Arch. Ryb. Pol.	Archives of Polish Fisheries	Vol. 3	Fasc. 1	85 - 94	1995

THE INFLUENCE OF pH ON EMBRYONIC DEVELOPMENT OF COMMON CARP (*Cyprinus carpio* L.)

Barbara Jezierska, Małgorzata Witeska

Agricultural Pedagogical University in Siedlce

A B S T R A C T. Eggs of *Cyprinus carpio* were incubated in acidic and alkaline water. The effect of pH on embryonic development was observed. Acidification and alkalization caused disturbances in cleavage and blastula formation and delay of hatching. At pH 4.5 and 10.5 there were no live eggs in 24 h after fertilization. Mortality during the development indicate that the most sensitive stages are: embryos in the first period after fertilization, hatching larvae and newly hatched larvae. This study indicates that embryonic development of carp is possible within the range of pH 5.0-9.5 although at the extreme pH levels certain disturbances and increased mortality occured.

Key words: EGGS OF CARP, INCUBATION, pH

INTRODUCTION

The early life stages represent a sensitive part of the life cycle of fish. There are data confirming that the effect of water pH on fish depends on the age and developmental stage (Lloyd and Jordan 1964, Swarts et al. 1978, Muniz and Leivestad 1980, Frenette and Dodson 1984).

Thus, even a short-term change of pH may adversely influence fish population, especially if occurs during spawning or embryonic and larval development. The sensitivity depends on the species (Daye 1980, Rombough 1982, Norrgren and Degerman 1993).

An experiment was performed to study the effect of low and high pH on embryonic development of carp (*Cyprinus carpio* L.), to determine the pH range in which development is possible and to determine the most sensitive stages.

MATERIAL AND METHODS

Eggs and milt of the common carp were obtained during spawning artificially induced by pituitary injection in the hatchery of the Inland Fisheries Institute in

Żabieniec (Poland). Eggs and milt were obtained in three consecutive spawnings, in May 1993. They were transported to the laboratory in a cold box at about 5°C.

The embryonic development was observed in several treatments in eggs that were fertilized by the „dry" method. Products from one pair of spawners were used in each of three replicates. Eggs were spread in the incubation sieves, about 300 eggs in each, mixed with milt and rinsed in the water. The sieves were placed in the aquaria with water of various pH. Temperature was maintained at the level of 20±1°C. Water saturation with oxygen was about 80%.

The effect of the following pH values was tested: control - 8.0; acidic water - 4.5, 5.0, 5.5 and alkaline water 9.0, 9.5, 10.0, 10.5. Water was acidified and alkalized every 6 hours by addition of 3% sulphuric acid or 3% sodium hydroxide. Such frequency of pH control assured that fish stayed 75% of time in adjusted pH (Jezierska 1988).

Observations using a binocular microscope started 1 h after fertilization. For the observations, sieves holding the fertilized eggs were placed in Petri dishes filled with the incubation water.

The following embryonic stages of development were distinguished: 1. 2 blastomers, 2. 4 blastomers, 3. early blastula, 4. blastula, 5. appearance of the embryo, 6. body segmentation,7. brain vesicles, 8. eye pigmentation, 9. heart movements, 10. body pigmentation, 11. blood coloration, 12. start of hatching, 13. mass hatching.

One group of eggs developed in pH 8.0 was transferred to pH 4.5 at stage 8.

Survival of the embryos and larvae was calculated during development. The percent of fertilized eggs was determined 24 h after fertilization. Non-fertilized eggs (white and opaque) were removed from the sieves. Live, fertilized eggs were transparent and yellowish. About 40 h after fertilization black eyes of the embryos became visible. The percent of the „eyed eggs" was calculated in relation to the number of fertilized eggs. After the end of hatching the number of larvae was noted and the percent of normal, deformed and dead hatch was calculated.

