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EFFECT OF LOW WATER pH ON THE EMBRYO AND LARVAL DEVELOPMENT OF THE PIKEPERCH (*Stizostedion lucioperca* L.) - HISTOLOGICAL OBSERVATIONS

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ABSTRACT. The embryos of the pikeperch, *Stizostedion lucioperca* L., were incubated continuously from fertilisation, at 7.2-7.4 (control) and 5.0-5.2, at 14-16°C. The subsequent larvae in these environments were maintained at these levels for 7 days after hatching. The investigations revealed histopathological changes caused by low pH level in the cells and tissues of the following organs: integument, brain, eye, heart, gill, intestine and yolk sac.

Key words: PIKEPERCH, LARVAL DEVELOPMENT, LOW pH

INTRODUCTION

The acidification of water in natural reservoirs could result from the peat water inflow, mine deposit wash and some industrial effluents. However, the main cause of the water acidification are acid rains resulting from atmospheric pollution. In Europe, acidification is observed in Norway, Finland, Sweden, Denmark, Holland, Germany, Czech Republic, Slovakia and Poland.

In Poland, on about one third of the territory, the rain pH amounts to 4.1 and in over half of the country to 4.3. The highest acidification of water in Poland was observed in the mountain protection areas: in the High Tatra, Karkonosze, Świętokrzyskie Mountains and also in the Upper-Silesian Industrial Region (Symposium on Atmospheric Pollution and Water Degradation 1991).

The acidification of water reservoirs causes serious damages to the ecosystems. Acid rains disturb the hydrochemical regime of a reservoir by changing water quality and act toxically on its biota (Högland 1961). The increasing process of the water reservoir degradation affects the state of the fish population which clearly declines with the pH drop (Lance 1981) and simultaneous increase aluminium concentractions (Vuorinen et al. 1993).

The degree of fish sensitivity to adversep changes depends on the species, stage of development and physiological state. The lowest resistance is usually observed during early development but there are differences between species shortly after hatching, that is during the most crucial periods of the fish life (Trojnar 1977a), and Daye and Garside (1980) demonstrated increased mortality rate of the fertilised fish ova and abnormalities in the embryonic development of Salmonidae during their incubation at pH 3.7-5.6.

The aim of this experiment was to analyse by histological methods the causes of high mortality rate of pikeperch larvae (*Stizostedion lucioperca* L.) shortly after hatching when the eggs were incubated in water at a low pH since the time of fertilisation.

MATERIAL AND METHODS

Pikeperch larvae for this study were obtained from the experimental fish culture station of Warsaw Agricultural University SGGW at Łąki Jaktorowskie. The ova were obtained from one female. Incubation of fertilised ova was performed in water at 14-16°C and at pH 5.0-5.2. The water pH level of 5.0 was reached using 2 mol l^{-1} H_2SO_4. Control group was incubated in water of the same temperature at pH 7.2-7.4.

Larvae for the investigation were taken from the period of mass hatching. Postembryonal development took also place in water of 14-16°C at pH 5.0-5.2 (experimental group) and pH 7.2-7.4 (control group). Each 20-litre aquarium held 350 newly-hathed larvae (three replicates per treatment). During the experiment the temperature, pH and water oxygen content were controlled. Water in aquariums was aerated continuously and oxygen level was about 80-90% of saturation.

Every 8 hours during 7 days after hatching, 10 larvae were collected for histological examinations both from the experimental and the control group.

Material collection and methods of histological procedures were described in an earlier work (Ostaszewska 1989). Standard histological procedures were employed and the material was embedded in paraffin. Slices 6 μm thick were stained with azan, haematoxylin-eosin. The number of erythrocytes, mucous cells was estimated from a series on histological cross-section. Observations were carried out with a light microscope.

The method of pathogenesis interpretation based on comparison of larvae deve-

loping in water at pH 5.0-5.2 with larvae developing in water at pH 7.2-7.4 was used in this study. Observations included the cells and tissues of the following organs: integument, brain, eye, heart, gills, yolk sac, intestine and swimbladder.

