blastodisc which very soon "moved" sidewise. This was caused by translocation of the gravity centre, resulting in a change of the internal egg statics (Fig. 4a, b, c).

Fig. 4. Formation of the blastodisc (a, b – view from above) and its subsequent sliding to lateral position (c – side view).



Cleavage - The first division took place 46 h° since fertilisation (Fig. 5a), and the subse-quent ones (4 blastomeres) - after 65 h° (Fig. 5 b). The eggs entered the morula stage already after 115 h° (Fig. 6). The subdividing blastodisc and the forming embryo were located later-ally in relation to the yolk ball throughout the embryogenesis.

Fig. 5. Cleavage:
a – 2-blastomere stage (side view)
b – 4-blastomere stage (view from above)

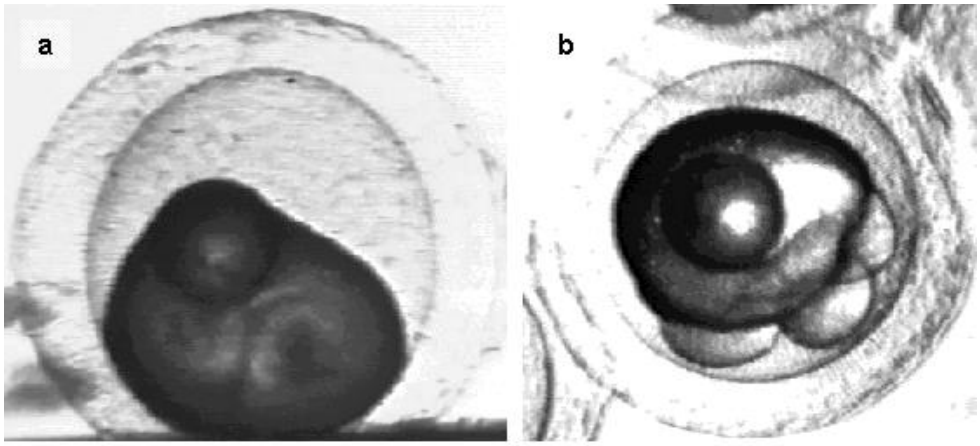
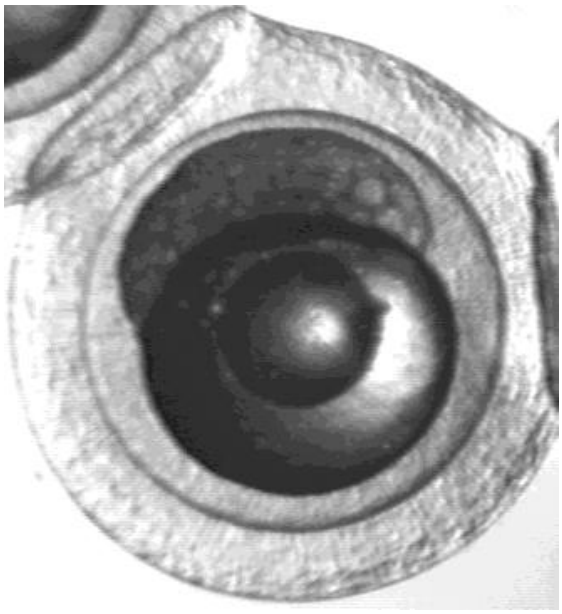
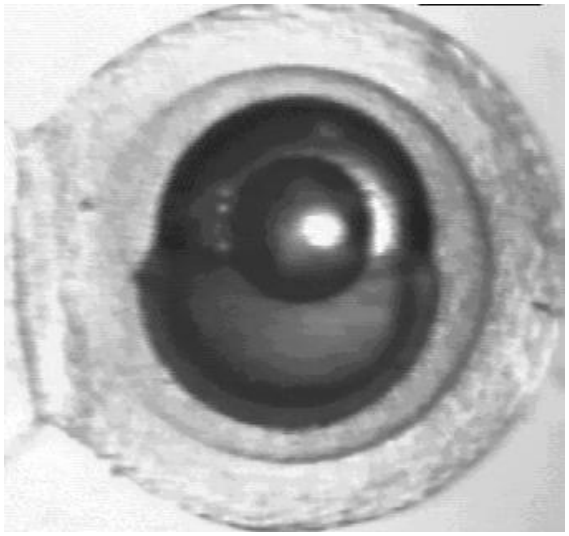


Fig. 6. Morula (view from above).



