ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XXVII, Fasc. 2 Szczecin 1997

Aleksander *WINNICKI*, Agata *KORZELECKA*

Fish embryology

MORPHOMECHANICAL ASPECTS OF THE DEVELOPMENT OF THE BLEAK (*ALBURNUS ALBURNUS* L.)

MORFOMECHANICZNE ASPEKTY ROZWOJU UKLEI (*ALBURNUS ALBURNUS* L.)

Department of Anatomy and Embryology of Fish, Agricultural University of Szczecin, Poland

Morphomechanical study on the changes taking place in the course of the embryonic development of the bleak (*Alburnus alburnus* L.) conducted on the live fish in vertical and horizontal light beams, revealed that the oocyte (yolk mass) occupies some 30% of the entire egg's volume, while the re-maining 70% is occupied by the perivitelline space.

The blastodisc and subsequently the embryo and the larva before the hatching always take up lateral position in the egg. No lipid droplets (structural fat), commonly occurring in the eggs of the other teleost fishes are present in the yolk mass of the bleak eggs.

Biological justification of the above extraordinary phe-nomena as well as their adaptive importance is discussed.

INTRODUCTION

Distribution of the bleak on the entire European sub-continent has been well known and described (Berg 1946; Baruš and Oliva 1995). It inhabits not only open and secluded inland waters (Szczerbowski 1993), brackish areas of the Baltic Sea, but also the maritime estuaries (Młyniec 1986; Baruš and Oliva 1995).

The biology of this fish has also been well known. It matures in 2nd or 3rd year of life (Nikolskij 1950; Krzykawski 1968; Baruš and Oliva 1995). It spawns 2 or 3 times laying a total of 3000 up to 10 500 eggs measuring 1.5–1.9 mm (Kugel 1942). The bleak is an active fish, particularly sensitive for oxygen deficiencies in the water (Starmach et al. 1976). Consequently it roams in the pelagic zone of lakes (Szczerbowski 1993). For its reproduction, in this number also spawning, it selects the coastal areas (shoals covered with water vegetation protruding above the surface). It deposits its eggs on living plants or on plant debris to which the eggs instantly adhere strongly. The spawning takes place at the time of well-pronounced surf, which is linked to intensive wave activity.

The available information on the development of the bleak is limited to the pre-spawning period, pre-embryonic phase (Richard and Kestement 1996), and to the latter period of ontogenesis, including the larval stages. The latter are relatively well known (Krzykawski 1968). The embryogenesis, however, has not been hitherto studied except for the number of the eggs deposited, their dimensions, duration of the embryonic development (Młyniec 1986; Szczerbowski 1993).

The above-mentioned voids in the literature, combined with the promising, preliminary observation conducted last year, prompted us to do more-detailed study on the morphomechanical phenomena occurring in the course of the embryonic development of this fish. We also undertook the challenge of explaining its biological justification. A favourable circumstance in the present study was a new methodology developed, enabling constant monitoring and recording structural changes in vertical and horizontal light beams (Winnicki et al. 1997). The above feature made possible to record and 3-D localisation of the newly formed structures of the embryo, throughout the entire period of embryogenesis from the cleavage to the hatching.

MATERIAL AND METHODS

The study was conducted in June and July 1997 in the village of Izdebno, near Sieraków in a fish farm situated by lake Krzemień and owned by Mr. Henryk Bak.

The roe of the bleak (*Alburnus alburnus* L.) was collected on evenings from artificial substrate (small branches of juniper placed in the spawning areas—between coastal reeds) at the depth of 20–30 cm at the time of well pronounced surf and intensive wave activity. The substrate was taken out of the water every 15 minutes. The roe acquired was swollen, almost totally fertilised (98%) and at the stage of two blastomeres.

The eggs were gently detached from the juniper leaves and transferred: initially to Petri dishes and subsequently to custom-made plastic chambers. The latter were 80-mm long, 203-mm wide and were equipped with specially designed shelves. The chambers were attached with their flat surface to the stage of a horizontally positioned microscope. This allowed observations in a horizontal beam of light. The other, vertically positioned, microscope was used to observe eggs in small Petri dishes.