The meaurement of yolk sac size has been determined with the help of a ligh microscope and for the calculating of its volume the elongate elipsoid formula has been used:

$$N = 0{,}5236 \cdot l \cdot h$$

where:
l - length, h - height.

RESULTS

INTEGUMENT

Histopathological changes were already observed in the epithelial integument one day after hatching of the larvae which developed at pH 5.0-5.2. The integumentary tissue had hyperplastic mucous cells with early nuclear pycnosis. There was the delamination and separation of the integumentary tissue of the head. At the same time the exfoliation of the epidermal mucous cells was observed and they became devoid of mucus (Fig. 1). This was accompanied by an accumulation of large amount of mucus on the body surface with a sinultaneous reduction of the size and number of mucous cells. In addition, as a result of exposure to acid environment, those cells had very flattened irregular shapes compared to the cells of the control larvae (Fig. 2).

BRAIN

The brain of four days after hatching and older larvae incubated in water at pH 5.0-5.2 was metaplastic. Apart from this, nuclear pycnosis and partial cellular necrosis was observed in all regions of the brain (Fig. 3). The brain tissue in younger larvae was normal (Fig. 4).

EYE

Low pH during embryonal development resulted in the disturbances of the

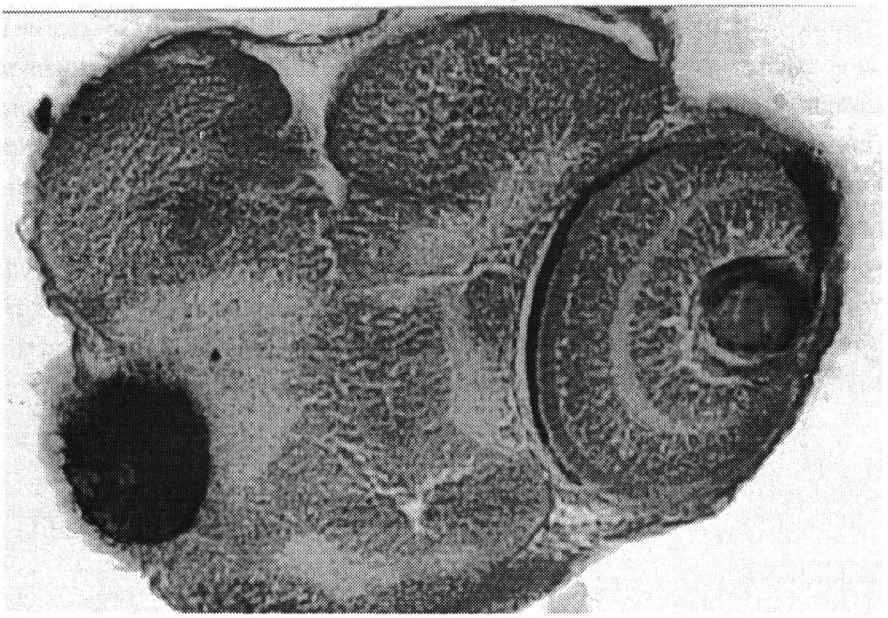

Fig. 1. One day after hatching larva, pH 5.0-5.2, x 6 000. Plastic mucous cells (mc) with ea[illegible]nuclear pycnosis. Sloughing and separation of the epidermal integument (ep) covering the head. „Shredding" of cells (mc) devoid of mucus. Haematoxylin and eosin stain.

