MORPHO-PHYSIOLOGICAL PROCESSES IN EMBRYONIC DEVELOPMENT OF ODESSA BARB *PUNTIUS PADAMYA* KULLANDER & BRITZ, 2008 (TELEOSTEI: CYPRINIDAE) IN THE OPTIMAL AND REDUCED PH

Agata Korzelecka-Orkisz
Agata Gajewska
Adam Tański
Krzysztof Formicki

Department of Hydrobiology, Ichthyology and Biotechnology of Reproduction,
The Westpomeranian University of Technology in Szczecin, Poland
kformicki@zut.edu.pl

Abstract

This study describes the structures of ruby barb (*Puntius padamy*), morpho-physiological changes during embryogenesis from activation to hatching under optimal conditions (25°C; pH 7.0), the effect of acidic pH (5.8) on the early developmental stages of the species, the relationship between pH water and fry survival. Water pH quite significantly influenced the rate of embryogenesis – the differences in the rate of development had already emerged at the stage of eight blastomeres. The total duration of embryogenesis from fertilization to the moment of mass hatching of larvae under pH 7.0 was 650°H (27 hours) and under pH 5.8 was 960°H (40 hours). The percentage of hatching inside pH 5.8 water was 10%, but 60% inside pH 7.0 water. Water pH quite significantly affected somatic motility of embryos.

Keywords

pH, spawning, ruby barb, *Puntius padamya*

INTRODUCTION

The odessa barb *Puntius padamya*, also known as "ruby barb" is one of the most interesting fish of the carp family, popular in the aquarium cultures. Its origin is not fully understood (Dazkewitsch 1973a, 1973b, Stallknecht 1973, Mills 2002, Arunkumar and Tombi Singh 2003, Axelrod et al.. 2008). Most probably it was presented for the first time on Odessa fish bazaar about 1971 - 1972. Its name - "Odessa barb" originates precisely from the city of Odessa (Hochstrasser 1980, Nieuwenhuizen 1984, Sterba 1988, Menon i in. 2000, Vishwanath and Laisram 2004;

Kullander and Fang 2005, Rüber i in. 2007). Kullander and Britz in 2008 described it as a new species (Kullander 2008, Kullander and Britz 2008,).

Odessa barb is a quiet gregarious fish. Adults grow to a length of up to 8 cm (Frey 1990). The body is of pale-yellow silver color, two dark spots run along its sides: one just behind the gillnet, the second above the anal fin (Mills 2002). All fins of the odessa barbs are have an orange-red hue (Krzykawski et al., 2001). The fish tolerates a wide temperature range, 14-25°C, however, only at 20°C are the specimens fully stained (Frey 1990, Dreyer and Keppler 1996, Kahl et al.in. 1997).

Sexual dimorphism in odessa barb is clearly marked. Mature males have a broad red band along the side of the body, they are more slender and have "dotted" fins (Frey 1990). Coloration of the males becomes more intense during spawning. Females during the breeding readiness period have the belly more prominent (Mills 2002).

Fish rub themselves most often among tiny illuminated cirriform vegetation (preferably in the morning sun). After superb chase, they freely release the eggs and this can take several hours because the barbs spawn in batches. Individual fertility is about 150 eggs (Frey 1990, Talwar and Jhingran 1991, Pertovicky 2003). The yellowish spawn is equipped with a sticky cover, with the help of whose it attaches itself to the vegetation. Development takes about 24 hours at 25°C. The larvae have glands that secrete sticky substance allowing it to attach itself to items such as tank wall, and after about 5 days, they begin to actively swim (Talwar and Jhingran 1991, Petrovicky 2003).

Their reproductive strategy is very similar to that adopted by the closely related species of cherry barb *Puntius titteys*. This species also lays eggs on aquatic vegetation and this is facilitated by the stickiness of the casing. Development of cherry barbs takes 19-36 hours, depending on temperature. The average volume of the cherry barbs egg is 0.97 ± 0.09, and yolk sphere inside them measure, on average, 0.40 ± 0.06. Large perivitellar space constitutes, which creates favorable conditions for the embryo to breath and allows for free movement, makes up to 60% of the egg volume. After about 1.5 h of development, thick-cell morula appears, closing of blastopor takes place after 4.5 hours of development. Like in most carp, the yolk sac in cherry barbs is

divided into two branches: the bigger front branch known as proximal and the smaller rear known as caudal. The first somatic contractions are observable after just some 10 hours from activation, after 14.5 h the heart starts operating and attain a rate of 72 beats per minute just before hatching (Korzelecka-Orkisz et al 2009b).