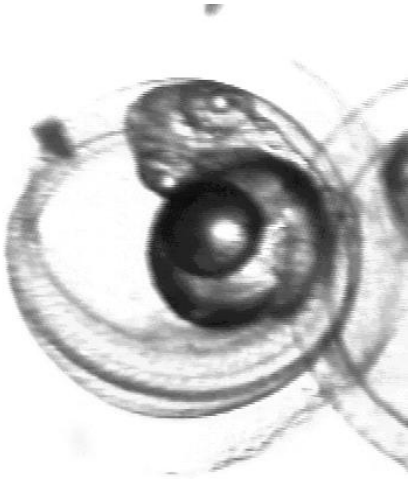
Gastrulation - Commenced after 312 h°, and epiboly reached half of the yolk ball after 350 h° (Fig. 7).

Fig. 7. Gastrulation – 1/2 epiboly (view from above)



Organogenesis. At the beginning of the second day of development, when 400 h° have passed, the embryo appeared. Twelve hours later it had a fairly well developed head, somites became noticeable, while the caudal part was slightly bent back from the yolk ball, and the first tail movements (at the beginning rare and irregular - every few minutes) became noticeable. As the time passed, the embryo became more and more mobile. After about 70 D° (1680 h°) caudal part of the embryo began to grow very rapidly and extended, so that its tip reached the head. Although yolk ball decreased in size along with intensive embryo development, fat droplets did not change in size (Fig. 8a, b).

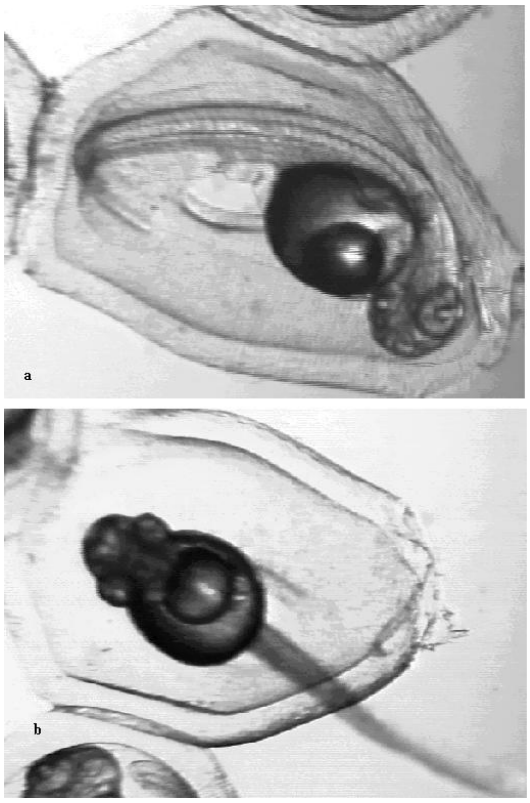
Fig. 8. The embryo – 90 D° (view from above) – considerable degree of yolk sac resorption is noteworthy, as well as the presence of a large fat sphere (practically unchanged since the beginning of egg development).



Heart (primordium) appeared after about 60 D°; at first it contracted very slowly - about 20 beats/min. Fully developed heart was much more efficient (40 heartbeats/min).

Mobility of the embryos increased systematically, reaching the peak just prior to hatching. It consisted of a series of contractions, with short breaks between them (Fig. 9a, b).

Fig. 9. Hatching (view from above)
a - immediately prior to - elongated egg , b - the embryo begins to leave the egg membranes



Hatching of the first larvae took place after 110 D°. Length of the hatchlings was 3.5 mm on the average.

DISCUSSION

The results of our studies, in which lateral viewing was introduced in course of permanent observations of perch embryogenesis, seem to add some new facts to the knowledge on the subject, similarly as had our earlier studies devoted to morphology and mechanics of teleost fish development [13, 28, 29]. This is especially true of the findings related to the topography and spatial structure of the egg complex, used in attempting to find out some causalities between conditions in the surrounding environment and the presence, structure and transformations of the respective egg complex components.