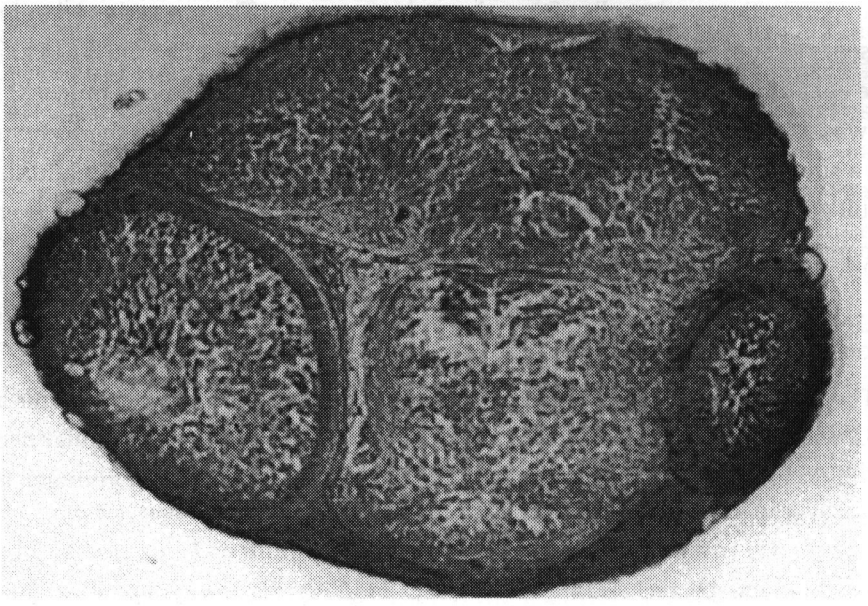

Fig. 2. One day after hatching larva, pH 7.2-7.4, x 6 000. No clear pathological changes in epidermal (ep) and mucous cells (mc). Epithelium (ep) correctly covers the head. Haematoxylin and eosin stain.

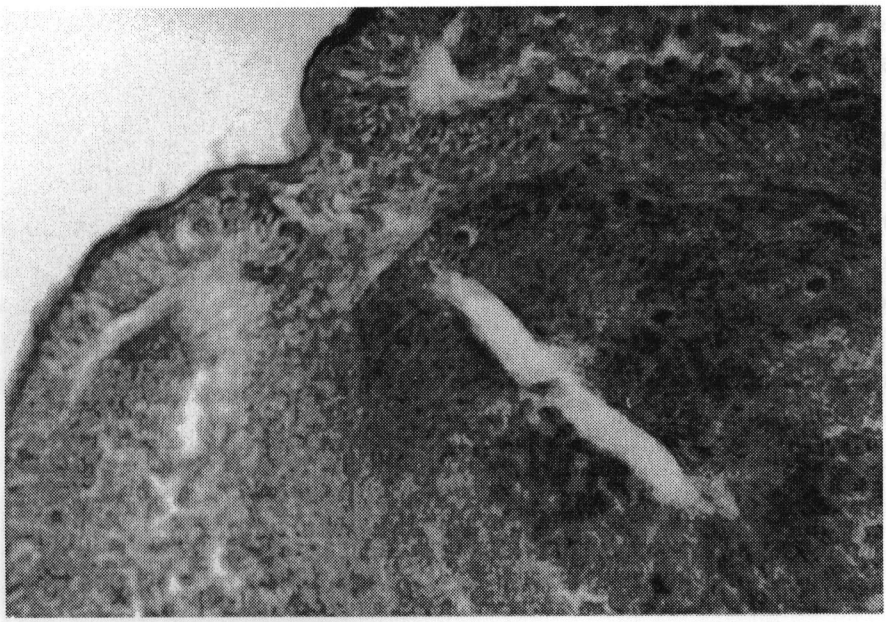

Fig. 3. Four days after hatching larva, pH 5.0-5.2, x 15 000. Nuclear pycnosis (p) with partial brain necrosis. Strongly fused cells of pituitary gland (pg) of irregular shape. Loose arrangement of the optic lobe cells (ol) form abnormal clefts. Haematoxylin and eosin stain.

