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THE INFLUENCE OF WATER pH ON THE EMBRYONIC DEVELOPMENT OF GRASS CARP, *CTENOPHARYNGODON IDELLA*

Nabil K. EL-Fiky
Zoology Department, Faculty of Science, Tanta University

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ABSTRACT

This study was conducted to evaluate the effect of water pH on embryonic development of grass carp (*Ctenopharyngodon idella*). Eggs of grass carp were incubated in different levels of acidic and alkaline water. The general embryonic development was described briefly. Moreover, details were given as to the epiboly stage.The effect of the following pH values was tested: acidic water (4.5, 5.0 and 5.5) and alkaline water (9.5,10.0, and 10.5) on the embryonic development. 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 20h after fertilization. Mortality during the development indicate that the most sensitive stages were embryos just after fertilization and at hatching period. This study indicates that embryonic development of grass carp was possible within the range of pH 5.5 - 9.5, although at the extreme pH levels, certain disturbances and increased mortality occurred. The changes and anomalies during development might have caused larva mortality during hatching. Survival of the embryos was calculated during development The percent of hatched eggs was calculated in relation to the number of fertilized eggs. The highest percentages of dead embryos were noticed at the extreme pH (4.5) and pH (10.5). The highest percentage of eyed embryos were recorded at pH (5.5) and pH (10.0).The quality of the hatched larvae in abnormal levels of pH was worse compared to the control. They died gradually from the beginning of rearing in neutral water.

Light and scanning electron microscopy (SEM) were used to study the effect of abnormal pH on the development of free neuromasts and structure of the eye. Excessive number of free neuromasts were found on the head region of hatched larvae incubated at alkaline pH. Differentiation of retina was inhibited at the

same condition. Acidic pH caused partially delaminated lens epithelium and reduce the number of chromatophores .

However, in the view of the results obtained, it can be stated that, even a short-term change of pH (resulting from industrial sewege) may adversely influence fish population, especially if occurs during embryonic development.

INTRODUCTION

The relationships between organisms and their environment are extremely complex, and it is difficult to design experiments that simultaneously take into consideration all the major biological, chemical and physical factors that may influence the embryonic development. PH is one of the most important factors, and its effect on embryonic development rate is unknown. PH may also affect reaction of fish organism to other environmental factors (Korwin-Kossakowski, 1988; Korwin-Kossakowski and Jezierska, 1985). The early life stages represent a sensitive part of the life cycle of fish. 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 and Jezierska and Witeska, 1995). 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 and Garside, 1980a; Rombough, 1982; Norrgren and Degerman, 1993). High water pH very rarely occurs in nature. In common carp ponds temporary alkalization may occure during hot summer, usually due to algal blooms, reaching sometime pH over 10.0 (Alabaster and Lloyd, 1980). Industrial sewege may also cause water alkalization. High pH (9.5-10) may result in high mortality and developmental disturbances in various fish species (Daye and Garside, 1975; 1977;1980a,b; Jezierska, 1988; Korwin-Kossakowski, 1992). Water pH is one of the most environmental factors affecting fish. Excessive acidification as well as alkalization are detrimintal to fish development (Jezierska and Witeska, 1995; Ostazewska and Wojda, 1997 and Ostazewska, *et al.* 1999). According to EIFAC criteria (1971)), safe water pH for fish ranges from 6.5 to 8.5. pH and nitrite are the main quality parameters in management of fish culture activities, their acute toxicity values for fishes are lacking. Safe chronic exposure levels are largely unknown. Since nitrite and pH affect not only the survival, but also growth rate and disease

resistance, these factors may interact and impose a concerted effect on embryonic development (Jezierska and Witeska, 1995) and growth of fish larvae (Yusoff *et al.* 1998).