Both vertical and horizontal views permitted observation of even very small structures under 100× magnification (like single blood cells in the last phase of embryogenesis) and monitoring the dynamics of changes in the spatial distribution of the newly formed structures of an embryo and the mechanics of this changes.

The microscopes used (Biolar) were equipped with special objectives (Nikon 2×) and a digital camera (CCD). They provided sufficient resolution and quality of the picture, previewed on a Sony monitor and recorded on a VCR. It (the instant preview) enabled prompt slowing down of the tape where it was necessary and even bringing it to a complete stop where more detailed analysis was needed. It also enabled saving digitised pictures in a computer disc.

The temperature of the water (taken from the lake—15-m distance) was identical as that on the spawning ground (22–25°C).

The aim of the present study was to study the embryonic development including:

- Measurements of the egg and the yolk mass giving information on the size and shape of the perivitelline space and its volume.
- Shape and size of the blastodisc, morula, and blastula.
- Spatial distribution of the structures of the embryo formed on the surface of the yolk ball.
- Shape changes of the yolk mass in the late phase of the embryogenesis.
- Hatching mechanism of the larvae well as the associated dynamic and structural phenomena.

RESULTS

The percentage of fertilised eggs both on the artificial substrate and on the plant debris (even on the pieces of foil and paper) in the spawning ground was very high amounting to 95–99%.

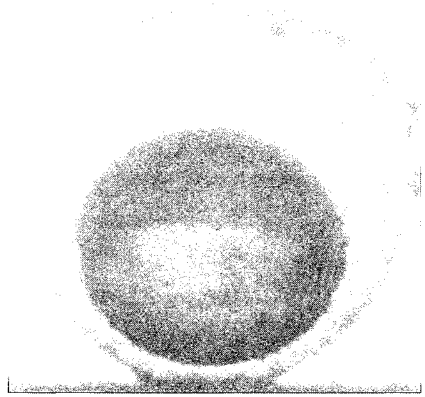


Fig. 1. Flattening of the egg and yolk mass (lateral view)

Length of the incubation period, calculated in degree-days (D°) and for higher accuracy in degree-hours (h°) (at the temperature of 20–23°C) was 58 D° or 1410 h°.

Egg swelling (intake of the water by the eggs after activation) was very quick and as early as after 15 minutes it could be observed that the process was terminated.

Chorions are slightly corrugated at the time of their deposition into water. Along with the water intake they become straighten and tense.