RESULTS

Observations of first stages of embryonic development revealed some abnormalities in embryo formation. Most interesting of them concerned cleavage. In strongly acid (pH 4.5) and stongly alkaline (pH 10.0 and pH 10.5) water some changes in the second division were observed. At the stage of 4 blastomers cells were uneven and

TABLE 1

Duration of embryonic development of carp in various pH levels (ranges of values of 3 replicates)

Stage	Time (h)					
	control	acidic water		alkaline water		
	8.0	5.5	5.0	9.0	9.5	10.0
1	1.2-1.3	1.3-1.4	1.4-1.6	1.3-1.4	1.3-1.5	1.3-2.0
2	1.6-2.1	2.0-2.2	2.1-2.5	1.6-2.2	1.7-2.5	1.7-2.5
3	3.8-4.3	4.0-5.3	4.2-4.8	3.8-4.6	3.9-4.6	4.0-4.4
4	4.2-4.8	5.0-5.7	4.8-5.7	4.3-4.7	4.4-5.0	4.5*
5	15.5-16.0	16.1-16.5	16.2-18.1	16.2-17.5	16.2-17.5	17.5
6	24-25	24-27	24-27	25-26	24-27	26
7	32-34	34-36	34-38	34-36	34-36	35
8	40-44	42-44	44-44	42-44	42-45	44
9	43-44	44-46	44-46	44-47	44-47	47
10	57-59	58-62	60-62	58-62	58-62	62
11	63-65	64-75	64-75	64-75	64-75	75
12	72-74	72-93	86-93	84-93	64-93	78
13	89-114	94-110	94-107	98-114	97-110	98-116

* - *only one sample*

were placed irregularly on the top of the yolk. Blastomers at the later stages of development, up to blastula formation, revealed similar disturbances. In pH 4.5 the blastula consisted of numerous small cells with larger ones placed on them. In some cases cells were fused.

Eggs incubated in pH 9.5 and 5.0 revealed disturbances at 8 cells stage. They were uneven and placed on the yolk in an irregular way. In addition, at pH 5.0 cells tended to separate from the yolk.

Percent of fertilized eggs (measured after 24 h) was low in all the experimental groups and did not exceed 40%. In pH 4.5 and 10.5 no fertilized eggs were observed.

The duration of embryonic development under various pH conditions is shown in Table 1. In all the experimental groups duration of the stages was similar until the start of hatching. The hatching process was considerably delayed in acidic water and

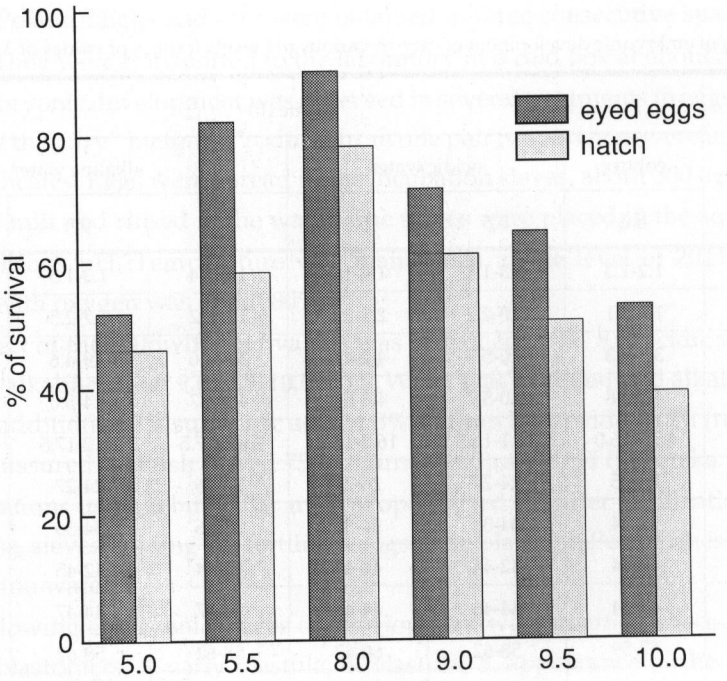

Fig. 1. The effect of pH on survival (average from 3 replicates)

in alkaline water. In the control first larvae hatched about 73 h after fertilization, while in acidic water after 84-93 h and in alkaline water after 64-93 h.

Separate group of embroys kept at pH 4.5 from eyed stage started to hatch after about 99 h. It is interesting that mass hatching appeared only a little later and the duration of hatching was shorter in acidic water than in controls and in alkaline treated embryos.

The effect of water pH on the survival of embryos until the stage of „eyed egg" and hatching is shown in the Fig. 1. In the control mortality during both periods was similar and low (up to 10%). At pH 5.5 was higher and also similar in both periods. At pH 5.0 mortality attained almost 50% and most of it occured in early stages of development. At all alkaline pH levels mortality was much higher until the „eyed" stage. Fig. 1 shows also that acidification and alkalization reduced the percent of hatching, comparing to the controls.