As has been shown earlier by other authors [18, 24, 30], egg envelope was not a uniform structure and consisted of two separate components: relatively thin zona radiata (6 μ according to Thomopuolos), adhering directly to the egg cell in a similar way as in all teleosts, and a thick amorphous structure covering zona radiata and called jelly envelope, which according to Retzius [18] and Wintrebert [30] was produced by follicular cells of the ovary. In contradistinction to other teleost fish (not only salmonids), zona radiata of perch is very susceptible to mechanical pressure (it is very weak) and can be easily broken. This seems quite understable considering that there is no need for this membrane to be very strong as perch eggs are additionally protected by thick jelly envelope. Contrarily to what might be expected, this thick jelly envelope does not prevent oxygen penetration because physically bound water of a gel in the salvatation layers ensures relatively rapid diffusion of this neutral gas. Moreover, the gel maintains fairly stable physico-chemical parameters of its water, considerable amounts of which pass to the egg through this specific filter during egg activation and just before hatching. The jelly envelope plays many roles, the major one being to evenly distribute the eggs in the lace-like jelly band, so that the developing eggs can be suspended in water in order to ensure optimal conditions of gas exchange. It seems that the jelly envelopes, and the bands thus formed, are also some kind of a camouflage and protection against small organisms [30]. Diameter of perch eggs cited in the introduction is probably given for the egg with its mem-brane and the jelly envelope (Table 1). Our measurements suggest that it would be more ap-propriate to give the size of the egg only (egg cell and its membrane), while thickness of the jelly envelope should be measured separately. This is so because size and condition of the embryo are to a certain extent determined by the size of egg itself with its zona radiata .

Period when perivitelline space was formed lasted in our experiments for 50 min. in 14°C, as was observed also by Thomopoulus [24]. Large perivitelline space, occupying about 50% of egg volume, ensured rapid embryonic development and the room for well-formed and physically fit larvae. Lateral positioning of the blastodisc in perch eggs and its causes have not been analysed as yet. Due to plasma gathering in one place, considerable uplifting (receiving colliculus) oc-curred at the generative pole. Since the specific weight of plasma is greater than of the yolk, the egg statics became disturbed, and the receiving culliculus and the forming disc tilted until they touched the inside of the egg membrane (Fig. 4c). The same phenomenon takes place in stickleback eggs [29]. Presence of fat drops in perch eggs is quite striking. They occupy up to 7% of the egg cell volume. Because fat droplets are not integrated with the blastodisc, they cannot act as floats pushing the disc up towards the pole, as is the case in salmonids and many other freshwater fish. Thus, their presence in the egg has no biological or physiological justification. One might even say that they are useless. In view of this, the fact that they are nevertheless present in perch eggs, similarly as in stickleback eggs, may suggest a relatively short historical period since the times when perch ancestors had left marine environment, in which the fat droplets had played a totally different role than in salmonids,

viz. they gathered at the vegetative pole to turn the egg so that the blastodisc would be directed downwards, and then they pushed it up towards the top. Moreover, presence of the fat droplets in perch eggs and their unchanging volume throughout the embryogenesis (0.035 mm^3) may also reflect the differences in the rate of evolutionary changes - more rapid as regards functional changes (different location of the disc), and slower with respect to the egg structure.

It is noteworthy that eggs did not change their size since 50 min. post the fertilisation until a few hours before hatching. Only then the eggs increased in size. This may be caused by the passage of embryo metabolites into the perivitelline space, resulting in an increase of osmotic pressure, so that the perivitelline space would absorb water. The latter phenomenon is additionally favoured by membrane digestion caused by the hatching enzyme [30]. Hence, the jelly envelope plays the role of a water reservoir for the egg. It filters the water which is then ab-sorbed into the perivitelline space.

Increasing egg size (Fig. 10) distends the egg membrane, facilitating its breaking up. Rapid increase of the egg size just prior to hatching was also noted by Wintrebert [30] and Berg [4], but they did not analyse these facts.