The pH of water has a highly significant effect on living organisms. Environmental acidification resulting inter alia from anthropogenic pollution is noticeable in almost every corner of the world. Study on the impact of low-pH water on the juvenile stages of herring (*Clupea harengus* L.) have showed that although no linear correlation between the level of acidification and the total length, weight, yolk sac size and size of otoliths of newly hatched larvae was demonstrated, however, concentration of RNA at hatching was reduced, which consequently resulted in lower concentrations of protein and thus may lead in future to reduced weight of developing fish (Franke and Clemmesen 2011). Also, in fish of the *Lebistes reticulatus* species subjected to a 24-hour exposure to water of pH 4.11-4.39, the result was modification of fish metabolism, which was manifested by an increase in lipid content of faces, by 4-36% per mg of dry weight, as compared with the energy content of food. This phenomenon occurred only in juveniles (Urban-Jezierska 2002).

Since it is difficult to predict the reaction of fish and other aquatic organisms to changing water pH, an attempt was made to draw a possible scenario for the next 50-100 years by subjecting the orange clownfish, *Amphiprion percula* to the actions of these factors. Acidification did not affect the duration of the embryogenesis, eggs survival rate and brood size of this species, however, it did have an impact on the size and weight of larvae – larvae were 15 to 18 per cent longer and 47 to 52 per cent heavier in acidified water compared with those in control setting (Munday at al. 2009).

In Germany, many pits left over from brown coal mines were flooded, and because residues from mining affected the water pH, therefore, the range of tolerance at juveniles stages of the *Tinca tinca* was examined. All embryos in water of pH 3.50-4.75 died before hatching or shortly after hatching. In water of pH 5.50, only 3 - 4% survived, which was taken as the threshold limit which can be tolerated by *Tinca tinca* embryo (Duis 2001).

Adult fish are better able to adapt to unfavorable environmental conditions caused by low water pH (Brown and Sadler 1989), although sensitivity of species to changing pH varies. Lowering water pH to 6.0, in the absence of other adverse factors, generally do not cause any adverse effects on fish organism. Temporal further reduction of pH by 1 degree does lead to noticeable, permanent damage, although maintaining lower pH for a long period would damage delicate structures of the gills, which reduces the possibility of oxygen uptake by this organ. At pH below 5.0, they are less mobile and tend to swim to the surface. At this water pH, symptoms of acid effects can already be observed – skin are covered with large amounts of mucus while gills are darker. Acidification increases the susceptibility of fish to other diseases and reduces their rate of development (Kocyłowski and Miączyński 1960).

Although odessa barb is a popular aquarium fish and is often used in laboratory experiments (Prakash and Kapoor 1976, Taki 1978, Satyanarayan and Ramakant 2004, Sahoo et al.. 2007, Sevilla 2007), there is no literature on both embryogenesis and impact in which adverse environmental conditions, including reduced pH, has on this fish. This has aroused our interest, hence we decided to follow the process of embryogenesis under optimal conditions and pH, compared to conditions under slightly reduced pH, and on this basis try to explain biological sense of observed differences, and show relationship existing between specific morpho-mechano-physiological processes and the chemical parameters (water acidic) of the environment in which natural reproduction takes place. During the experiments, changes taking place in the egg of developing embryo were observed and recorded, the heart rate and embryonic motility were monitored, in addition, the state of newly hatched specimens was analyzed.

MATERIALS AND METHODS

Studies on embryonic development of the odessa barb *Puntius padamya* were carried out from April 2007 to May 2009, in an aquarium room belonging to the Department of Hydrobiology, Ichthyology and Biotechnology of Reproduction ZUT in Szczecin.

Preparation of material - material for testing were obtained from specimens that had been breed from juvenile stage to sexual maturity. After 7 months of pre-

rearing, the best specimens were selected (10 males and 7 females). Prior to breeding, spawners were conditioned for 7 days. Water of adequate physical and chemical parameters was prepared 48 hours before releasing spawners into a 25-liter glass spawning aquarium, with a 1:1 ratio of distilled water to tap water, aerated for 72 hours (water temperature - 25 ±05°C, water pH - 7 ± 0.5, total hardness - 8°n).