The hatching of normal, dofermed and dead larvae are shown in Fig. 2 where the frequency abundance of these groups is shown in relation to the pH level. In the

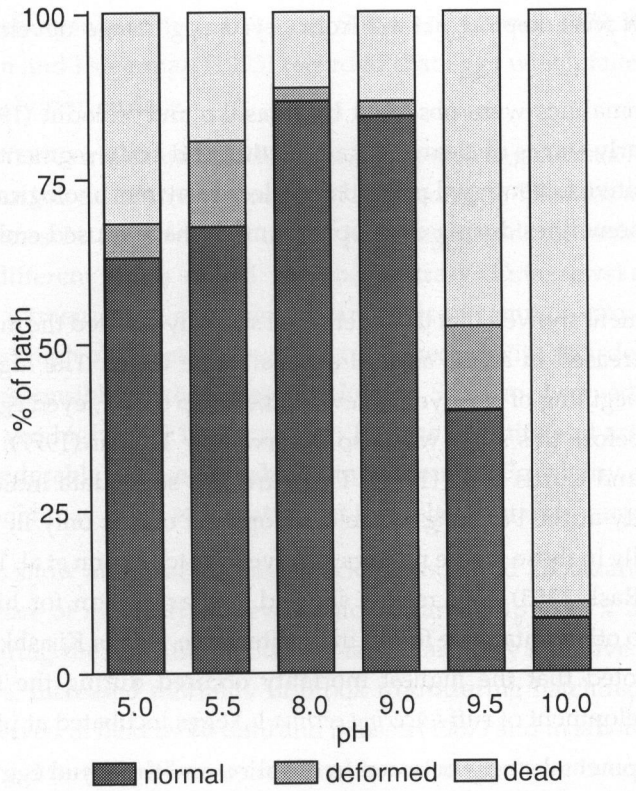

Fig. 2. Share of normal, deformed and dead hatch in various pH values (average from 3 replicates)

controls only 11% of the larvae were dead and 2% of them deformed while at pH 5.0 - there was 32% of dead larvae and 5% deformed. At pH 9.0 the share of dead and deformed larvae was similar to that in the control group but in pH 9.5 both values were much higher. At pH 10.0 only 9% of hatched larvae were normal. 2% was deformed and the remaining 89% were dead.

In the separate group of embryos, kept at pH 4.5 from the „eyed egg" stage up to 90% of newly hatched larvae were dead or deformed.

DISCUSSION

The results of the presented study indicate that water pH affects cleavage and blastula formation. At pH 4.5 and pH 10.5 eggs did not developed longer than 24 h but

the eggs which were kept at pH 4.5 from „eyed egg" stage developed and even hatched.

Similar anomalities were observed by Kijashko and Volodin (1978). They also noted that at early stages of cleavage, gastrulation and body segmentation embryos were most sensitive to abnormal pH and revealed many morphological changes. The changes and anomalities during development might have caused embryonic mortality.

The experiment showed that the level of pH strongly affected the mortality of carp embryos. It increased in acidic as well as in alkaline water. The highest mortality occured at the begining of embryonic development, up to the „eyed egg" stage. Most egg mortality before this stage was also observed by Trojnar (1977), Hulsman and Powles (1983) and Curtis et al. (1989). There are also some data indicating that the highest mortality at the begining of the development occurs only in the lowest pH values, especially in those where no eggs survive to hatch (Runn et al. 1977, Daye and Garside 1979, Rask 1983). Our results showed similar pattern for high pH values (9.0-10.0) but no other data were found in the literature, except Kijashko and Volodin (1978). They noted that the highest mortality occured during the first period of embryonic development of ruff (*Acerina cernua* L.) eggs incubated at pH 9.0.

The developmental stages between fertilization and the „eyed egg" stage appear to be most sensitive to acid and alkaline water. This was also confirmed for rainbow trout that were subjected to ammonia intoxication (Solbe and Shurben 1989).

The results revealed that pH did not affect the duration of the development up to the begining of hatching when a delay occured in all experimental pH levels, comparing to the control. Similar effects - prolonged duration of development of the eggs with decreasing pH were observed by Daye and Garside (1979) and Rask (1983).