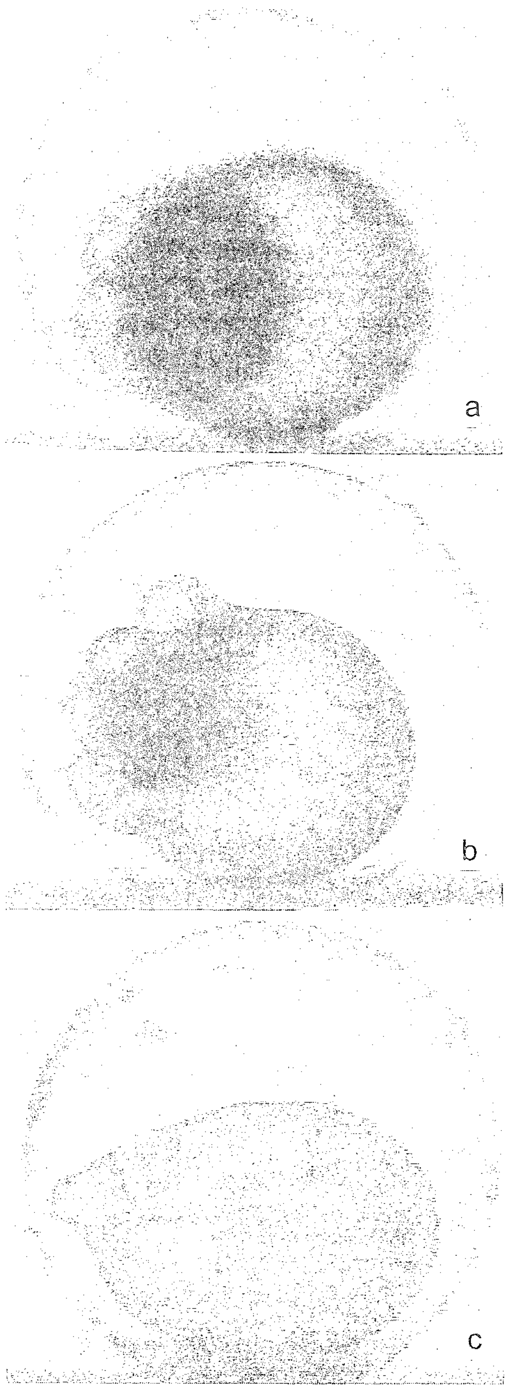


Fig. 2. Lateral arrangement of the embryo structures in the egg of the bleak a) 2-balstomere stage; b) 4-blastomere stage; c) fully-formed embryo (lateral view)

Shape of eggs is spherical, although slight flattening can be observed (egg height is not equal its horizontal diameter).

Egg size. The horizontal diameter is from 1.52 to 1.88 mm (1.59 mm in average); vertical diameter is from 1.40 to 1.60 (1.50 mm in average). Consequently the flattening (h÷l ratio) is 0.94.

The egg (yolk ball) has spherical shape (Fig. 1) and it is more flattened (h÷l = 0.89) than the entire egg.

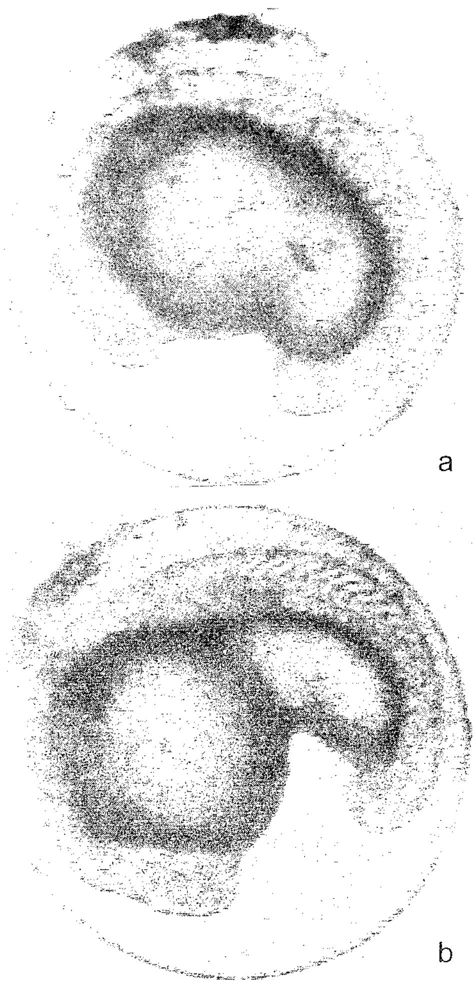
Lipid droplets, considered to be a common structural element of the eggs of the bony fishes are not present in the eggs of the bleak!

Blastodisc—of substantial size occupies lateral position. Such position is also assumed later by the blastula, morula, and the embryo until the time of hatch (Fig. 2).

Perivitelline space—of substantial size. Size of the entire egg of the bleak is 1.88 mm^3 and the size of the yolk mass is as little as 0.50 mm^3. Consequently the volume of the perivitelline fluid is 1.38 mm^3 and it constitutes 73.4% of the entire egg volume. The yolk mass occupies the remaining 26.6% of space.

After 288 h° traces of the embryo become visible. After 391 h° the head and the primordia of the miomeres can be seen. A little bit earlier the caudal part of the embryo becomes detached from the yolk vesicle.