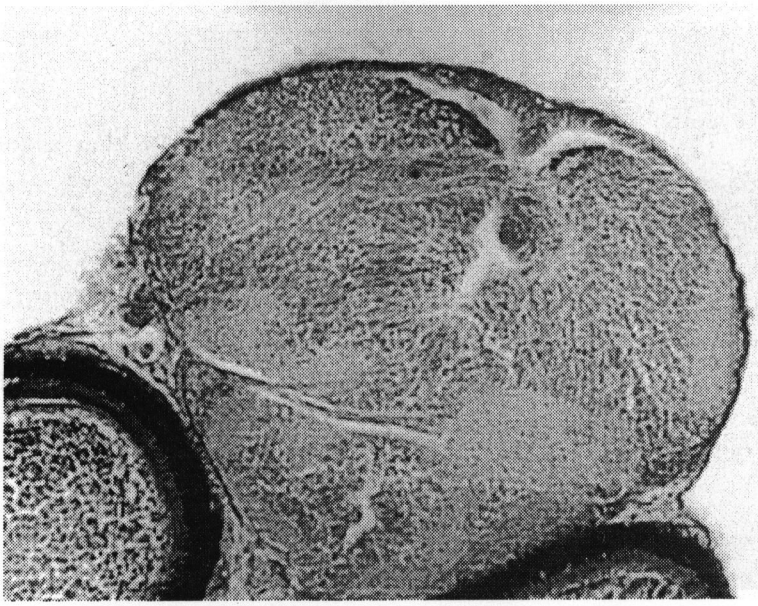

Fig. 4. Four days after hatching larva, pH 7.2-7.4, x 6 000. Normal cellular structure of all visible brain regions: pituitary gland (pg) and hypothalmic infundibulum (h), optic lobes (ol) and cerebral ventricles (cv). Haematoxylin and eosin stain.

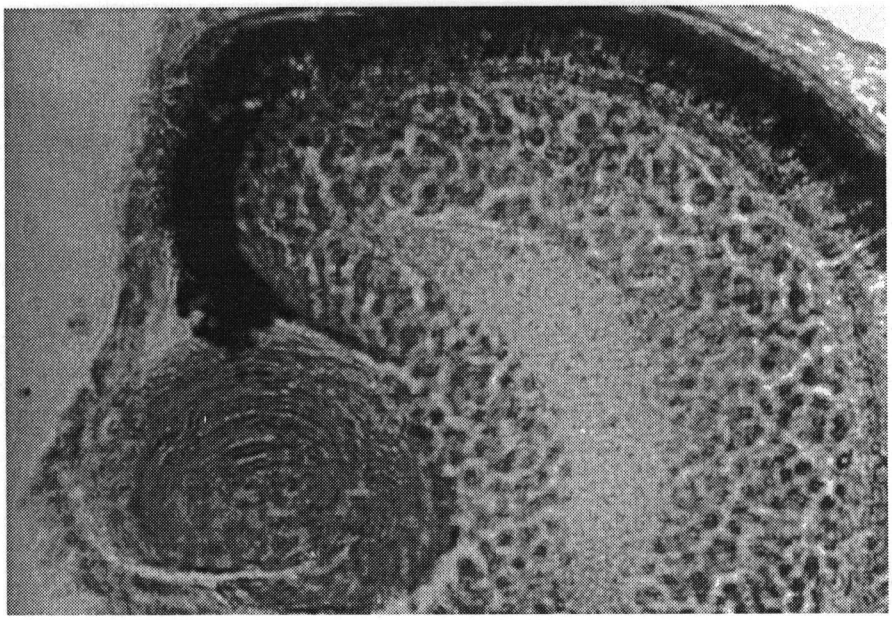

Fig. 5. Six days after hatching larva, pH 5.0-5.2, x 15 000. Optic lens (l) with sloughed and necrotic epithelium (ep). Anaplasia, dysplasia and nuclear pycnosis of the retina (r) and surrounding cells. Lack of free surface between the pigmentary integument and distal segments of photoreceptors. Haematoxylin and eosin stain.