Runn et al. (1977) found out that the main limiting factors of the development are the mechanisms involved in the hatching process. These mechanisms are connected with digesting of the egg envelope by the chorionase. The delay of hatching in our experiment might have been caused by the reduction of chorionase activity. Investigation of this enzyme from several species of fish has shown that the activity of the enzyme is pH dependent with maximum activity occuring around pH 8.0. A reduction in pH decreases this activity (Yamagami 1973, Hagenmaier 1974, Waiwood and Haya 1983, Kugel et al. 1990). Peterson et al. (1980) noted that low pH might have induced some changes in the structure of the chorion making it more difficult to break.

Larvae remain encapsulated and can't hatch (Daye and Garside 1979, Kwain and Rose 1985). Norrgren and Degerman (1993) recorded that eggs which failed to hatch had an intact inner chorion surface.

Runn et al. (1977) indicated that the transfer of perch eggs from a lower pH to pH 7.3 resulted in an increase in the frequency of hatched eggs. This was attributed to the lack of differences in the structure of the hatching glands in embryos that were incubated at different levels of pH. On the contrary, Ostaszewska (1983) found changes in the ultrastructure of hatching gland cells in common carp embryos incubated at low and high pH. These changes were connected with retarded development of gland cells responsible for chorionase production. She also observed that at low pH: 5.1-5.7, 4.1-4.7 and high pH 9.1-9.7 and 10.1; 10.7 the quantity and activity of chorionase were considerably reduced in relation to the control. Possibility of such changes in our experiment may be an explanation for the delay of start of carp hatching.

The results show also that increasing acidification and alkalization caused proportional increase of mortality of newly hatched larvae up to 89% at pH 10.0. It is known that during the hatching period the larvae are very sensitive to the environmental impacts. Increased mortality of brook trout during hatching period in acid water was observed in field by Jordahl and Benson (1987) and in laboratory by Kwain and Rose (1985). Mortality of newly hatched larvae might have been caused by low or high pH. many data indicate that freshly hatched larvae are more sensitive to the environmental factors than the embryos. Daye and Garside (1977), Daye (1980) and Rombough (1982) noted that alevins were more sensitive to acidification than embryos. Newly hatched larvae are also more sensitive to other toxicants (Holt and Arnold 1983, Hetland et al. 1991, Whale et al. 1992, Norrgren and Degerman 1993). Thus, it is possible that the sensitivity of the larvae to alkaline pH levels in our experiments was also higher and 89% of them died just after hatching in pH 10.0. It is probable that the difference in relative sensitivity occured because the newly hatched larvae were exposed directly to the water, whereas the embryos were shielded by egg envelope and perivitelline fluid.

It is interesting that at moderately changed pH (5.5 and 9.5) the percent of deformed larvae increased but at extreme pH levels at which the carp development is possible (5.0 and 10.0) the share of deformed hatch was low. Some authors confirm that deformations may result from various disturbances caused by low pH. They possibly were: disturbances and extension of hatching process (Trojnar 1977, Peterson

et al. 1980) and small volume of the eggs which might have been a factor preventing normal movements and reducing the diffusion of embryo metabolites (Runn et al. 1977, Kugel et al. 1990).

The results of this paper indicate that the embryonic development of carp is possible within the pH range 5.0-9.5 but at pH 5.0 only about 50% of eggs hatched and 40% of the newly hatched larvae were dead or deformed. At pH 9.5 about 50% of eggs hatched and 60% of them were dead or deformed.

Mortality during the development indicates that the most sensitive stages are: newly hatched larvae, embryos in the first period after fertilization until eyed stage and hatching larvae.

ACKNOWLEDGEMENTS

Special thanks to Dr. Zygmunt Okoniewski and his staff from the hatchery of the Inland Fisheries Institute in Żabieniec for the experimental material, for their advice and kind help.

REFERENCES

Curtis F.R. et al. 1989 - Role of exposure duration in hydrogen ion toxicity to brook (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*) - Can.J. Fish.Aquat.Sci. 46: 33-40.

Daye P.G. 1980 - Attempts to acclimate embryos and alevins of Atlantic salmon, *Salmo salar* and rainbow trout, *Salmo gairdneri* to low pH - Can.J. Fish.Aquat.Sci. 37: 1035-1038.