At the same time changes in the shape of the yolk vesicle occur. The latter initially spherical becomes elon-



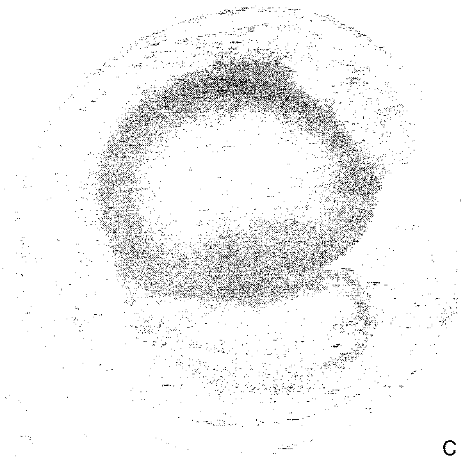


Fig. 3. Division of the yolk vesicle into two (anterior and posterior) uneven parts as a results of ectoplasm contractiona) beginning; b) intermediate phase; c) completion of the division (top view)

gated due to contraction of the ectoderm (primordia of the skin covering the yolk ball). The narrowing quickly occurs in the posterior part of the yolk mass leading to the change of shape within 1–2 hours. Now the yolk mass consists of two sections: anterior one consisting of some 3/4 of the weight and the posterior representing some 1/4 of the weight (Fig. 3). The narrowing is very distinct and it persists where, as later determined, the anus will form—until the moment of hatching.

In the meantime, both compartments of the yolk vesicle change their shape from spherical, through oval to elongated (almost cylindrical) by the end of embryogenesis, so the newly hatched larva has slim shape, without large, bulky "luggage" like in the other teleost fishes (Fig. 4).

The embryos in the ova grow very quickly, become elongated (particularly their caudal section and they wrap themselves around the yolk vesicle touching with their back the inside of the chorion in the equatorial plane (!). Immediately

Fig. 4. Anterior part of the newly hatched larva with elongated yolk vesicle in the final phase of resorption

before hatching the end of tail is located near the head (Fig. 5). The embryos are active. Initially their movements are rare (8 per minute). By the end of the incubation the intensity and the frequency of the wavy contractions of the body increase and are estimated for 20–25 per minute. The movements of the embryo initially single, later become short series of contractions separated by intervals (up to 10 seconds).

After o series of strong movements, in the calm period the body (torso and tail) rests in the lateral position and the embryo tightly touches with its head and back (as if sprung back) the inner side of the chorion.

Immediately before the hatching the embryo assumes interesting spatial position. It is bent (at the earlier observed constriction point of the yolk vesicle) at the angle of 90° in such a way, that relatively straight body, aligned horizontally, takes up a section equal 1/3 of the internal diameter of the egg, while the caudal part burdened with the lighter vesicle—is distinctly arc-like bent as far as to the head (Fig. 6). At the time of the perforation of the chorion, the caudal part apparently cuts the chorion in its equatorial area and the newly hatched embryo instantly assumes straight position. It takes a few additional movements