Fig. 10. Volume increase complex (perch egg with jelly band) of development

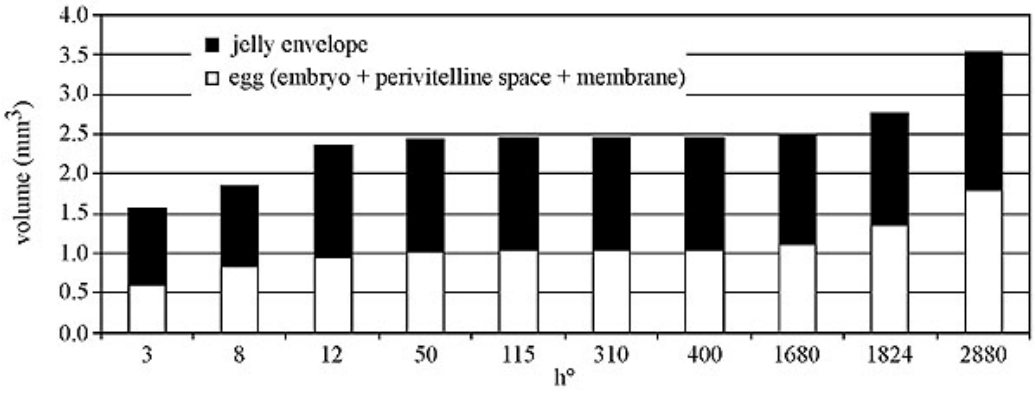
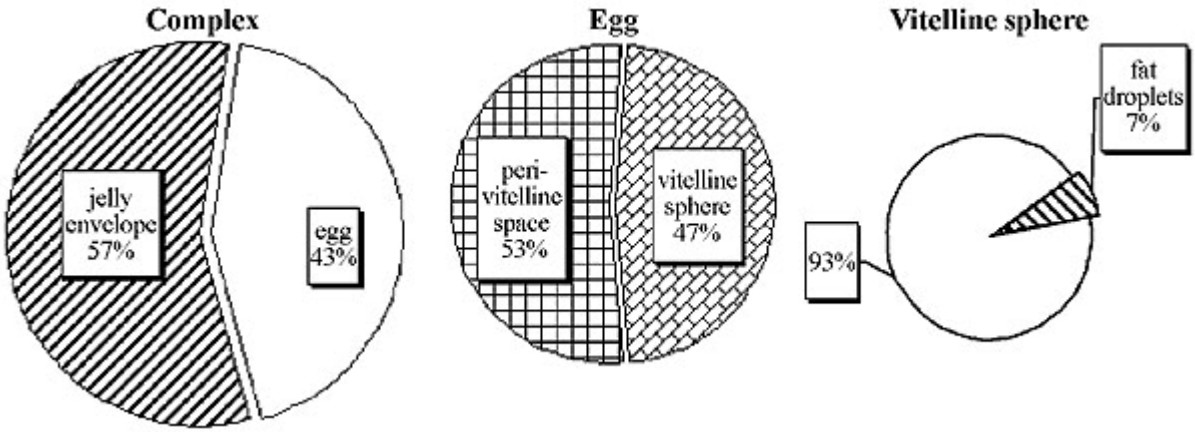


Fig. 11. Element percentage rate in perch egg (jelly band, egg, vitelline sphere, structural fat)



CONCLUSIONS

It seems that the results obtained in course of the studies, viewed over a broader theoretical background of evolutionary processes, lead to the following conclusions:

1. Lateral placing of the blastodisc suggests recent "marine past" of perch ancestors.

2. Fat droplets present during perch embryogenesis but not integrated with the blastodisc do not play any role and seem to be a specific relict of the "marine past" of this fish. Lipids contained in these droplets are used in the energetic processes only when the hatched larvae leave the egg membranes.

3. Outer, fairly thick jelly envelopes, adjoining similar envelopes of other eggs, form lace-like bands which protect the eggs and the developing embryos from mechanical injuries, make it possible to "suspend" the eggs in the water column, ensure optimal conditions for gas exchange, constitute a reservoir as well as a filter for water absorbed by the eggs, and play a masking role.