Daye P.G., Garside E.T. 1977 - Lower lethal levels of pH for embryos and alevins of Atlantoc salmon, *Salmo salar* L. - Can.J. Zool. 55: 504-1508.

Daye P.G., Garside E.T. 1979 - Development and survival of embryos and alevins of the Atlantic salmon, *Salmo salar* L., continuously exposed to acidic levels of pH, from fertilization - Can.J. Zool., 57: 1713-1718.

Frenette J.J., Dodson J.J. 1984 - Brook trout (*Salvelinus fontinalis*) population structure in acidified Lac Tantare, Quebec - Can.J. Fish. Aquat.Sci., 41: 865-877.

Hagenmaier H.E. 1974 - The hatching process in fish embryos. IV. The enzymological properties of a highly purified enzyme (chorionase) from the hatching fluid of the rainbow trout (*Salmo gairdneri*) - Comp. Biochem. Physiol. 49 B: 315-324.

Hetland R.M., Ronnestad I., Fyhn H.J., 1991 - Ammonia toxicity in eggs and larvae of the turbot *Scophthalmus maxim* - European Society for Comparative Physiology and Biochemistry (13 Conference), Antibes 6-10 Oct. 1991.

Holt G.J., Arnold C.R. 1983 - Effects of ammonia and nitrite on growth and survival of red drum eggs and larvae - Trans.Am.Fish.Soc. 112: 314-318.

Hulsman P.F., Powles P.M. 1983 - Mortality of walleye eggs and rainbow trout yolk-sac larvae in low-pH waters of the La Cloche Mountain Area, Ontario - Trans.Am.Fish.Soc. 112: 680-688.

Jezierska B. 1988 - The influence of water pH early stages of carp (*Cyprinus carpio* L.) - Rozprawa naukowa 25, WSRP Siedlce (in polish).

Jordahl D.M., Benson A. 1987 - Effect of low pH on survival of brook trout embryos and yolk-sac larvae in west Virginia Streams. Trans.Am. Fish.Soc. 116: 807-816.

Kijashko V.I., Volodin V.M. 1978 - Vlijanie aktivnoj reakcii sredy na embrionalnoje razvitie ersha *Acerina cernua* (L.) - Vopr. Icht. T. 18: 779-781

Kugel B., Hoffman R.W., Friess A. 1990 - Effects of low pH on the chorion of rainbow trout *Oncorhynchus mykiss*, and brown trout, *Salmo trutta* F. *fario* - Jour.Fish.Biol. 37: 301-310.

Kwain W., Rose G.A. 1985 - Growth of Brook trout *Salvelinus fontinalis* subject to sudden reductions of pH during their early life history - Trans.Am.Fish.Soc. 114: 564-570.

Lloyd R., Jordan D.H.M. 1964 - Some factors aftecting the resistance of rainbow trout, *S. gairdneri* R. to acid waters - Int.J. Air Wat.Pollut. 8: 393-403.

Muniz I.P., Leivestad H. 1980 - Acidification - effects on freshwater fish: 84-92 - In: Ecological impact of acid precipitation (Ed.) Drablos D.A. Tollan)) - Proc.Int.Conf. Sandefjord Norway

Norrgren L., Degerman E. 1993 - Effects of different water qualities on the early development of Atlantic salmon and brown trout exposed in situ - Ambio 22: 213-218.

Ostaszewska T. 1990 - The effect of water pH on the development of hatching gland cells in *Cyprinus carpio* L. - Proceedings of FAO-EIFAC Symposium on Production Enhancement in Still-Water Pond Culture Praque, 15-18 May 1990.

Peterson R.H., Daye P.G., Metcalfe J.L. 1980 - Inhibition of Atlantic salmon (*Salmo salar*) hatching at low pH - Can.J. Fish.Aquat.Sci. 37: 770-774.

Rask P.J. 1983 - The effect of low pH on perch, *Perca fluviatalis* L. I. Effects of low pH on the development of eggs of perch - Ann.Zool.Fennici 20: 73-76.

Rombough P.J. 1982 - Effects of low pH on eyed embryos and alevins of Pacific salmon - Can.J. Fish.Aquat.Sci. 40: 1575-1582.

