

EMBRYOGENESIS OF THE STINGING CATFISH, *HETEROPNEUSTES FOSSILIS* (ACTINOPTERYGII: SILURIFORMES: HETEROPNEUSTIDAE)

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Background. The stinging catfish, *Heteropneustes fossilis* (Bloch, 1794), has recently raised interest among fish farmers, ornamental fish keepers, and pathologists. Its natural populations are threatened due to habitat loss and high fishing pressure. A number of factors may influence the reproductive success of this. The aim of this study was to assess the effect of one of such factors—the water hardness—on the course of the embryogenesis, the structure of the egg shell, the general morphology, and the behaviour of the hatched larvae.

Materials and Methods. The fertilised eggs were incubated at a constant temperature of $23 \pm 0.2^\circ\text{C}$ in water of different hardness: 0°GH (soft), 9°GH (moderately soft), 18°GH (moderately hard). Egg membranes of activated eggs were viewed under a scanning electron microscope. Also egg membranes strength and egg deformations were determined 1.5 h after fertilisation. Images of eggs and newly hatched larvae, recorded with the observation sets described above, were measured and analysed.

Results. The study showed the eggs were surrounded by thin, translucent, $\sim 5 \mu\text{m}$ thick membranes equipped with numerous outgrowths on the external surface and porous on the inner side. The following observations were made within 828 h° (degree-hour) at $23 \pm 0.2^\circ\text{C}$: the highest volume of eggs ($1.16 \pm 0.092 \text{ mm}^3$) was typical for eggs incubated in soft water (0°GH), while the lowest egg volume ($0.99 \pm 0.113 \text{ mm}^3$) was recorded in eggs incubated in moderately soft water (9°GH); yolks spheres were greenish in colour, the embryonic disc and the embryo itself being reddish; the embryo performed diverse movements (quasi-peristaltic, cardiac muscle contractions); after few hours of hatching, the pigments developed resulting in colour appearance; club-like primordial barbels were formed 24 h after hatching; the larvae commenced feeding on day 3 post hatching.

Conclusion. Water hardness influences embryonic and larval development and the effects may be diverse. Low water hardness is recommended for egg incubation. However, the situation changed when the egg membrane protection ceased to exist at hatching. Increasing water hardness a few hours prior to the expected hatching time prevents larval deformation.

Keywords: stinging catfish, *Heteropneustes fossilis*, embryogenesis, eggs, embryonic motorics, embryonic morphometry, water hardness

INTRODUCTION

The stinging catfish, *Heteropneustes fossilis* (Bloch, 1794), known also as the liver catfish or the fossil catfish, has increasingly attracted attention of fishermen and ornamental fish keepers (Robins et al. 1991, Kahl et al. 1997). In contrast to Europe, the stinging catfish is of high commercial value in Asia. However, in Europe, the species has recently gained interest of fish farmers, particularly those operating in discharge canals of power plant cool-

ing waters. The global interest in the species is understandable, because—from the Far to the Middle East—the species is a valued food product due to its tasty, high quality meat rich in protein and low in fat (22.8% and 0.6%, respectively); in addition, the meat is a good source of dietary iron (226 mg per 100 g of body weight). Those qualities make it a food recommended as a diet component for patients recovering from illnesses (Pandey et al. 2001).

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In addition to the species' value for fish farmers, the stinging catfish—due to some structural and physiological peculiarities—has become interesting also for the ornamental fish trade. Recently, the species has become a study object for pathologists focusing on transmittable zoonoses.

The stinging catfish occurs naturally in tropical waters of south Asia, general in bodies of water abundant in vegetation, oxygen-depleted due to decomposition of the plant material, and heated by the sun throughout the water column (Munshi et al. 1976, Pandey 1978, Pethiyagoda 1991, Kahl et al. 1997, Jayaram 1999).

In its native habitat, the stinging catfish attains 70 cm of total length. The sexual dimorphism is distinct; the males are smaller and have a clearly pointed genital papilla. The female fecundity is body size-dependent and varies from 3 thousand to 45 thousand eggs (Wheeler 1977, Baensch and Riehl 1985, Rahman 1989).

Under natural conditions, the stinging catfish reproduces from July to August. The reproductive activity is temperature dependent, with 22°C being the reproductive temperature optimum. The eggs are tended by both parents who perform fanning movements with their fins to refresh the water around the eggs (Sheel and Singh 1981, Saxena and Sandhu 1994). Egg incubation was found to take from 18 to 24 h, depending on the water temperature. The knowledge on embryonic development of the stinging catfish has been fragmentary, focusing on gamete morphology and duration of the embryogenesis in relation to some environmental factors, such as the water temperature and the oxygen content (Altman and Dittmer 1962, Thakur et al. 1974). The newly hatched larvae, on average 2.72 mm total length, carry a fairly large yolk sac which becomes completely resorbed on the fourth day after hatching. The larvae commence feeding on their third day after hatching (Singh et al. 1989, Roy and Pal 1986, Alok et al. 1993).

One of the factors having meaningful effect on the embryonic development of this fish is the water hardness. *Clarias gariepinus* incubated in hard water (more than 200 mg · L⁻¹ of CaCO₃) had more malformed larvae (in relation to control). Also soft water (0–10 mg · L⁻¹ of CaCO₃) has not been considered as a potential optimal medium for developing embryos (Molokwu and Okpokwasili 2002).

Taking into the consideration the results of the above-mentioned studies it would be interesting to learn about the morphogenesis of this fish. This could help to understand the biological nature of the changes observed.

The presently reported study describes the structure of the egg membranes (from fertilization to hatching) and the embryo morphology, as well as the behaviour of the larvae from hatching until complete yolk sac resorption. Particular attention was paid to the effects of water hardness on the course of the embryogenesis. The acquired knowledge may translate into practical means affecting cultivation procedures employed by fish growers.

MATERIALS AND METHODS

The study was carried out in June 2007 at the con-

trolled-temperature laboratory of the Division of Fish Anatomy, Hydrobiology, and Biotechnology of Reproduction, West Pomeranian University of Technology in Szczecin.

Eggs and sperm were collected from three females measuring 17–25 cm (TL) and four males (10–18 cm TL), respectively. Gametes were collected from spawners which had been injected twice with carp pituitaries (a total amount of 5 mg). The second injection was applied 8 h after the first. Ovulation occurred 12 h after the second injection. The eggs were fertilised using the dry method. The sperm, collected from the genital papilla with a syringe equipped with elastic tubing, was diluted in the immobilising fluid consisting of 200 mM NaCl (Linhart et al. 1987, Linhart et al. 2004) and was used to fertilise the eggs. The fertilised eggs were transferred to rectangular baskets constituting a part of a hydromechanical device, ensuring constant aeration and water temperature during the incubation in 60 L aquaria. To prevent lumping and to provide enough oxygen, the eggs from three females were arranged separately in baskets in a single layer.