After spawning, the adults were transferred to breeding aquarium, while the eggs were transferred to a 0.5-liter mini aquariums with water parameters as shown in Tab.1

Table 1. Experimental treatments

	Temperature [°C]	Water pH	Hardness of water [°n]
Variant no 1 (control)	25±0,5	7,0±0,5	8
Variant no 2	25±0,5	5,8± 0,2	8

Embryonic and larval development

The water in the tanks containing the spawn was constantly aerated, and the lower pH was maintained using Tropical-pH minus (Tropical Company), a medium used in aquarium. The content of hydrogen ions was monitored every 3 hours using CX-401 multimeter (Elmetron).

Observations and recording of ruby barb embryonic and larval development were facilitated by two sets of equipment consisting of:

– a Nikon TE-2000S microscope, a Sony CCD camera coupled with a screen and an S-VHS recorder, and a computer (with the NIS Elements software);

– an SMZ 1500 stereomicroscope with a Trol–8100/9100 microprocessor regulator and a Nikon DS. Fi-1 color camera coupled with the computer screen.

The frequency of heart contractions and intensity of somatic movements of the embryos were analyzed, while the process of larvae hatching as well as further development of newly hatched specimens were observed. At the morula stage, and during early organogenesis, percentage of fertilization was calculated.

The length of embryonic development was determined using temperature-degree-hours Unit [°H] which is the sum of temperatures in the respective hours of development.

Using Multiscan v.13.01 computer program for image analysis, for reproducing of recorded image, precise measurements of two diameters (short and long) of the egg cell and egg were performed. The values obtained were used for calculation:

1. in embryos:

- of the surface area of the egg and egg cell (S); $S = 4 \cdot \pi \cdot r \; \pi \cdot r^3$
- of the volume of the egg and egg cell (V); $V = 4/3 \cdot \pi \cdot r^2$
- of the size of perivitellar space.

2. in larvae:

- of total length (l. t.)
- of volume of double chamber sac (Vs), which comprised of volume of the front part (Ve) and volume of the rear part (Vw), according to the formula:

$$Vs = Ve + Vw = (\pi/6 \cdot l_1 \cdot h_1) + [\pi \cdot (h_2/2)^2 \cdot l_2] \text{ [mm}^3\text{]}$$

(Blaxter i Hemple 1963, Bonisławska 2001),

where: l_1 – length of the front part of the yolk sac, l_2 – length of the rear part of the yolk sac, h_1 – width of the front part of the yolk sac, h_2 – width of the rear part of the yolk sac

Results were analyzed statistically using Excel and Statistica v. 8.0 programs.

Results

The size of eggs and yolk spheres

The pH of water inside which the embryos developed did not, in any significant way, statistically affect the size of the eggs and yolk spheres.

Odessa barb eggs were spherically shaped and their sizes were quite varied, the diameter of the largest developing egg in a 7.0 pH solution was 1.12 mm and the smallest 0.96 mm (average 1.06 ± 0.06 mm). The diameter of the largest yolk spheres measured 0.76 mm and the smallest 0.60 mm (mean 0.67 ± 0.06 mm). Eggs developing in water of pH 5.8 were slightly smaller (Tab. 2).

Table 2. Size of ruby barb (*Puntius padamya*) eggs and yolk sphere (n = 30; $\bar{x}$ ±sd = mean and standard deviation)

	Diameter [mm]		Volumne V [mm3]		Surface S [mm2]		Coefficient S/V	
	Egg	Yolk sphere	Egg	Yolk sphere	Egg	Yolk sphere	Egg	Yolk sphere
Variant no 1 (control) (pH 7,0)	1,06 ± 0,06	0,67 ± 0,06	0,63 ± 0,11	0,16 ± 0,05	3,53 ± 0,41	1,44 ± 0,28	5,68 ± 0,36	8,97 ± 0,89
Variant no 2 (pH 5,8)	1,05 ± 0,03	0,64 ± 0,05	0,61 ± 0,10	0,14 ± 0,05	3,46 ± 0,43	1,13 ± 0,27	5,71 ± 0,31	9,38 ± 0,98

Perivitellar space constituted up to 74% of the total egg volume, this guarantees free movement for developing embryo.

Stages of embryogenesis and comparison of the rate of development of odessa barb embryos in water of pH 7.0 and pH 5.8

Water pH quite significantly influenced the rate of embryogenesis (Tab. 3). Odessa barb eggs expanded in volume for 15 minutes after activation (6°H). During this time of ectoplasm, fertilization cone, in the form of the growing bulge at the animal poles of the egg cell, evolved. The differences in the rate of development had already emerged at the stage of eight blastomeres. The third cleavage furrow in the control setting appeared 15 minutes earlier (at 36°H). Along with progressing embryonic development, this difference became more pronounced, the eggs from the control setting entered 1/3 epiboly stage 1.5 hours earlier (at 72°H), it developed to ½ two hours earlier (at 76°H) while closing of blastopor took place 3 hours earlier (at

84°H). At ¾ epiboly, the body of the embryo fast emerging over the surface of yolk sphere was already visible.