Runn P., Johansson N., Milbrink G. 1977 - Some effects of low pH on the hatchability of eggs of Perch, *Perca fluviatilis* L. - Zoon 5: 115-125.

Solbe J.F. de L.G., Shurben D.G. 1989 - Toxicity of ammonia to early life stages of rainbow trout (*Salmo gairdneri*) - Wat. Res. 23: 127-129.

Swarts F.A., Dunson W.A., Wright J.E. 1978 - Fenetic and environmental factors involved in increased resistance of brook trout to sulfuric acid solutions and mine acid polluted waters - Trans.Amer.Fish.Soc. 107: 651-677.

Trojnar J.R. 1977 - Egg hatchability and tolerance of brook trout (*Salvelinus fontinalis*) fry at low pH - J.Fish.Res.Bd.Can. 34: 574-579.

Waiwood B.A., Haya K. 1983 - Levels of chorionase activity during embryonic development of *Salmo salar* under acid conditions - Bull. Environ.Contam.Toxicol. 30: 511-515.

Whale G.F., Sheahan D.A., Kirby M. 1992 - Assessment of the value of including recovery period into chronic toxicity test guidelines for rainbow trout, *Oncorhynchus mykiss* (Walbaum) - Abstract for the EIFAC Symposium on sublethal and chronic toxic effects of pollutants on freshwater fish, 19-22 May 1992. Section B: Factors affecting fish populations.

Yamagami K. 1973 - Some enzymological properties of a hatching enzyme (chorionase) isolated from the freshwater teleost, Oryzias latipes - Comp.Biochem.Physiol. 46: 603-611.

STRESZCZENIE

WPŁYW pH NA ROZWÓJ ZARODKOWY KARPIA

Zapłodnioną ikrę karpia inkubowano w wodzie o pH 8,0 (kontrola) oraz w wodzie zakwaszonej (pH 4,5, 5,0, 5,5) i zalkalizowanej (pH 9,0, 9,5, 10,0, 10,5), w temperaturze 20±1°C.

Przy najwyższych wartościach zakwaszenia i alkalizacji wystąpiły zmiany już na etapie bruzdkowania. Zaobserwowano nierównomierne rozmieszczenie, nierówną wielkość, zlewanie się lub odrywanie blastomerów. Procent zapłodnienia, liczony po 24 godzinach, był niski we wszystkich grupach i nie przekraczał 40%. W pH 4,5 oraz 10,5 nie zaobserwowano jaj zapłodnionych. Odczyn wody nie wpłynął znacząco na czas trwania poszczególnych etapów rozwoju (tab. 1) aż do początku wykluwania. Wykluwanie w wodzie zakwaszonej i zalkalizowanej było opóźnione w porównaniu z kontrolą. Czas wykluwania larw był natomiast w wodzie zakwaszonej krótszy niż w kontroli i środowisku alkalicznym. Odczyn wody wywarł wpływ na śmiertelność zarodków w czasie rozwoju (rys. 1). Wzrastała ona proporcjonalnie do wielkości zakwaszenia i alkalizacji.

Odczyn wody wpłynął także na jakość larw (rys. 2). Ze wzrostem zakwaszenia, a zwłaszcza alkalizacji zwiększył się procent larw zdeformowanych lub martwych.

Otrzymane wyniki wskazują, że świeżo wyklute larwy są wrażliwsze na zakwaszenie i alkalizację wody niż zarodki w osłonkach jajowych. W okresie embrionalnym najwrażliwsze są wczesne stadia rozwojowe, do etapu zaoczkowania, a także ryby w stadium wykluwania. Przypuszcza się, że zakłócenia w procesie wykluwania larw związane są z wpływem odczynu wody na gruczoły wyklucia i ich produkt - chorionazę. Uzyskane dane wskazują także, że rozwój zarodkowy karpia jest możliwy w zakresie pH 5,0–9,5, przy czym w skrajnych wartościach tego zakresu wykluwa się jedynie 50% larw, a u wielu z nich obserwuje się deformacje ciała.

Adres Autorów:

Dr hab. Barbara Jezierska
Dr Małgorzata Witeska
Katedra Fizjologii Zwierząt
Wyższa Szkoła Pedagogiczno-Rolnicza
08-110 Siedlce
ul. Prusa 12