The eggs were incubated at 23 ± 0.2°C in water of different hardness: soft (0°GH = 0 mg CaCO₃ · L⁻¹), moderately soft (9°GH = 160.7 mg CaCO₃ · L⁻¹), and moderately hard (18°GH = 321.5 mg CaCO₃ · L⁻¹) (°GH = Degrees of General Hardness).

Egg membranes of activated eggs were fixed in buffered 4% formaldehyde, dehydrated in gradually more concentrated solutions of ethanol and acetone, and dried in critical point liquid CO₂. They were subsequently dusted and viewed under a scanning electron microscope (SEM) (Fei Quanta 200). The observations were photographically documented.

Embryonic development was recorded live with a set consisting of a light microscope (Nikon ECLIPSE TE 2000S) and a dissecting microscope (Nikon SMZ 1500) coupled with a microprocessor control (Trol-8100/9100) and a colour digital camera (Nikon DS Fi-1.), a screen (LG), a video recorder (JVC-HR-S7700), and a computer (PC). One set made it possible to view the egg from above, the other providing a side view of the developing embryo; the two observation sets, taken together, made it possible to record details of changes in spatial distribution of various structures in the developing embryo.

Duration of embryonic development was determined using thermal units—accumulated degree hours (h°), calculated as the sum of water temperature each hour during the development period. All types of motility in the developing eggs were recorded and subsequently analysed. The number of somatic movements and the number of heart contractions per minute were recorded and analysed and measured with the specialised Nikon Imaging System-Elements BR.

Egg membranes strength and egg deformations were determined 1.5 h after fertilisation, using a universal material testing machine (Zwick/Roell Z 2.5) with adjustable crosshead pressure. Simultaneous measurement of three parameters: height (mm), deformation (%),

and strength (g) began as the crosshead touched the egg surface. As the fertilisation cone began to form, diameters of the egg and the oocytes they contained were measured and, after averaging, the volumes (V) of the eggs were calculated. The precision of the apparatus was 0.001 g.

Immediately after hatching, the total larval length (TL), yolk sac height (h), and yolk sac length (l), were measured. The yolk sac volume (V) was calculated with the formula

$$V = \frac{\pi}{6} lh^2$$

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and the number of heart contractions per minute were recorded. Images of eggs and newly hatched larvae, recorded with the observation sets described above, were analysed and measured with the specialised Nikon Imaging System-Elements BR.

The data were processed with the Statistica® v.8.0 PL software. Each sample analysed consisted of 60 specimens.

The presently reported study has been approved by the Local Ethical Committee for Experiments on Animals in Szczecin (Regulation No. 8/2007 and Licence No. 4/2004).

RESULTS

The stinging catfish eggs had thin, transparent, 5 ± 0.2 μm -thick egg membranes which were covered by gelatinous material. On the outside, the membranes were outfitted with numerous processes; the internal surface of the membrane was porous (Fig. 1–4). The perivitelline space, formed within 40 min after activation, occupied mean

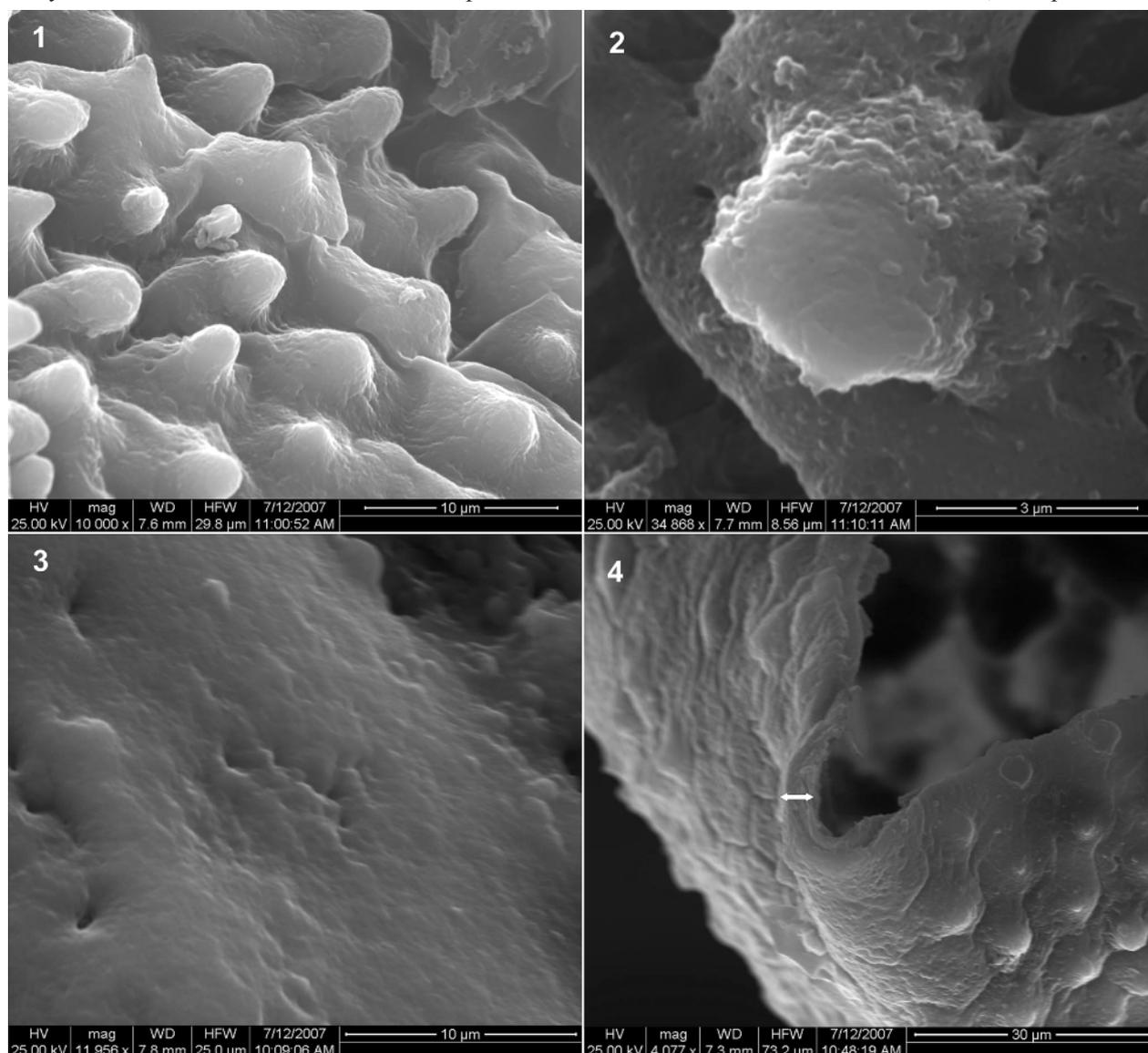


Fig. 1–4. Egg membrane of *Heteropneustes fossilis* (SEM micrographs); **Fig. 1.** Outer surface of egg membrane (10 000 \times); **Fig. 2.** The process contributing to the stickiness of the zona radiata externa; the nearby pits facilitate penetration of oxygen through the egg membrane (34 868 \times); **Fig. 3.** Inner surface of egg membrane (11 956 \times); **Fig. 4.** Cross-section of egg membrane (4077 \times)

48.94% \pm 0.04% of the egg volume (min. 38.11%, max. 54.39%) The diameters of a hydrated egg and an egg cell were 1.27 ± 0.04 and 1.03 ± 0.03 mm, respectively. The stinging catfish egg and egg cell sizes varied rather considerably, as the difference between the largest and the smallest egg was about 60%.