Table 3. Embryogenesis of ruby barb (*Puntius padamya*) in water at two pH levels (7.0 and 5.8)

Etapy rozwoju	pH 7.0 (control)		pH 5.0	
	[°H]	time from fertilisation	[°H]	time from fertilisation
CLEAVAGE				
germ plate	6	0:15	6	0:15
2 blastomeres	18	0:45	18	0:45
4 blastomeres	28.2	1:10	28.2	1:10
8 blastomeres	36	1:30	42	1:45
Blastula	42	1:45	48	2:00
GASTRULATION				
1/3 epiboly	72	3	120	4:50
½ epiboly	76	3:10	123.8	5:10
¾ epiboly	79.7	3:20	139.2	5:50
blastopore closure	84	3:30	153.3	6:24
ORGANOGENESIS				
outline of the developing body of an embryo	114	4:45	186	7:45
embryo with emerging cephalic part	144	6	210	8:47
optic capsule	222	9:15	274.3	11:27
visible outlines of emerging encephalon vesicles	222	9:15	274.3	11:27
first myomeres	240	9:45	296.5	12:12
formation of two parts of the yolk sac	282	11:45	363.84	15:10
initial somatic movements	300	12:30	406.7	16:59
slow contractions of heart primordia	438	18:15	448.5	20:12
"trebling of embryo"	600	25	864	36:00
mass hatching	650	27	960	40:00
% fertilization	60 %		50%	
% survival	65%		10%	

The process of organogenesis, as a result of which different tissues and organs are formed, started earlier inside higher pH water and lasted about 10 hours shorter than in water of pH 5.8. First miomera (in the thoracic part) appeared at 240°H (after 9 hours and 45 minutes), while the first somatic movements were already observable

at 300°H (after 12.5 hours). In the caudal section, a diverticulum protruding from yolk sac, dividing it into two parts: the spherical shaped front part containing 2/3 of egg yolk, and less cylindrical rear part whose content underwent resorption as the tail part of the embryo extended, appeared at 364°H (after 15 hours and 10 minutes) in water of pH 5.8, and at 282 °H (after 11 hours and 45 minutes) in water of pH 7.0 (Tab. 3)

"Tremor of the embryos", short and repetitive (2-4 times a one minute) movements signalizing the moment of hatching of well-developed embryos, were observed at 600°H (after 25 hours) in water of pH 7.0 and at 864°H (after 36 hours) in water of pH 5.8. The embryos left the egg shell using the tail or head. The first hatching occurred after 630°H inside pH 7.0 water and after 910°H inside pH 5.8 water.

The total duration of embryogenesis from fertilization to the moment of mass hatching of larvae under pH 7.0 was 650°H (27 hours) and under pH 5.8 was 960°H (40 hours). The difference in the duration of embryonic development was 310°H. The percentage of hatching inside pH 5.8 water was 10%, but 60% inside pH 7.0 water.

Embryonic motility

In embryos developing inside water of lower pH, the first contractions of the heart were observed 450°H from the moment of fertilization, and heart rate was 32 contractions per minute. However, under higher pH heart movement appeared 440°H from the moment fertilization, and the frequency of heart contractions was slightly lower, 24 times per minute (Fig. 1).

In embryos developing inside higher pH water, the heart rate regularly and gradually increased and only shortly before hatching was there an observed slight decrease in number of contractions and leveled at 140 times per minute.

On the other hand, embryos developing inside lower pH water, just before hatching, there was an observed systematic, regular increase in the number of heart contractions that at the moment of hatching reached a level of 140 contractions per minute.