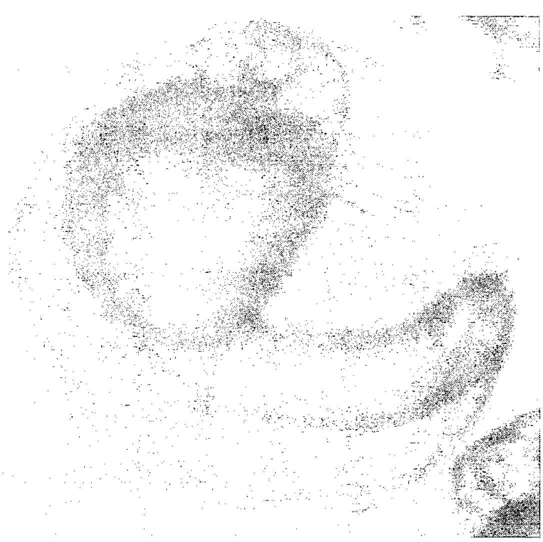


Fig. 5. Arrangement of the embryo in the egg before the hatching

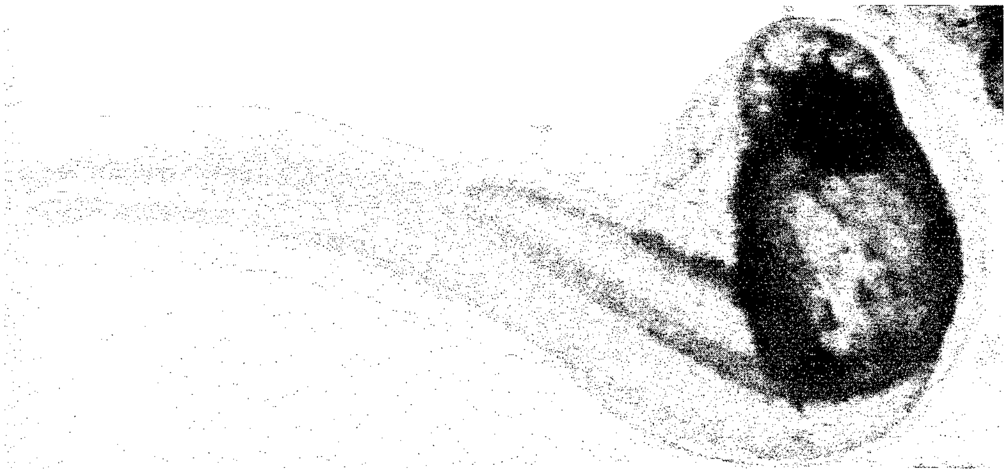


Fig. 6. Characteristically bent position of the hatching larva of the bleak

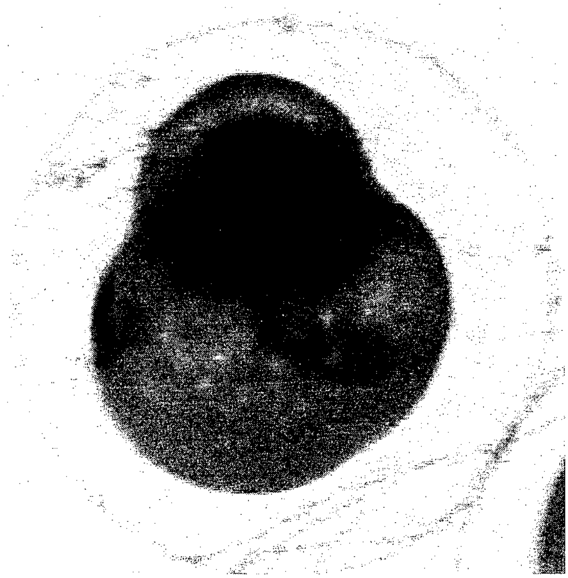


Fig. 7. Particularly distinct convexity of the blastodisc (top view)

for a larva to free itself completely from the envelopes, and after a brief rest it can efficiently move and swim (Fig. 4).

The larvae of the bleak are not "inoculated" at the moment of hatching, which means, that their optic cups lack pigment. Otherwise their eyes are morphologically perfectly developed and all elements of the optic refraction system are present. The larvae react to light. There are no melanophores on the head and the body. Newly hatched larvae measured in average 4.6 mm including 0.45 mm of the head.

DISCUSSION

The results acquired in the present work yielded, among previously known details (like the way and place of depositing eggs on the substrate, size and shape of eggs, incubation time) also a number of new (as we think) data concerning the embryonic development of the bleak, dynamics of the structural changes, and the morpho-physiological phenomena associated with these changes. The latter phenomena need separate description, explanation, and finding cause-result relationships taking place between the spatial distribution of the structures being formed and their mechanical role and physiological functions in a wider background of a species adaptation to the reproduction—the act of ultimate importance.

The first thing that captures attention is the size of the perivitelline space, constituting as much as 73.4% of the egg volume. The perivitelline space on the upper pole constitutes almost half of the egg height, so (taking into account exceptionally well pronounced convexity of the blastodisc (Fig. 7) it is much too big to protect it (disc) from all possible mechanical injuries. Consequently the perivitelline space cannot play a role in mechanical protection of the embryo against injuries.

The second, not less interesting phenomenon is a complete absence of lipid droplets in the yolk mass (inside and on the surface). Lipid compounds present in a lipid droplet in the state of dispersion play only their basic energetic role. They do not play any additional mechanical role as it occurs in the embryogenesis of the majority of teleost fishes consisting in elevating the blastodisc upwards (freshwater fishes) or elevating the entire yolk mass (marine fishes). The structural fat in a form of droplets does not exist in the bleak. If it would—and was connected with the blastodisc—it would elevate it upwards. This in turn would be unfavourable for the embryo being formed, because quickly growing and becoming longer embryo (at the moment of hatching almost 5 times longer, than the diameter of the yolk ball) taking up the upper position in the egg, should fold itself beneath the ball. This would be difficult because the ball settles on the bottom of the egg.