As shown by the data (Table 1), 1.5 h after fertilisation of the eggs, subjected to varying pressures, were deformed to a small extent only (32%), the egg membrane strength amounting to 2.5 g. Yolk spheres were green; the embryonic disc, which emerged during 30 min post activation, was reddish, as was the embryo itself. The stinging catfish eggs were devoid of structural lipids visible as droplets. As a result, the embryonic disc moved to the sides as much as the perivitelline slit size allowed.

The history of embryogenesis (from activation through hatching) and major concurrent structural changes are shown in Table 2 and Figs. 5–12 (the images were taken in the vertical light beam unless indicated otherwise). The course of the embryonic development was described based on the treatment with water of moderate hardness (9°GH).

Embryonic movements. Quasi-peristaltic movements: fairly intensive, proceeding only during yolk coverage; latitudinal shifting of an undulating groove on the yolk sphere surface causes the embryo to move within the perivitelline space (Figs. 11–12); the movement results in

mixing of the perivitelline fluid. No movement of this kind was observed after the blastopore closed.

Somatic movements were initially weak, intensifying with time; at the beginning, only the trunk muscles were moving, the remaining muscles undertaking movements later on.

Contractions of the heart (or rather of its primordia) appeared at 600 h°; initially rather slow (about 20 beats per min), their rate picked up gradually to reach about 80 beats per min just before hatching.

The embryos began to hatch at 720 h° and at 828 h°, more than 50% of the larvae were released from the eggs. The hatching terminated after 1104 h°. Most of the larvae exited the egg membranes tail first, although the head-first exit was fairly frequent as well. The newly hatched larvae had no pigment in the eye and on the body (Fig. 13). 24 h post hatch, the larvae show club-shaped primordia of the barbels. As soon as in a few hours after hatching, some pigment cells appeared on the head and on the skin fold surrounding the body. 36 hours post hatch, the pigment appeared in the eye (Fig. 14). On day 3 post hatch, the larvae commenced feeding (Fig. 15). The yolk sac was completely resorbed 4 days after hatching (Fig. 16).

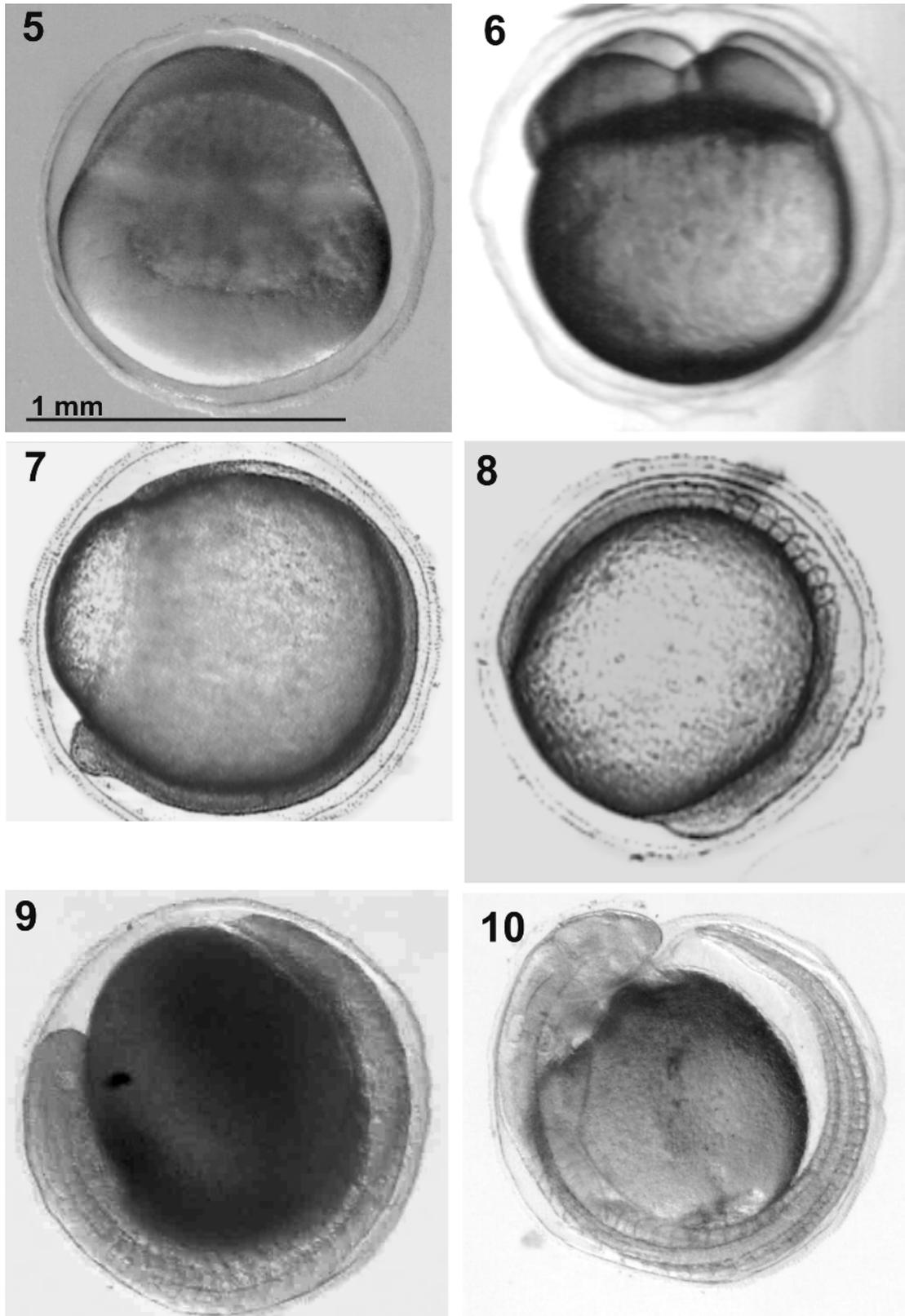
Effects of water hardness on the size of egg cell and eggs, the course of embryogenesis, and the larval size. Water absorption was at its fastest in the 0°GH water, the slowest absorption took place at 9°GH. The volume of

Table 1
Strength of egg membranes and egg deformations of *Heteropneustes fossilis* 1.5 h after fertilisation exposure in moderately soft water (9°GH)