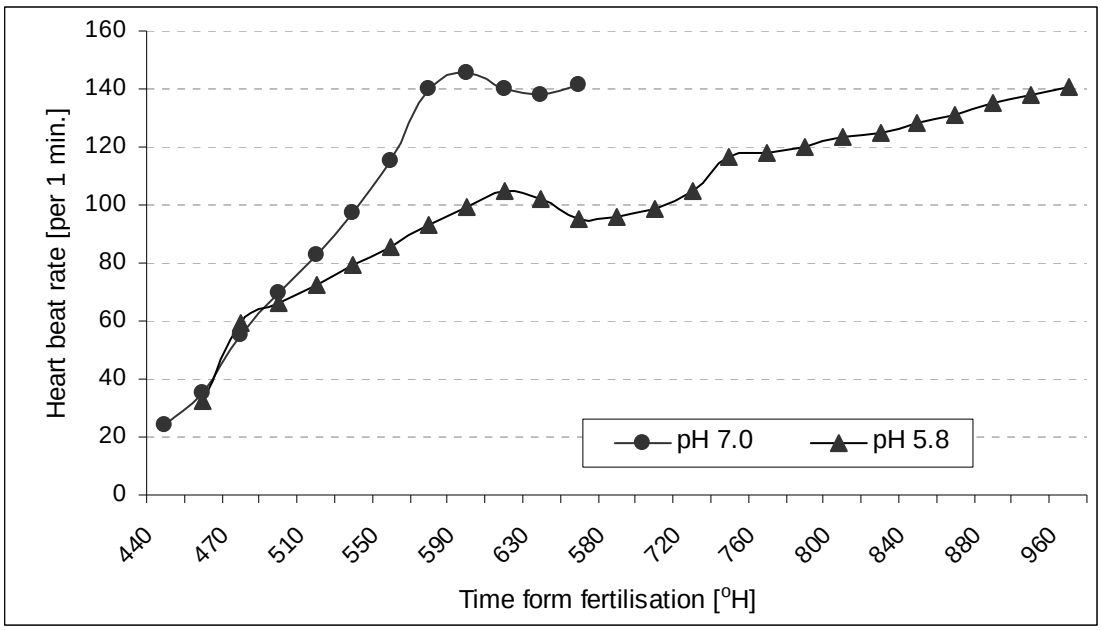

Fig. 1. Heart beat ruby barb (*Puntius padamya*) in water at two pH levels (7.0 and 5.8)

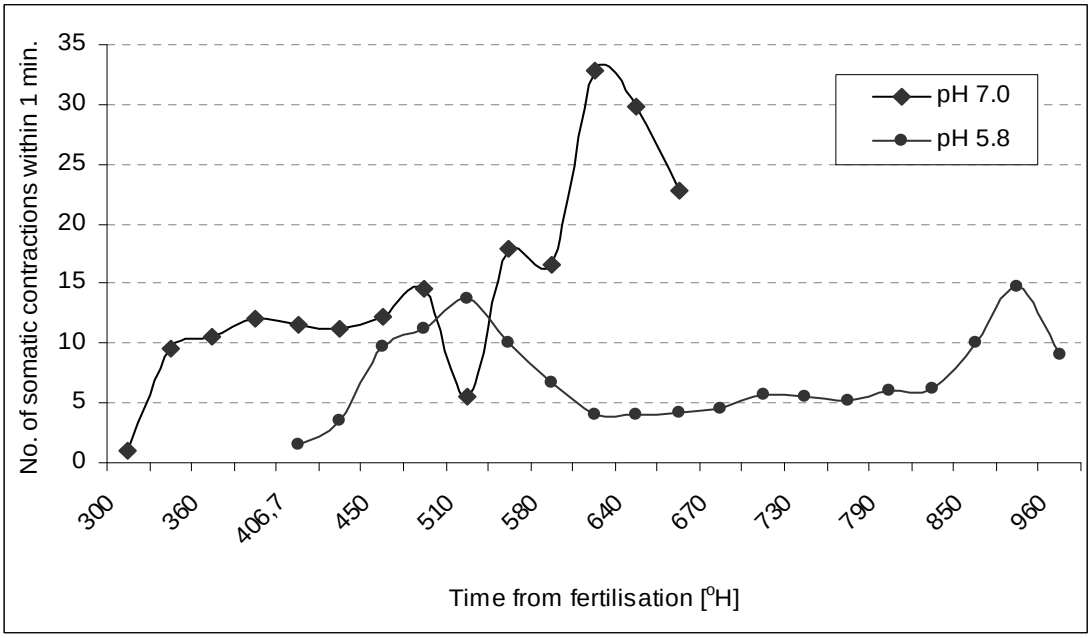

Fig. 2. Somatic motorics of ruby barb (*Puntius padamya*) in water at two pH levels (7.0 and 5.8)

Water pH quite significantly affected somatic motility of embryos. At pH 7.0 embryos began to move faster. The first somatic movements were observed at 300°H, while in water with a pH of 5, the embryos began to move 407°H from the moment of fertilization. At lower pH the number of somatic movements made per minute was

about half the number under pH 7.0. In both cases, just before hatching, there was an observed decrease in the intensity of somatic movements (Fig. 2).