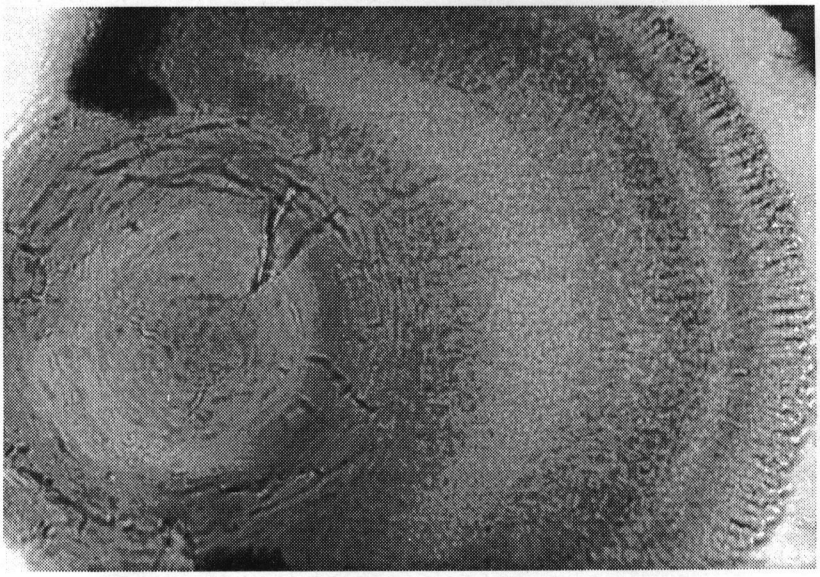

Fig. 6. Six days after hatching larva, pH 7.2-7.4, x 15 000. All normally developed inner structures of the eye structures. Clearly visible free space between the pigmentary integument (pi) and distal segments of photoreceptors (p). Haematoxylin and eosin stain.

eyeball structure. Numerous histopathological changes were observed in the eyes of all age groups of the larvae developing in water of low pH (Fig. 5). The greatest damages of the internal structures of the eyeball were observed in six day old pikeperch since they were exposed to a harmful pH level for the longest period. Lens epithelium of these larvae was necrotic and sloughed, and lens fibers were anaplastic. This effect was accompanied by dysplasia, anaplasia and pycnosis of nuclei of retinal and surrounding cells. This was connected with some disturbances of the differentiation of particular eye structures: 1/ distal segments of photoreceptors, 2/ outer nuclear layer, 3/ outer plexiform layer, 4/ inner nuclear layer, 5/ inner plexiform layer, 6/ fibrous cells of the lens, 7/non-nucleated layer of the lens. The next pathological change of the eyeball observed only in six day old larvae of the experimental group was a lack of free space between the pigmentary integument and the distal segments of the photoreceptors (Fig. 6).

HEART AND BLOOD

The first significant histopathological changes in the heart muscle were observed only in six day old larvae exposed to constant effect of pH 5.0-5.2. These larvae had transudate in all cells together with pericardial effusion which was accompanied by pericardiac exudates in the form of blood serum transudation into the tissues of the body cavity. Moreover, the heart muscle was characterised by poorly developed and leaky walls of the arterial bulb, atrium and ventricle with invisible valves between them (Fig. 7). Some changes also occurred in blood cells. Lysis took place in the heart ventricles followed by denaturation of necrotic erythrocytes and deformation of their oval shape - swelling. Heart muscle in the larvae from all age groups of the control lot was well developed (thick walls and large ventricles) and formed without any cellular abnormalities, and its space was filled with normal blood cells (Fig. 8).

GILLS

Also in gills of the pikeperch larvae some developmental abnormalities were observed as a result of exposure to low pH. Dilation of arterioles and capillaries occurred in the filaments and lamellae in all age stages of larvae incubated at pH 5.0-5.2. Moreover, the larvae maintained at this pH level had general epithelial