	Strength of egg membranes		Egg elasticity [%]
	[g]	[N]	
$\bar{x} \pm SD$	2.553 ± 1.193	0.026 ± 0.012	32.067 ± 6.608
max	5.021	0.050	43.438
min	1.311	0.013	22.149

Table 2
The course of embryogenesis of *Heteropneustes fossilis* in time (degree hours d°) at $23 \pm 0.2^\circ\text{C}$ in moderately soft water (9°GH)

Developmental stage	Time from fertilisation [h°]
Formation of embryonic disc	11.5
Termination of swelling	15.3
2 blastomere stage	20
4 blastomere stage	27
8 blastomere stage	36
16 blastomere stage	42
Blastula	86.2
1/4 epiboly	130
3/4 epiboly	189
Blastopore closure: embryo with emerging cephalic part and 4 distinct myomeres	226
11 myomeres in the trunk; outlines of emerging myomeres in the trunk region; visible outlines of the emerging encephalon vesicles	328
Initial somatic movements (1–2 movements per minute)	476
Slow contractions of heart primordia (20 beats per minute)	543
Mass hatching (50% of larvae released from egg membranes)	770



Figs. 5–10. Stages of embryonic development of *Heteropneustes fossilis*; **Fig. 5.** Formation of perivitelline space after water imbuement and concentration of maternal cell material at the animal pole 30 min after fertilization embryonic disc 30 min. after fertilization; **Fig. 6.** Four-blastomere stage; **Fig. 7.** 80%–85% epiboly with the blastopore wide open. The yolk-sac syncytium shows still lapping cells at the fringes; **Fig. 8.** Development of the cephalic part; the somites are well developed along the latter part of the body axis but not fully reached the frontal region; The optic capsule is vaguely visible and the eye capsules are not yet completed **Fig. 9.** Formation of somites were already almost completed in the previous stage; visible outline of the eye and lens as well as the ophthalmic optic capsule; **Fig. 10.** Embryo at the moment of hatching

incubated eggs was related to water hardness as well: those eggs incubated at 0°GH were the largest (mean volume of $1.16 \pm 0.092 \text{ mm}^3$); the lowest volume ($0.99 \pm 0.113 \text{ mm}^3$) was shown by the eggs incubated at 9°GH. Although water hardness-related differences in egg volume were observed since the beginning of the experiment, significant differences between all the treatments began to be visible 78 h° after activation (Table 3).

The embryonic development was observed to take the longest at 18°GH: the mean duration was 828 h°, i.e., by as much as 58 h° longer than in the remaining treatments. The embryo survival rate at 0 and 9°GH was higher (about 80%) than that in the hardest water (18°GH)—54%.

The larvae hatched from eggs incubated at different water hardness levels differed in the volume of their yolk sacs and in the total body length. The longest ($3.584 \pm 0.219 \text{ mm}$) were the larvae that hatched from eggs developing at 18°GH, but at this treatment their yolk sacs (mean volume of $0.427 \pm 0.094 \text{ mm}^3$) were the smallest (Fig. 17). On the other hand, those larvae that developed from embryos exposed to 0°GH were the shortest ($3.409 \pm 0.133 \text{ mm}$), but carried the largest yolk sacs ($0.469 \pm 0.064 \text{ mm}^3$) (Fig. 18). The differences between the experimental treatments (water hardness) with respect to the mean larval length were significant.

Observations on the larvae developing in water of different hardness showed the number of larvae with swollen yolk sacs and deformed abdomens to increase with decreasing hardness.

DISCUSSION

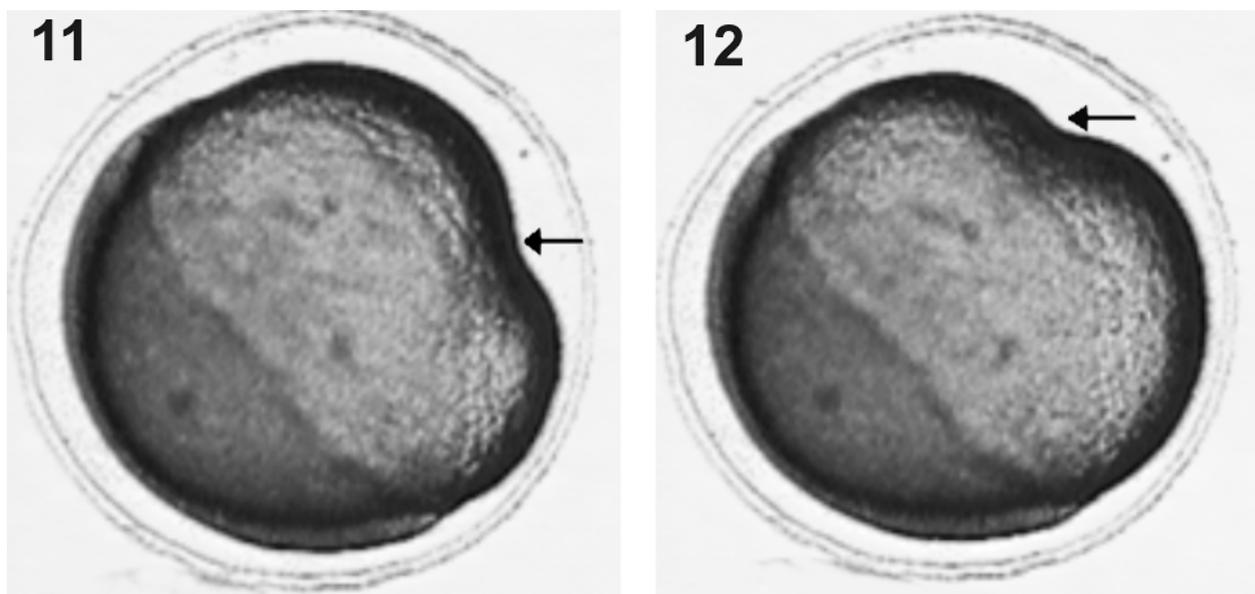
The results obtained illustrate structural changes taking place in the stinging catfish embryonic development from egg activation until early post-embryonic stage. The first peculiarity observed concerns the structure of the egg membrane, an egg component which ensures environmental stability to the embryo, the stability being crucial for the subsequent development (Thomopoulos 1953, Winnicki et al. 1970). The viscous layer surrounding the membrane allows the egg to attach to the substrate, thus protecting the egg from unfavourable conditions prevailing in the bottom mud. Attachment to the substrate facilitates brood protection and is a defence against microorganisms, which guarantees the permanence of the egg membrane itself (Riehl and Patzner 1998, Davenport et al. 1986, Morrison et al. 1999). At higher temperatures, required by the stinging catfish embryo to develop, activity of protozoans and bacteria is very high, which can occasionally result in total destruction of the viscous layer (Adams et al. 2003). On the other hand, the club-like processes on the