Larvae

The larvae were less mobile after leaving the egg shells. They attached themselves to the tank walls with the help of sticky secretions from the cement glands situated in front of the head. In the cephalic part of the larvae, all parts of the brain were visible, a labyrinth with otoliths could be seen on the rear side of the head. From back to the anus ran the skin fold from which, during development, dorsal, anal and caudal fins evolved. Initially pigmented cells were not visible on the body of the larvae.

The pigment in the eyes appeared 24 hours after hatching. Thirty-five hours after hatching, melanophores developed on the dorsal part of the larvae body were observed.

Larvae developing inside water of pH 5.8 measured, on average, 2.99 mm ± 0.15 mm (x ± SD) and had a yolk sac, volume 0.13mm^3 ± 0.01mm^3. However, larvae incubated in water of pH 7.0 mm measured, an average, 2.92 ± 0.07 mm and had a yolk sac, volume 0.21mm^3 ± 0.03mm^3. Thus, larvae at pH 5.8 had smaller yolk sac, but were larger in size, while the larvae of pH 7.0 were smaller and had larger yolk sac. No development anomalies were observed in hatched larvae. Survival rate of newly hatched larvae developing inside water of pH 5.8 was lower, and the larvae lived only for 3 days.

DISCUSSION

Results obtained from the study provided much information on both the course of embryogenesis, as well as morphological and physiological changes during embryonic and larval development of odessa barb (*Puntius padamya*) as well as the impact of water pH on these processes.

The range of pH of the water in which embryonic development of this barb species proceeds smoothly is narrow, 6.5 - 7.0 (Frey 1990, Riehl 1991). Changing the pH may not only alter the rate of embryogenesis, as in the above studies, but also

cause sharp drop in survival rate, or even lead to hundred per cent mortality. The phenomenon of survival of odessa barb embryos, in spite of unfavorable water conditions, pH 5.8, is interesting. This may be related to the egg casing which fulfills the function of protection or could be related to perivitellar fluid, which could also fulfill this role. Egg casing is a semipermeable structure, although currently there is no full scientific consensus among researchers as to when and to what extent it retains this property. It is permeable to water, depending on the species of fish, for several minutes after egg fertilization, because during this period the volume of the eggs increase Bonisławska et al. 2004, Korzelecka-Orkisz et al. 2009a, 2010). This time period can be extended under the influence of magnetic field (Sadowski et al. 2007). The egg casing is not permeable to high-molecule compounds (Riehl 1978), but does allow permeation of gases and oxygen to the space around the embryo and excretion of carbon dioxide to the outside (Stehr and Hawkes 1979). This means that low-molecule compounds may get inside and this may have adverse affect on the developing embryo that at this stage is very delicate (Munday et al. 2009).

Protective and neutralizing role is also provided by perivitellar fluid. Steroids (Schreck et al. 1991) and other substances such as metabolites excreted by the embryos are, to some extent, able to neutralize toxins penetrating into the interior. A large, 70%, of space, and hence a large amount of fluid is guaranteed to operate for the benefit of embryos.

The lower pH under which the embryos developed significantly slowed the rate of embryogenesis, hence delayed respective stages, and influenced motility. The first movements in embryos incubated in pH 5.8 water appeared 4.5 hours later compared to embryos developing under pH 7.0. Also, the rate of contractions was lower. Low somatic motility was somewhat offset by a rapid heart rate. The high rate of heart contractions allow for more efficient distribution of oxygen in the body of the embryo, for availability of oxygen is a prerequisite to "using up" energy stocks accumulated in the yolk (Korzelecka 1999).

The observed difficulties of the embryos leaving the egg shells may be associated with adverse effects of lowed pH on the activity of hatching enzyme,

which must digest the egg casing for the embryo to be able to break it (Korwin-Kossakowski 2011).

After leaving the egg casings, the larvae exposed to action of lower pH water, with gills not fully formed, for oxygen to the bloodstream goes through a network of blood vessels found shallow on the skin, were not able to survive. Adverse environmental conditions such as low water pH, has proved to be lethal factor during larval development.