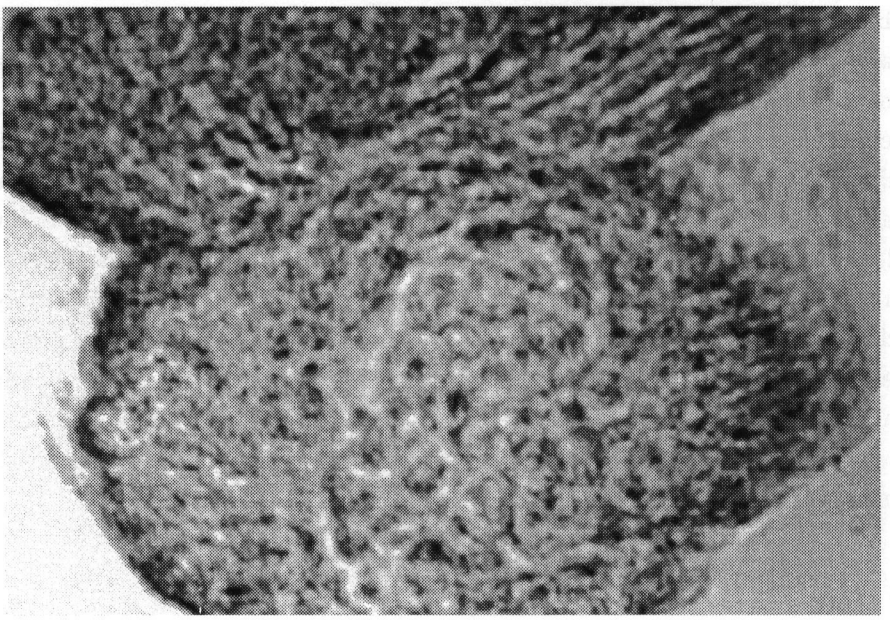

Fig. 7. Six days after hatching larva, pH 5.0-5.2, x 15 000. Heart muscle with leaky walls of the arterial bulb atrium and ventricle. Denaturation of necrotic erythrocytes. Haematoxylin and eosin stain.

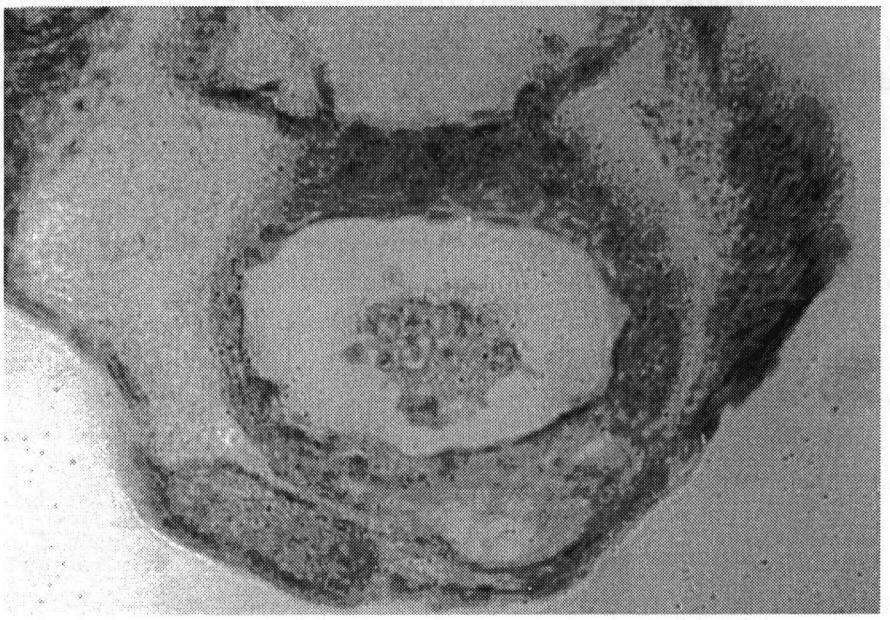

Fig. 8. Six days after hatching larva, pH 7.2-7.4, x 15 000. Well developed heart muscle - thick walls and large ventricles. Haematoxylin and eosin stain.