Table 3

Embryonic changes in the volume [mm^3] of oocytes and eggs of *Heteropneustes fossilis* incubated in water of different hardness ($n = 60$)

Time from fertilisation [h°]	Yolk sphere volume			Egg volume		
	Treatment			Treatment		
	0°GH	9°GH	18°GH	0°GH	9°GH	18°GH
7	0.641 ^a	0.673 ^a	0.693 ^a	1.203 ^b	1.040 ^a	1.082 ^a
21	0.493 ^a	0.500 ^a	0.525 ^a	1.160 ^b	0.997 ^a	1.048 ^a
78	0.501 ^a	0.499 ^a	0.576 ^b	1.145 ^c	0.978 ^a	1.078 ^b

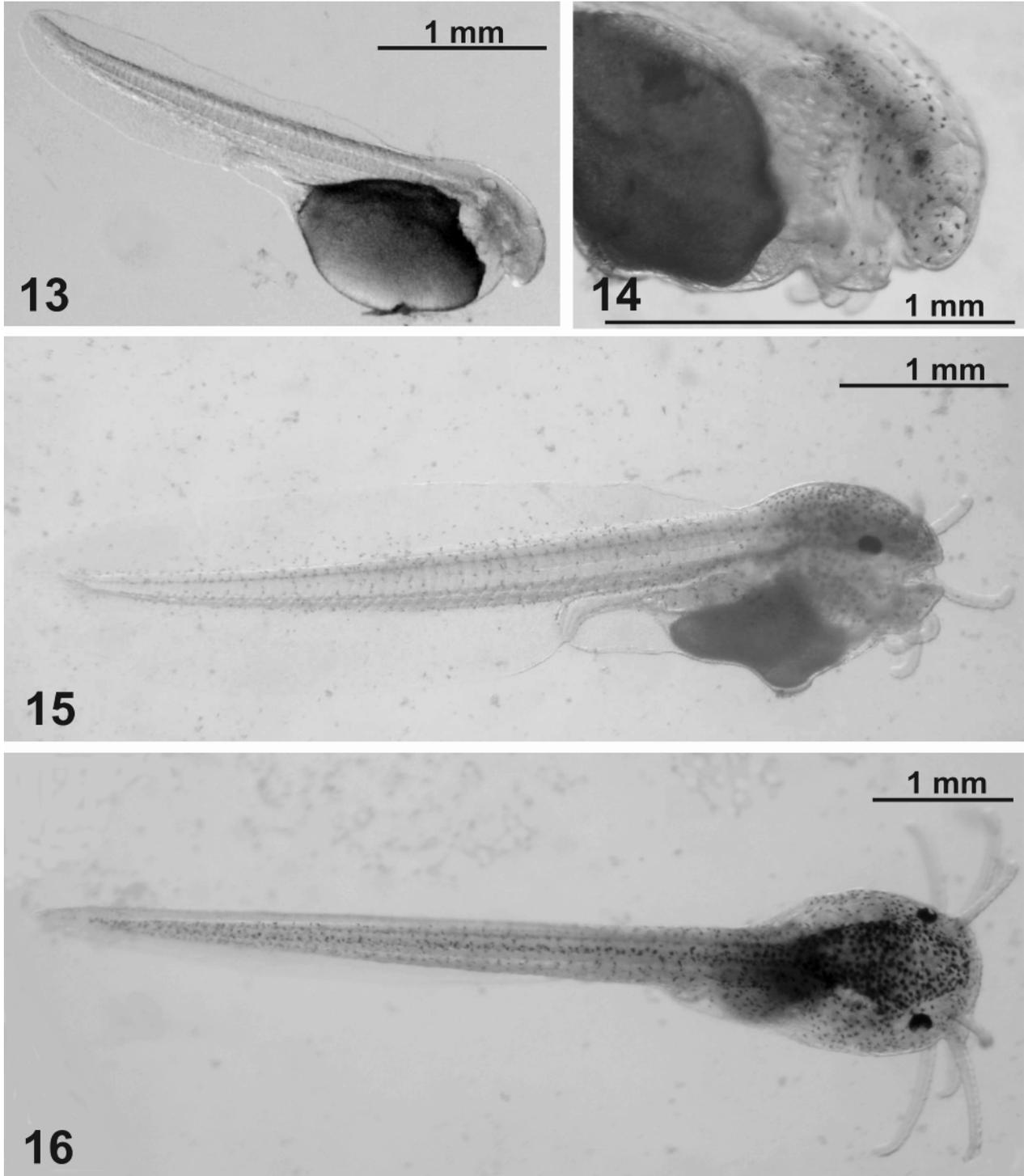
*values denoted with different letters in superscript are significantly different; Duncan's multiple range test ($P < 0.05$).



Figs. 11–12. The 1/2 epiboly stage in *Heteropneustes fossilis*; the moving quasi-peristaltic wave is visible

outer surface of the egg membrane ensure that, despite adherence to the substrate, the egg experiences some gas exchange because the water, flowing between the processes, rinses the entire egg surface. Similar processes are present on the inner surface of the egg shells of cyprinid fishes such as vimba bream or white bream (Szulc et al. 2008) which may be related to the similar environmental conditions favoured for egg development by those fish.

Another noteworthy peculiarity is the coloration of both the yolk spheres (greenish) and the embryonic disc and the embryo itself (reddish). The translucent, greenish-coloured eggs blend with the background consisting of plants, abundant in swamps, ponds, and marshes, i.e., in habitats where the species breeds. Although the eggs are protected by the parent fish, this camouflage of a kind serves as an additional protection from predators; thus it increases the survival rate,



Figs. 13–16. Larvae of *Heteropneustes fossilis*; **Fig. 13.** A newly hatched larva; **Fig. 14.** Anterior part of a 36-h-old individual with developed barbels; **Fig. 15.** Progressive resorption of the yolk sac in a larva; **Fig. 16.** A feeding larva

and—perhaps—facilitates the parental care. The lack of pigment, both in the eyes and on the body, is a frequent natural camouflage whereby a fish larva can disappear among the vegetation until it loses its burden of the yolk sac (Winnicki and Korzelecka 1997, Korzelecka and Winnicki 1998, Korzelecka-Orkisz et al. 2005).