References

1. Arunkumar L., Tombi Singh H., 2003. Two new species of puntiid fish from the Yu River system of Manipur. Journal of the Bombay Natural History Society. 99: 481-487.
2. Axelrod H.R., Emmens C.W., Burgess,W.E., Pronek N., Axelrod G.S., 1980. Exotic tropical fishes expanded edition. T.F.H. Publications, Neptune, pp.1302
3. Bonisławska M. 2001. Wpływ temperatury na tempo i przebieg embriogenezy u ryb. Temperature effects on the rate and course of embryogenesis in fish. PhD Thesis, Agricultural University of Szczecin. (In Polish.)
4. 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; 51–72.
5. Blaxter J.H.S., Hemple G., 1963. The influence of egg size on herring larvae (*Clupea harengus*). J. Cons. Int. Explor. Mer., 28: 211–240.
6. Brown D. J. A., Sadler K., 1989. Fish survival in acid waters. In: Acid toxicity and aquatic animals (eds R. Morris, E. W. Taylor, D. J. A. Brown & J. A. Brown), Cambridge, UK: Cambridge University Press. pp. 31–44.
7. Dazkewitsch W., 1973a. Eine schöne neue Barbe kam von Moskau nach Berlin. Aquarien-Terrarien. 20: 75.
8. Dazkewitsch W., 1973b. Noch eine Barbe aus Odessa. Aquarien-Terrarien 20: 365.
9. Dreyer S., Keppler R., 1996. Akwarium słodkowodne. OW „Mulico", Warszawa.
10. Duis K., 2001. Toxicity of acidic post-mining lakewater to early life stages of tench, *Tinca tinca* (Cyprinidae), Water, Air, and Soil Pollution 132: 373–388
11. Franke A., Clemmesen C., 2011. Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.) Biogeosciences Discuss., 8, 7097-7126
12. Frey H., 1990. Akwarium słodkowodne. Wyd. Sport i turystyka, Warszawa.
13. Hochstrasser G., 1980. Die Odessabarbe – ein Geheimnis? Die Aquarien- und Terrarien-Zeitschrift 33: 87.

14. Kahl W., Kahl B., Dieter V, 1997. Atlas ryb akwariowych. Ponad 750 gatunków ryb. Delta W–Z, Warszawa.

15. Kocyłowski B., Miączyński T. 1960. Choroby ryb i raków. PWRiL. Warszawa

16. Korwin-Kossakowski M., 2011. Fish hatching strategies: a review. Rev Fish Biol Fisheries, 22 (1): 225-240

17. Korzelecka A.. 1999. Motoryka embrionalna u ryb kostnoszkieletowych. [Embryonic motorics of teleost fishes]. PhD Thesis, Agricult. Univ. Szczec. [in Polish]

18. Korzelecka-Orkisz A., Bonisławska M., Pawlos D., Szulc J., Winnicki A., Formicki K., 2009a. Morphophysiological aspects of the embryonic development of tench *Tinca tinca* (L.), Electronic Journal of Polish Agricultural University (EJPAU) 12(4), #21.

19. Korzelecka-Orkisz A., Smaruj I., Pawlos D., Robakowski P., Tański A., Szulc J., Formicki K. 2010. Embryogenesis of the stinging catfish, *Heteropneustes fossilis* (Actinopterygii: Siluriformes: Heteropneustidae). Acta Ichthyol. Piscat. 40 (2): 187–197.

20. Korzelecka-Orkisz A., Tański A., Kaczmarek M., Brysiewicz A., Sobociński A., Formicki K. 2009b. Embryogenesis of cherry barb (*Puntius titteya* Deraniyagala 1929). Trudy Analiz i Prognozirovanije Sistem Upravlenija, Sankt-Petersburg, Izdatielstwo SZTU, 55–64.

21. Krzykawski S., Więcaszek B., Keszka S., Antoszek A, 2001. Systematyka krągłoustych i ryb. Przewodnik do ćwiczeń. Wyd. AR, Szczecin.

22. Kullander, S.O., 2008. Five new species of Puntius from Myanmar (Teleostei: Cyprinidae). Ichthyological Exploration of Freshwaters, 19: 59–84.

23. Kullander S. O., Britz R., 2008. *Puntius padamya*, a new species of cyprinid fish from Myanmar (Teleostei: Cyprinidae). Electronic Journal of Ichthyology, 2: 56 – 66

24. Kullander, S.O., Fang, F., 2005. Two new species of Puntius from northern Myanmar (Teleostei: Cyprinidae). Copeia, 290-302.