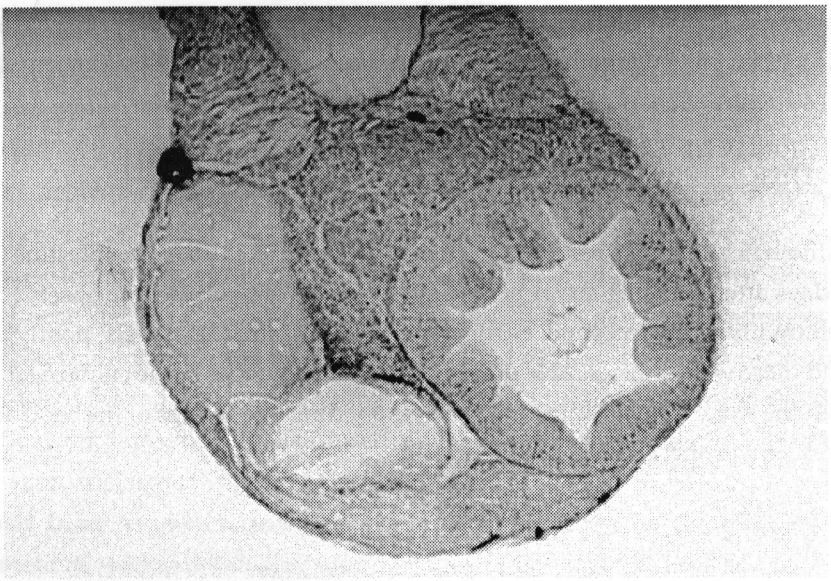

Fig. 9. Six days after hatching larva, pH 5.0-5.2, x 6 000. Deformation and damage of the columnar epithelium (cep) with its weakly expressed structure. Intestine (i). Haematoxylin and eosin stain.

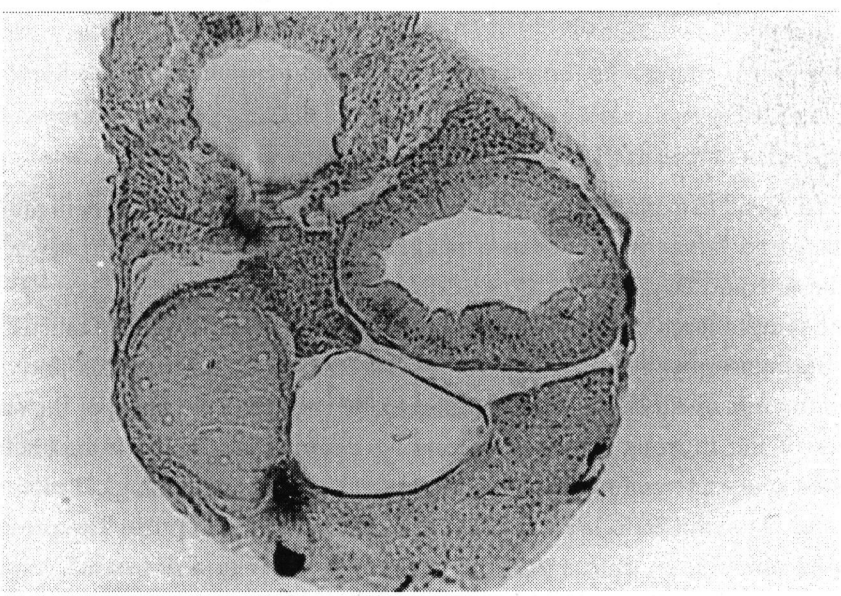

Fig. 10. Six days after hatching larva, pH 7.2-7.4, x 6 000. Intestine with regular edges and clearly visible normally formed cells of columnar epithelium (cep). Haematoxylin and eosin stain.

dysplasia and some sloughing of epithelium which was accompanied by abundant secretion of mucus on greatly reduced gill surface. In the control no abnormalities in the gill structure were observed.

INTESTINE

Pathological changes resulting from low pH occurred also in the intestine (Fig. 9). In six days after hatching larvae some significant deformations were observed in the epithelium lining the intestine. Particular epithelium structures were poorly developed and there were considerable deformations and damages to the cylindrical epithelium. At the same time the intestine in corresponding age groups of the control larvae was well and properly developed (Fig. 10).

YOLK SAC

The delay of the yolk sac resorption was noted already in the one day old larvae incubated in water at pH 5.0-5.2. This prolonged the resorption time which, at the lowered pH, finally occurred five days after hatching. In the control larvae total resorption of the yolk was observed on the fourth day of the larval life.

DISCUSSION

It is difficult to explain the direct reasons of the decreased development rate of fish and increased larvae mortality as a result of water acidification. However, a few hypotheses could be given.