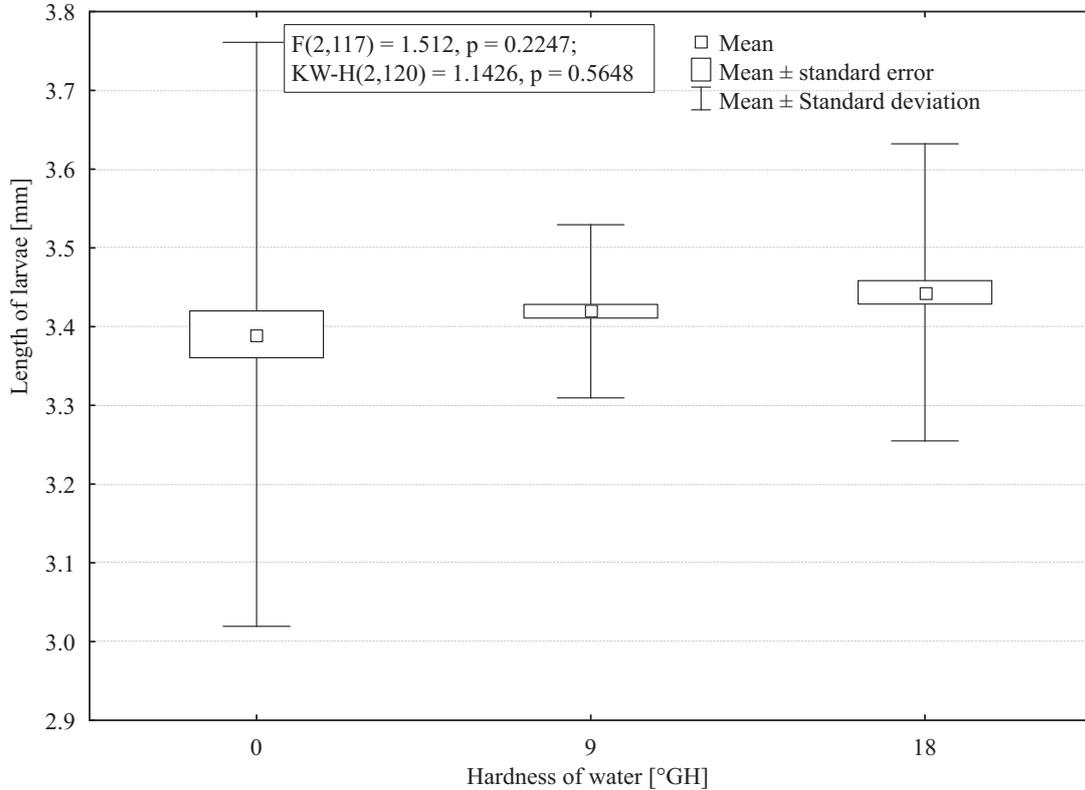


Fig. 17. Total length of *Heteropneustes fossilis* larvae immediately after hatching kept in water differing in hardness; results of ANOVA ($n = 120$; $F(2;117) = 1.512$; $P = 0.2247$)

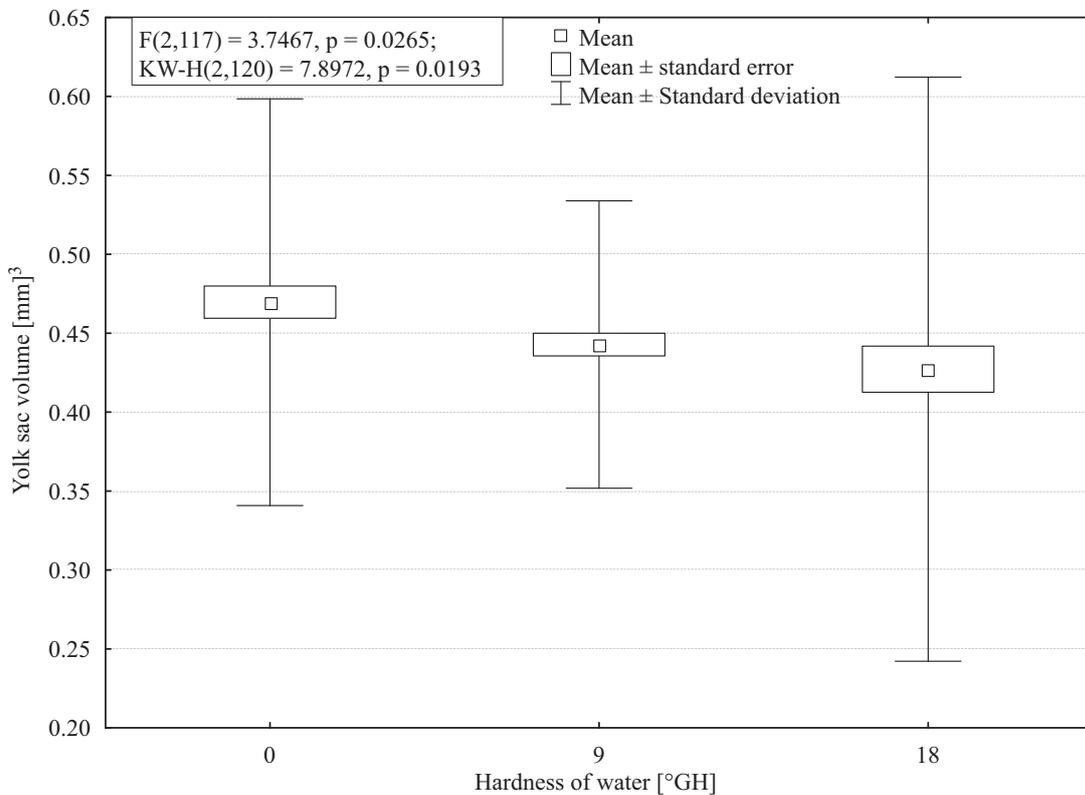


Fig. 18. Yolk sac volume of *Heteropneustes fossilis* larvae immediately after hatching kept in water differing in hardness results of ANOVA ($n = 120$; $F(2;117) = 3.7467$; $P = 0.0265$)

The stinging catfish lays eggs which are small, compared to the size of eggs of other catfish species: the wels catfish, *Silurus glanis* L., (native to Europe) or the introduced brown bullhead, *Ameiurus nebulosus* (Lesueur, 1819), and the north African catfish, *Clarias gariepinus* (Burchell, 1822). The eggs of wels catfish measure from 1.4 (Baruš and Oliva 1995) to 2.5 mm (Kryżanowski 1949); after swelling, when the perivitelline space has been formed, the egg diameter may increase even up to 3.5 mm (Kryżanowski 1949). Still larger are the eggs laid by the brown bullhead: the diameter of the swollen eggs is 3–4 mm (Frank 1956). The differences are still more distinct in the egg volume. Consequently, the eggs of the fossil catfish are as much as 10 times smaller than those of the brown bullhead. The difference is reflected in the rate of embryonic development, as at a comparable temperature (20.6–23.3°C), the brown bullhead embryo may take up to 9 days to develop (Scott and Crossman 1973), while the mere 1.5 days are necessary for the stinging catfish embryo.

For the reasons mentioned above, the larvae of *Heteropneustes fossilis*, measuring 3.5 mm are also by half smaller from those of *Silurus glanis* or *Clarias gariepinus* having larvae on the average 6–7 mm long. The size increments of *Heteropneustes fossilis* are also lower than those of other fish.

The perivitelline space, occupied about 50% of the egg volume, thus providing the embryo with enough space for movements. These, while facilitating the mixing the perivitelline fluid and oxygenation of the fast growing embryo, are an intensive training for the muscles (Tański et al. 2000, Bonisławska et al. 2004, Winnicki et al. 2004, Korzelecka-Orkisz et al. 2005, Korzelecka-Orkisz et al. 2009).