The lateral position enables the embryo to wind around the ball without major obstacles if it is to take up position in the equatorial plane.

Such specific demand for undisturbed development and growth resulted in such a voluminous perivitelline space in the bleak. Presence of structural fat under the blastodisc (embryo) would make impossible assuming such position so the species “got rid of it”.

Interesting phenomenon of dividing the yolk vesicle into two compartments (anterior—bigger; posterior—smaller) is easy to explain. By the end of the embryogenesis, the embryo is almost two times longer than the egg diameter. Consequently it has to assume arc-like position. The big head and the trunk ventrally connected with the yolk vesicle make such winding impossible. The narrowing is then an act allowing subsequent bending of the body which would be impossible with a uniform, undivided vesicle.

Lack of the pigment in the eyes and the skin of the newly hatched larva should be treated as adaptive camouflage protecting it against earlier hatched, feeding young fry of the predators (perch, pike, ruffe).

Taking into account all of the above mentioned differences observed during the reproduction, occurring in the embryonic period and typical for the bleak morphomechnical phenomena it is evident that they are not coincidental but they constitute a logical chain of causes and results responsible for the continuity of the species. Choosing the time and place of reproduction is not a matter of coincidence.

Relatively low individual fecundity (few hundred to few thousand eggs) should be associated with relatively high water temperature (shortening the embryonic development) and good oxygenation. The former demand, however, can be problematic in the case of bleak, as it is very often associated, in our climatic conditions, with algal blooms causing destabilisation in diel oxygen content. The only feasible stabilising factor can be a wave activity. It causes pronounced surf area on the windward side of the body of water. The surf contributes also to protection of the fine-grain roe attached (glued) to the substrate—by whirls and turbidity.

Portioned spawning repeated 2 or 3 times provides further safety. Sufficient numbers of the offspring would end up in the water even if the other spawning attempts fail, as the result of various unpredictable causes.

Broad perivitelline space provides conditions for unrestricted movements for an embryo. This in turn promotes good mixing of the perivitelline fluid, allowing O_2 to reach the surface of the embryo. This would explain the series of rapid movements observed.

At the intervals between the movements the hatching enzyme is being applied on the inner side of the chorion at the places where it is firmly touched by the head and tail (Wintrebert 1912; Hayes 1942; Baburina and Buznikov 1957; Winnicki 1970). In the consequence the chorion is digested in the strictly defined plain, which is followed by its perforation and tearing off by the fully formed larva.

The achievement of the latter goal is indirectly promoted by the described earlier specific shape of the yolk vesicle. The newly hatched larva, due to its slim shape, is ready for intensive swimming in the water.

Lack of the pigment in the eyeballs and on the body makes the larva almost invisible or totally invisible for predators.

CONCLUSION

1. Perivitelline fluid in an activated eggs of the bleak constitutes 70% of the volume, while the yolk ball—less than 30%.
2. The blastodisc and all subsequent developmental stages of the embryo up to the fully formed larva before the hatching always assume lateral position in the egg, which is permitted by the voluminous perivitelline space.
3. Eggs of the bleak completely lack "structural" fat in the form of lipid droplets. Such droplets, in the eggs of other fishes stabilise the blastodisc on the upper pole of the egg (freshwater fishes) or the lower pole (marine fishes).
4. The yolk mass in the middle of the developmental period of the bleak divides into two parts, the proximal and the caudal ones, which enabled the larva to assume bent position and fitting into the space limited by the chorion.

REFERENCES

Baburina E. A., G. A. Buznikow, 1957: Železy vyluplenija embrionov kubanskogo rybca kak istočnik gialuronidazy i fermienta vyluplenija [Hatching glands of the embryos of the zanthe as the source of gialuronidase and the hatching enzyme]. Dokl. AN SSSR, **113**: 1387–1391. (In Russian).

Baruš V., O. Oliva, 1995: Michulovci (Petromyzontes) a ryby (Osteichthyes) [Lampreys (Petromyzontes) and fishes (Osteichthyes)]. Vol. 2, Academia, Praha. (In Czech).