The symptom which is easiest to notice is the increased secretion of mucus. In this study the presence of mucus was observed first of all on the body surface and on gills. Large amounts of mucus were accompanied by a gradual decrease of the size and number of the mucous cells and increasing deformation of the integument. The increased mucus secretion was also determined using the histological methods by Daye and Garside (1976, 1977, 1980) and Vinogradov et al. (1978). The presence of increased amounts of mucus was directly connected with a decreased respiratory capacity of the fish. The gas exchange became more difficult by reducing the oxygen diffusion by mucus secreted on gills and body surface. In the extreme cases a very high acidification can lead to coagulation of mucus covering the gill epithelium or to

protein denaturation in the epithelial cells (Opuszyński 1983). This phenomenon together with increased mortality of the larvae was observed by Alabaster and Lloyd (1980) and Malte (1986).

In case of the fish the acidification of environment leads to the acidification of blood, i.e. acidemia (Vinogradov et al. 1978). In the present experiment, two successive defence reactions were observed in response to an exposure to low levels of pH. The first reaction was an increase of the number of erythrocytes. Red blood cells released from the storage organs levelled the difficulties in oxygen transport to the cells which was accompanied by a dilation of blood vessels. The second reaction was an increase of the cell volume, „swelling" of erythrocytes thus increasing the possibilities of oxygen transport. It was caused by the disturbances of the ion balance and dehydration of plasma (Daye and Garside 1980).

Apart from an enhancement of mucous cell secretion on the gills and body integument as well as a swelling and damages of erythrocytes, the acidic environment also caused some histopathological changes in such organs as the intestine, brain, eyeball and heart.

Abnormalities in the structure of the intestine cylindrical epithelium were also observed by Shrivastava and Dwivedi (1979) at the hatching of carp. It can be assumed that such changes can hinder absorption and impair the alimentary tract development. Necrosis in all regions of brain as a result of exposure to low pH was also detected by Daye and Garside (1980). Pericardial effusion was observed in the heart muscle as well as the reduction of its size. Changes of similar character in the heart muscle of white suckers (*Catostomus commersoni*) incubated at low pH had been earlier reported by Trojnar (1977b). Acidic environment caused histopathological changes in the eyeball (e.g. the lack of differentiation of particular structures together with cellular deformation), which are detrimental for pikeperch because sight is the most important sense of predatory fishes for getting food.

The effect of acidic water on the larval development of pikeperch was also shown in the prolonged resorption of the yolk sac. Similar observations were made by Nelson (1982) and Korwin-Kossakowski (1988) and Vuorinen et al. (1993). Nelson (1982) linked this phenomenon with slowing up the protein synthesis in acidic environment. Korwin-Kossakowski (1988) explained the delayed resorption of the yolk sac in carp larvae incubated in water at low pH by a lack of motor activity resulting from not

filling up the swimbladder on time.

The prolongation of the larval development with simultaneous sublethal changes which occur during the incubation in acidic water are obviously harmful. The larval period, when all organs are not yet fully developed, is the most crucial period in the life of fish. Prolongation of that period, when the larvae are not fully mobile and their body size is relatively small increases their mortality rate which is anyway high that period.

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STRESZCZENIE

WPŁYW NISKIEGO pH WODY NA ROZWÓJ EMBRIONALNY I LARWALNY SANDACZA (*Stizostedion lucioperca* L.) - OBSERWACJE HISTOLOGICZNE

W Zakładzie Ichtiobiologii i Rybactwa SGGW przeprowadzono badania nad rozwojem embrionalnym i larwalnym sandacza w dwóch zakresach pH wody 5,0-5,2 (grupy doświadczalne) i pH 7,2-7,4 (grupy kontrolne). Temperatura wody wynosiła 14-16°C. Podczas doświadczenia pobierano próby do badań histologicznych. Celem pracy było zbadanie struktury komórek i tkanek larw rozwijających się w wodzie o niskim pH. Obserwacje dotyczyły następujących organów: nabłonka, mózgu, oka, serca, skrzel, woreczka żółtkowego i jelita.

Stwierdzono, że niskie pH wody podczas rozwoju powoduje deformację nabłonka, wzmożone wydzielanie śluzu, martwicę komórkową w mózgu, zaburzenia w rozwoju oka i mięśnia sercowego, nieprawidłowości w budowie nabłonka cylindrycznego jelita oraz wydłużoną resorbcję woreczka żółtkowego.

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