The size of the perivitelline space of this catfish is similar than that of the European perch (Korzelecka-Orkisz et al. 1998). It is on the other hand much smaller than that of cyprinid fishes, e.g., tench, *Tinca tinca*, where it constitutes 70% of the egg volume (Korzelecka-Orkisz et al. 2009). It is larger than the perivitelline space in northern pike, where it takes up as little as 30% of the egg volume (Tański et al. 2000). The differences in the size of the perivitelline space among individual fish species are caused by various environmental factors affecting the embryogenesis and also by the pace of embryogenesis and by diversified phenomena such as embryonic locomotory activity.

Another issue that is worth considering is the effect of water hardness on the eggs as well as on the embryonic and larval development. As shown by this study, and as demonstrated by the experience of ornamental fish breeders dealing with the species, the effects may be diverse (Molokwu and Okpokwasili 2002, Blanksma et al. 2009). Firstly, the rate of water absorption through the egg membranes changed with water hardness, which impinged on the egg size: the egg size increased as the water hardness decreased. However, the embryo survival rate was not affected; quite in contrary, incubation was successful at low (or zero) hardness, which could have been caused by the inhibition of protozoans and other aquatic microor-

ganisms under low hardness conditions. However, the situation changed when the protection provided by the egg membranes ceased to exist. Then, the water with low (or null) concentration of ions would easily overcome the barrier formed by the skin, whereby water would be accumulated in the yolk sac and/or in other parts of the larva's body. The ornamental fish keepers, solve the problem, by increasing water hardness a few hours prior to the expected hatching (Molokwu and Okpokwasili 2002).

The barbels develop shortly before the larva commences active feeding. This is related to the need of finding the food and to analyse its utility for consumption by the taste buds developing on the barbells, similarly as in other catfish species (Whitear 1990, Mukai et al. 2010), cyprinids (Sado and Kimura 2002), or gadids (Harvey and Batty 1998).

It seems that the results study on reproduction this kind of fish may also contribute to the success of future culturing of the stinging catfish, not only because of nutritive qualities of this interesting species—but also—in view of a real danger posed by careless handling of the fish in an aquarium culture.

REFERENCES

- Adams S.L., Kleinans F.W., Mladenov P.V. Hessian P.A.** 2003. Membrane permeability characteristics and osmotic tolerance limits of sea urchin (*Evechinus chloroticus*) eggs. *Cryobiology* **47** (1): 1–13.
DOI: 10.1016/S0011-2240(03)00063-4.
- Alok D., Krishnan T., Talwar G.P., Garg L.C.** 1993. Induced spawning of catfish, *Heteropneustes fossilis* (Bloch), using d-Lys⁶ salmon gonadotropin-releasing hormone analog. *Aquaculture* **115** (1–2): 159–167.
DOI: 10.1016/0044-8486(93)90366-7.
- Altman P.L., Dittmer D.S.** 1962. Growth, including reproduction and morphological development. *Biological Handbook Federation of American Societies for Experimental Biology*. Washington, DC.
- Baensch H.A., Riehl R.** 1985. *Aquarien atlas*. Band 2. Mergus, Verlag für Natur- und Heimtierkunde GmbH, Melle, Germany.
- Baruš V., Oliva O.** (ed.) 1995. *Fauna ČR a SR. Míhulovci (Petromyzontes) a ryby (Osteichthyes)*. [Cyclostomes (Petromyzontes) and fishes (Osteichthyes).] Vol. 2. Academia, Praha. [In Czech.]
- Bonisławska M., Korzelecka-Orkisz A., Winnicki A., Formicki K., Szaniawska D.** 2004. Morphophysiological aspects of the embryonic development of ruffe, *Gymnocephalus cernuus* (L.) under different thermal conditions. *Acta Ichthyologica et Piscatoria* **34** (1): 51–74.
- Blanksma C., Eguia B., Lott K., Lazorchak J.M., Smith M.E., Wratschko M., Dawson T.D., Elonon C., Kahl M., Schoenfuss H.L.** 2009. Effects of water hardness on skeletal development and growth in juvenile fathead minnows. *Aquaculture* **286** (3–4): 226–232.
DOI: 10.1016/j.aquaculture.2008.09.026.
- Davenport J., Lønning S., Kjorsvik E.** 1986. Some mechanical and morphological properties of the chorions of marine teleost eggs. *Journal of Fish Biology* **29** (3): 289–301.
DOI: 10.1111/j.1095-8649.1986.tb04946.x.