25. Menon, A., Rema Devi, K. & Vishwanath, W., 2000. A new species of Puntius (Cyprinidae: Cyprininae) from Manipur, India. Journal of the Bombay Natural History Society, 97: 263-268.

26. Mills D., 2002. Ryby akwariowe. Wiedza i życie.

27. Munday P. L., Donelson J. M., Dixson D. L., Geoff G. K., 2009. Endotropical marine fish, Proc. R. Soc. B 276, 3275-3283

28. van den Nieuwenhuizen, A., 1984. Überraschung bei Habarane. Ist das nun die Odessabarbe oder nicht? Die Aquarienund Terrarien-Zeitschrift. 37: 321-325.

29. Petrovicky I., 2003. Ryby akwariowe. Delta W–Z, Warszawa.

30. Prakash Kapoor C., 1976. Studies on the oogonial cycle in *Puntius ticto* (Ham.). I. Formation of primary oocytes. Acta Anatomica, 96 (1), 1–8.

31. Riehl R. 1978. Ultrastructure, development and significance of the egg membrane of teleost – Riv. It. Piscin. Ittiopat. 13: 113–121.

32. Riehl R. 1991. Structure of oocytes and egg envelopes in oviparous teleosts - an overview. Acta Biol. Benrodis, 3: 27-65 [in German].

33. Rüber, L., Kottelat, M, Tan, H.H., Ng, P.K.L., Britz, R., 2007. BMC Evolutionary Biology 7: 38. Schäfer, F. (2001). Das Geheimnis der Odessabarbe ist gelöst! Aqualog News 42: 1-2.

34. Sadowski M., Winnicki A., Formicki K., Sobociński A., Tański A., 2007. The effect of magnetic field on permeability of egg shells of salmonid fishes Acta Ichthyologica et Piscatoria) 37 (2)

35. Sahoo P.K., Nanda P., Barat, A., 2007. Karyotypic analysis of *Neolissocheilus hexagonolepis* (McClelland), *Puntius ticto* (Ham.) and *P. chola* (Ham.) (Family: Cyprinidae, Pisces), Cytologia 72 (4), 409–413.

36. Satyanarayan S., Ramakant S., 2004. Bioaccumulation Kinetics and Bioconcentration Factor of Chlorinated Pesticides in Tissues of *Puntius ticto* (Ham.) Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes, 39 (2), 321–332.

37. Schreck C.B., FitZpatrick M.S., Feinst G.W., Yeoh C.G, 1991. Developmental continuum between mother and offspring. Proc. Fourth Int. Symp. Reprod. Physiol. Fish, Norwisch, 256.

38. Sevilla, R.G., Diez, A., Norén, M., Olivier, M., Marc, J., Verrez-Bagnis, V., van Pelt, H., Favre-Krey, L., Krey. G, FishTrace Consortium & Bautista, J., 2007. Primers and polymerase chain reaction conditions for DNA barcoding teleost fish based on the mitochondrial cytochrome b and nuclear rhodopsin genes. Molecular Ecology Notes, 7: 730-734.

39. Stallknecht, H., 1973. Die Odessa-Barbe gehört zum Barbus ticto-Formenkreis. Aquarien-Terrarien, 20: 366.

40. Stehr C.M, Hawkes J.W., 1979. The comparative ultrastructure of the egg membrane and associated pore structures in the starry flounder *Platichthys atullatus* (Pallas) and pink salmon *Oncorhynchus gorbuscha* (Walbaum). Cell Tissue Res. 2002: 347–356.

41. Sterba, G., 1988. Aquarienkunde. Urania-Verlag, Leipzig, 487 pp.

42. Taki, Y., Katsuyama, A. & Urushido, T., 1978. Comparative morphology and interspecific relationships of the cyprinid genus Puntius. Japanese Journal of Ichthyology, 25: 1-8.

43. Talwar, P.K. and Jhingran A.G., 1991. Inland fishes of India and adjacent countries. 1: 291–292.

44. Urban-Jezierska, E. 2002. Effect of acidification on feeding in guppy fish (*Lebistes reticulatus* vel *Poecilia reticulata*), Polish Journal of Ecology, 50, 1, 25-43.

45. Vishwanath, W. & Laisram, J., 2004. Two new species of Puntius Hamilton- Buchanan (Cypriniformes: Cyprinidae) from Manipur, India, with an account of Puntius species from the state. Journal of the Bombay Natural History Society, 101: 130-137.

Рецензент проф. Юзвяк З.