Berg L. S., 1949: Ryby presnych vod SSSR i sopredelnych stron [Freshwater fishes of the waters of the U.S.S.R. and the neighboring countries]. Izd. AN SSSR. Moskva–Leningrad. (In Russian).

Bourdin J., 1926: Le mécanisme de I'éclosion chez les Téléostéens. I. Etude biologique et anatomique. C.R. Soc. Biol., **95**: 1149–1151.

Hayes F. R., 1942: The hatching mechanism of salmon eggs. J. Exp. Zool., **83**: 357–373.

Krzykawski S., 1968: Obserwacje nad wylęgiem uklei (*Alburnus alburnus* L.) w jeziorze Legińskim [Observations on the hatching of the bleak (*Alburnus alburnus* L.) in lake Legińskie]. Zesz. Nauk. WSR Olsztyn: **24**, 649: 613–621. (In Polish).

Kugel G., 1942: Untersuchungen über den Uklei. Zietschr. f. Fischerei: **15**, 2: 225–262.

Młyniec B., 1991: Ukleja (*Alburnus alburnus* L.) [The bleak (*Alburnus alburnus* L.)]. In: M. Brylińska (ed.) Ryby słodkowodne Polski [Freshwater fishes of Poland]. PWN, Warszawa. (In Polish).

Rinchard J., P. Kestemont, 1996: Comparative study of reproductive biology in single and multiple spawner cyprinid fish. I. Morphological and histological featurs., J. Fish. Biol., **49**, 5: 883–899.

Starmach K., S. Wróbel, K. Pasternak, 1976: Hydrobiologia [Hydrobiology]. PWN, Warszawa. (In Polish).

Szczerbowski J., 1993: Rybactwo sródlądowe [Inland fisheries]. IRŚ, Olsztyn. (In Polish).

Winnicki A., A. Korzelecka, A. Tański, 1997: New data on the reproduction of the three-spined stickleback (*Gasetrosteus aculeatus* L.). Arch. Ryb. Pol., (In press).

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.

Wintrebert P., 1912: Le mécanisme de l'eclosion chez 1a truite arc-en-ciel. C. R. Soc. Biol., **72**: 724–727.

Aleksander *WINNICKI,* Agata *KORZELECKA*

MORFOMECHANICZNE ASPEKTY ROZWOJU UKLEI (*ALBURNUS ALBURNUS* L.)

STRESZCZENIE

Prześledzono przebieg rozwoju zarodkowego uklei (*Alburnus alburnus* L.) od pierwszego podziału (dwa blastomery) do pierwszych minut po wylęgu. W pionowej i poziomej wiązce światła pod mikroskopem (powiększenie 100×) sprzężonym z kamerą cyfrową CCD, monitorem, magnetowidem oraz komputerem, badano na żywo kształty, wymiary i przestrzenne rozmieszczenie wewnątrz jajowych struktur oraz dynamikę zmian.

Stwierdzono, że jaja uklei (kule żółtkowe) pozbawione są tłuszczu strukturalnego (w postaci pęcherzyków tłuszczowych) zapewniającego u innych ryb kostnoszkieletowych „pływalność" tarczki zarodkowej, ustawiającej ją w pozycji górnej jaja.

Tarczka zarodkowa, a zatem zarodek i dojrzała larwa tuż przed wylęgiem zajmuje zawsze pozycję boczną w jaju.

Stwierdzono również w okresie embriogenezy wyraźny podział kuli żółtkowej (woreczka żółtkowego) na dwa oddziały: proksymalny – większy i kaudalny – mniejszy oraz charakterystyczny przestrzennie kształt larwy przed wykluciem (ostre, pod kątem 90° zgięcie tułowia w linii odbytu.

Dyskutowany jest w pracy sens biologiczny i adaptacyjny tych zjawisk w tak znacznym stopniu odbiegających od przebiegu embriogenezy innych ryb kostnoszkieletowych.

Received: 5 November 1997

Authors' address

Aleksander Winnicki PhD DSc ProfTit
Agata Korzelecka MSc
Department of Fish Anatomy and Embryology
Agricultural University of Szczecin
Kazimierza Królewicza 4, 71-550 Szczecin, Poland