- Frank S.** 1956. Sumeček americký (*Ameiurus nebulosus* Le Sueur 1819) v našich vodách. [American catfish (*Ameiurus nebulosus* Le Sueur 1819) in our waters.] Časopis Národního muzea **125** (1): 35–47 [In Czech.]
- Harvey R., Batty R.S.** 1998. Cutaneous taste buds in cod. *Journal of Fish Biology* **53** (1): 138–149. DOI: 10.1111/j.1095-8649.1998.tb00116.x.
- Jayaram K.C.** 1999. The freshwater fishes of the Indian region. Narendra Publishing House, Delhi.
- Kahl W., Kahl B., Vogt D.** 1997. Kosmos Atlas Aquarienfische. Kosmos Verlag, Stuttgart.
- Korzelecka A., Bonisławska M., Winnicki A.** 1998. Structure, size and spatial distribution of perch (*Perca fluviatilis* L.) egg components during incubation. *Electronic Journal of Polish Agricultural Universities* **1** (1): #5 <http://www.ejpau.media.pl/volume1/issue1/fisheries/art-05.html>.
- Korzelecka A., Winnicki A.** 1998. Peculiarities of embryogenesis in *Scardinius erythrophthalmus* L., *Electronic Journal of Polish Agricultural Universities* **1** (1): #01. <http://www.ejpau.media.pl/volume1/issue1/fisheries/art-01.html>.
- Korzelecka-Orkisz A., Formicki K., Winnicki A., Bonisławska M., Szulc J., Biernaczyk M., Tański A., Wawrzyniak W.** 2005. Peculiarities of egg structure and embryonic development of garfish (*Belone belone* (L.)). *Electronic Journal of Ichthyology* **1** (2): 42–53.
- Korzelecka-Orkisz A., Bonisławska M., Pawłowski D., Szulc J., Winnicki A., Formicki K.** 2009. Morphophysiological aspects of the embryonic development of tench (*Tinca tinca* (L.)). *Electronic Journal of Polish Agricultural Universities* **12** (4): #21. <http://www.ejpau.media.pl/volume12/issue4/art-21.html>.
- Kryžanovskij S.G.** 1949. Ekologo-morfologičeskie zakonomiernosti razvitiia karpovykh, viunovykh i somovykh ryb (Cyprinoidei i Siluroidei). [Eco-morphological principles of the development of cyprinid, cobitid and silurid fishes.] *Trudy Instituta morfologii životnykh im. Sieviercova Akademiâ Nauk SSSR* **1**: 5–338. [In Russian.]
- Linhart O., Koužil J., Hamáčková J.** 1987. Increased rate of egg fertilization in artificial propagation of sheatfish (*Silurus glanis* L.) by means of suppressing the movements of spermatozoa with immobilization solution. *Aquaculture* **65** (3–4): 353–358. DOI: 10.1016/0044-8486(87)90247-X.
- Linhart O., Gela D., Rodina M., Kocur M.** 2004. Optimization of artificial propagation in European catfish, *Silurus glanis* L. *Aquaculture* **235** (1–4): 619–632. DOI: 10.1016/j.aquaculture.2003.11.031.
- Molokwu C.N., Okpokwasili G.C.** 2002. Effect of water hardness on egg hatchability and larval viability of *Clarias gariepinus*. *Aquaculture International* **10** (1): 57–64. DOI: 10.1023/A:1021395122919.
- Morrison C., Bird C., O'Neil D., Leggiadro C., Martin-Robichaud D., Rommens M., Waiwood K.** 1999. Structure of the egg envelope of the haddock, *Melanogrammus aeglefinus*, and effects of microbial colonization during incubation. *Canadian Journal of Zoology* **77** (6): 890–901. DOI: 10.1139/cjz-77-6-890.
- Mukai Y., Tuzan A.D., Lim L.S., Yahaya S.** 2010. Feeding behavior under dark conditions in larvae of sutchi catfish *Pangasianodon hypophthalmus*. *Fisheries Science* **76** (3): 457–461. DOI: 10.1007/s12562-010-0237-3.
- Munshi J.S.D., Pandey B.N., Pandey P.K., Ojha, J.** 1976. Oxygen uptake through gills and skin in relation to body weight of an air-breathing silurid fish, *Saccobranchus (=Heteropneustes) fossilis*. *Indian Journal of Experimental Biology* **14** (5): 557–560.
- Pandey B.N.** 1978. Seasonal variations in oxygen consumption of *Heteropneustes fossilis* (Bloch). *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **40**: 165–170.
- Pandey A.K., Nayak P.K., Singh B.N., Das R.C., Saha C.** 2001. Brood stock management, induced breeding and larval rearing of the Indian catfish, *Heteropneustes fossilis* (Bloch.). *Fishing Chimes* **21**: 80–81.
- Pethiyagoda R.** 1991. Freshwater fishes of Sri Lanka. The Wildlife Heritage Trust of Sri Lanka, Colombo.
- Rahman A.K.A.** 1989. Freshwater fishes of Bangladesh. Zoological Society of Bangladesh. Department of Zoology, University of Dhaka.
- Riehl R., Patzner R.A.** 1998. Minireview: The modes of egg attachment in teleost fishes. *Italian Journal of Zoology* **65** (Suppl.): 415–420. DOI: 10.1080/11250009809386857.
- Robins C.R., Bailey R.M., Bond C.E., Brooker J.R., Lachner E.A., Lea R.N., Scott W.B.** 1991. World fishes important to North Americans exclusive of species from the continental waters of the United States and Canada. American Fisheries Society Special Publication No. 21.
- Roy S., Pal B.C.** 1986. Quantitative and qualitative analysis of spawning behaviour of *Heteropneustes fossilis* (Bloch) in laboratory aquaria. *Journal of Fish Biology* **28** (3): 247–254. DOI: 10.1111/j.1095-8649.1986.tb05162.x.
- Sado T., Kimura S.** 2002. Developmental morphology of the cyprinid fish, *Candidia barbatus*. *Ichthyological Research* **49** (4): 350–354. DOI: 10.1007/s102280200052.
- Saxena P.K., Sandhu R.** 1994. Influence of temperature on ovarian recrudescence in the Indian catfish, *Heteropneustes fossilis*. *Journal of Fish Biology* **44** (1): 168–171. DOI: 10.1006/jfbi.1994.1014.
- Scott W., Crossman E.J.** 1973. Freshwater fishes of Canada. *Bulletin of the Fisheries Research Board of Canada* **184**: 1–996.
- Sheel M., Singh B.R.** 1981. O₂ uptake through water during early life of *Heteropneustes fossilis* (Bloch). *Hydrobiologia* **78** (1): 81–86. DOI: 10.1007/BF00011944.
- Singh B.R., Kohli M.P., Goswami U.C.** 1989. Studies on age and growth of an air-breathing catfish *Heteropneustes fossilis* (Bloch). *Journal of Inland Fisheries Society of India* **21** (2): 17–24.
- Szulc J., Winnicki A., Formicki K.** 2008. Środowiskowe uwarunkowania ultrastrukturalnej budowy osłonek jajowych ryb. [Influence of environmental factors on ultrastructure of fish egg membranes.] Pp. 245–249. In: Zakęs Z., Wolnicki J., Demska-Zakęs K., Kamiński R., Ulikowski D. (eds.) *Biotechnologia w akwakulturze*. [Biotechnology in Aquaculture.] Wydawnictwo Instytutu Rybactwa Śródlądowego, Olsztyn. [In Polish.]
- Tański A., Korzelecka A., Bonisławska M., Winnicki A., Formicki K.** 2000. New data on morphomechanical changes during embryogenesis of pike (*Esox lucius* L.).

- Folia Universitatis Agriculturae Stetinensis seria Piscaria **2000** (214/27): 207–213.
- Thakur N.K., Pal R.N., Khan H.A.** 1974. Embryonic and larval development of *Heteropneustes fossilis* (Bloch). Journal of Inland Fisheries Society of India **6**: 33–44.
- Thomopoulos A.** 1953. Sur l'oeuf de *Perca fluviatilis* L. Bulletin de la Société Zoologique de France **78**: 106–114.
- Wheeler A.** 1977. Das grosse Buch der Fische. Eugen Ulmer, Stuttgart.
- Whitear M.** 1990. Collagen turnover in regenerating barbell of a catfish. Archives of Histology and Cytology **53** (3): 283–286. DOI: 10.1679/aohc.53.283.
- Winnicki A., Korzelecka A.** 1997. Morphomechanical aspects of the development of the bleak (*Alburnus alburnus* L.). Acta Ichthyologica et Piscatoria **27** (2): 17–27.
- Winnicki A., Korzelecka-Orkisz A., Sobociński A., Tański A., Formicki K.** 2004. Effects of the magnetic field on different forms of embryonic locomotor activity of northern pike *Esox lucius* L. Acta Ichthyologica et Piscatoria **34** (2): 193–203.
- Winnicki A., Stańkowska-Radziun M., Radziun K.** 1970. Structural and mechanical changes in the egg membranes of *Salmo gairdneri* Rich. during the period of hatching of the larvae. Acta Ichthyologica et Piscatoria **1** (1): 7–20.

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