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REFERENCES

1. Anisimova I.M., W.W. Lavrovskij, 1983: Ichthyology. Wyžš. Škola, Moskva (In Rus-sian).
2. Bastl I., 1969: Spawning of pike-perch (Stizostedion lucioperca L.) in bottom nests in condition of the Orava reservoir (Northern Slovakia). Práce Labor. Rybárstva SAV, 2: 159-184.
3. Baruš V., O. Oliva, 1995: Lampreys (Petromyzontes) and fishes (Osteichthyes). Vol. 2, Academia, Praha (In Czech).
4. Berg L.S., 1949a: Commercial fishes of USSR. Piščepromizdat', Moskva (In Russian)
5. Berg L.S., 1949b: Freshwater fishes of USSR and neighbouring states. Izd. AN SSSR, Moskva-Leningrad (In Russian).
6. Bertin L., 1958: Sexualité et fécondation. In: Traité de zoologie [Grassé P.-P. (ed.)] Mas-son et Cie Paris, 13, 2: 1585-1653.
7. Čihař I., 1995: Freshwater fish-guide. Multico, Warszawa (In Polish).
8. Devillers Ch., 1951: Les mouvements superficiels dans la gastrulation du germe chez la Truite. C.R. Ass. Anat., 38: 418-423.
9. Długosz M., 1983: Histology of the perch (Perca fluviatilis L.) ovary's development in warmed up water from Lake Gosławskie. Rocz. Nauk Rol., Ser. H, 100, 1: 57-71 (In Pol-ish).
10. Dragin P.A., 1939: Spawning of fish in the Ilmen Lake. Inform. Biul. VNIORCh, No 3: 9-10 (In Russian).
11. Kryžanovskij S.G., 1953. Properties of the mature eggs of the teleost fishes. Vopr. Ich-tiol., No 1: 37-62 (In Russian).
12. Kokurewicz B., 1971: The thermal conditions and spawning of some species of fishes. IRŚ Olsztyn, Zakład Upowszechniania Postępu, 47; 1-16 (In Polish).
13. Korzelecka A., A. Winnicki, 1998: Peculiarities of embryogenesis in Scardinus erythro-phtalmus L. Electronic Journal of Polish Agricultural Universities, Annual I, vol. 1, http://www.ejpau.media.pl/Journal/english/series/volume1/fisheries/art.-02:1-8.html.
14. Kozikowska Z., 1961: Influence of the environment on morphology and biology of fish. Cisco, perch-selected elements. Ekol. Pol., A, 9: 541-678 (In Polish).
15. Kucharczyk D., R. Kujawa, A. Mamcarz, A. Skrzypczak, E. Wyszomirska, 1997: The perch's spawning in hatchery. Komunikaty Rybackie, No 2: 7-8 (In Polish).
16. Nikolski G., 1970: Detailed ichthyology. PWRiL, Warszawa (In Polish).

17. Mežnin F.I., 1978: Development of gonad's during early stages of the perch's (Perca flu-wiatilis L) ontogenesis. Vopr. Ichtiol., 18, 1: 84-100 (In Russian).
18. Retzius M.G., 1912: Zur Kennentnis der Hüllen und besonders des Follikelepithels an den Eiern der Wirbeltire. I Bei den Fischen. Biol. Untersuch., (n.s.) 17: 5-29.
19. Rodziewicz M., 1996: Biostructural conditions of ovulation and spawning of the perch. Praca magisterska, AR Szczecin (In Polish).
20. Rolik H., J.M Rembiszewski 1987: Fishes and Cyclostomes. PWN, Warszawa (In Polish).
21. Suvorov E., 1954: The base of ichthyology. PWN, Warszawa (In Polish).
22. Švátora M., 1981: Reproduction, genetics and hybridisation of fishes. In: Lampreys (Pet-romyzontes) and fishes (Osteichthyes) [Baruš V., O. Oliva., 1995 (ed.)] Academia, Praha, vol. 2 (In Czech)
23. Terlecki J., 1991: Perch (Perca fluviatilis L.). In: Freshwater fishes of Poland [M. Bryli-ńska ed]. PWN, Warszawa (In Polish).
24. Thomopoulos A., 1953: Sur l'oeuf de Perca fluviatilis L.. Bull. Soc. Zool. France, 78: 106-114.
25. Treasurer J.W., F.G.T. Hollyday, 1981: Some aspects of the reproductive biology of perch Perca fluviatilis L. A histological description of the reproductive cycle. J. Fish Biol., 18: 359-376.
26. Winnicki A., 1967: Plasticity and hardening of the rainbow trout egg membranes. Bull. Acad. Pol. Sci., Cl. II, 15 (12): 779-783.
27. Winnicki A., K. Formicki, A. Korzelecka, 1995: The spatial structure of the jelly-like protecting eggs after their evacuation from the perch's ovary. XVI Zjazd PTZool., Łódź. Streszczenia referatów: 179 (In Polish).
28. Winnicki A., A. Korzelecka, 1997: Morphomechanical aspects of the development of the bleak (Alburnus alburnus L.)]. Acta Ichth. Piscat., 27, 2: 17-27.
29. Winnicki A., A. Korzelecka, A. Tański, 1998: New data on breeding and early ontogene-sis of three-spined stickleback (Gasterosteus aculeatus L.). Arch. Ryb. Pol., 6, 1: 115-122.
30. Wintrebert P., 1926: L'eclosion de la Perche (Perca fluviatilis). C. R. Hebd. Soc. Biol., 2.
31. Załachowski W., 1997: Fishes. PWN, Warszawa (In Polish).
32. Żuromska H., 1961: Growth of perch (Perca fluviatilis L.) from lakes of Węgorzewo area. Rocz. Nauk Rol., B, 77: 603-639 (In Polish).

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