Hatching is a process, in which a developing fish embryo sheds its envelope, thus changing its life from intracapsular to a free-living type (Balon, 1975). In teleosts, the emergence of an embryo is achieved in two sequential steps: (a) enzymatic dissolution or softening of the proteinous layers of the egg envelope (chorion), and (b) rupturing of chorion remnants by mechanical action such as pressure exerted from within (movements, intial body tension) or mastication by the embryo (Daye and Garside, 1980a,b;Yamagami, 1988). The hatching enzyme (hatching chorionase) is produced by the embryo's unicellular hatching gland cells, where the enzyme is stored until the time of hatching (Yamagami, 1981,1988; Ostazewska ,1990 and Oppen-Bernsten, 1990). The mechanism of hatching process was controlled by (a) genetic differentiation of hatching glands and hatching proteinase synthesis. (b) physiologiacal 'chain of events', which would explain how the stimuli received by the embryo from its external and /or internal environments are linked with each other and transformed into stimulus (stimuli) triggering the activity of the hatching glands leading to the secretion of the hatching proteinase and finally resulting in embryonic decapsulation (Oppen-Bernsten, 1990). PH is one of the most effective factors in the external environment around the embryo that may affect the secretion of the hatching proteinase.

This study was undertaken to evaluate the effect of short term exposure of this water pH quality factor on the embryonic development of the grass carp (*Ctenopharyngodon idella*) to determine the most sensitive stages of development and to understand the role of the pH stimuli on the hatching process.

MATERIAL AND METHODS

Fertilized eggs of the grass carp (*Ctenopharyngodon idella*) were obtained during spawning artificially induced by pituitary injection in the hatchery station of Fowa, kafr EL-sheich governorate. The fertilized eggs were transferred in a glass like column with a strong continuous aeration to prevent sticking of the eggs together by their chorions. In the laboratory. the fertilized eggs were spread with plastic spoon in the incubation sieves, about 300

eggs in each, and maintained under strong aeration for moving the eggs to avoid the adhesion. The sieves were placed in the aquaria with water of various pH. Each group of eggs was incubated in three replicates. Temperature was maintained at level of 20±1° C.

Observations using a binocular microscope started two hours after fertilization, where fertilized eggs were placed in Petri dishes filled with the incubation water. From the end of epiboly onward, a number of embryos were dechorionated manually with watchmaker's forceps. The embro was mounted on a adepression slide without an overlying coverslip, in standard embryo medium (Westerfield, 1994) containing 1.5% methyl ceilulose. Sometimes, the embryo was mounted between coverslips, in1% agar, for positioning. The higher-magnification views showing parts of embryo were taken using an immersion objective. In the following, the general embryonic development was described briefly. Moreover, details were given as to the epiboly stage.

In the first expirement, the effect of the following pH values was tested: Control(7.5); acidic water (4.5, 5.0 ,5.5) and alkaline water (9.5, 10.0, 10.5). Water was acidified and alkalized every 12 hours by using 1% sulphoric acid or 1% sodium hydroxide. Such frequency of pH control assured that egg stages 80% of time in adjusted pH.. Non-fertilized eggs (white and opaque) were removed from the sieves. Live fertilized eggs were transparent and yellowish.Survival of the embryos was calculated during development. The percent of the hatched eggs was calculated in relation to the number of fertilized eggs.The dead eggs were divided into two groups.The first one included the eggs before the appearance of eye pigments (cleavage, blastula, epiboly, gastrula and segmentation stages). The second one included the eggs after the eye pigmentation (eyed eggs). The percentage of these groups were calculated in relation to the number of fertilized eggs. Newly hatched larvae were inspected and counted, then transferred to other sieves in clean water aquarium. The observation of hatching larvae allowed to evaluate their quality.

In the second experiment, the larvae obtained in the series I (hatched group) were used for further rearing. The aim of the experiment was to evaluate the effect of embryonic development conditions on further survival of the larvae. Fifty larvae from each group exposed to (pH 5.5 and 9.5) were transferred to small tanks, and reared at the same temperature though in normal pH (7.5), and

well aerated water. Survival of the embryos was calculated during development.

Samples for histopathological examination were taken. The samples were fixed in Bouin-Holland solution, embedded in paraffin, and cut into slices of 5 um thickness . Slices were treated according to standard histological procedure, and stained with haematoxylin and eosin.

For scanning electron microscopy (SEM), the dechorionated embryos and the newly hatched larvae were fixed overnight in 2.5% glutaraldehyde solution in phosphate buffer at pH 7.4, postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol, dried in a critical point dryer, mounted on stubs, and sputter coated with gold palladium in a polaron E5100 unit. Specimens were viewed with JEDL JSM. 5200 according to Kawase (1996) method.

RESULTS

Basic mechanisms of embryonic development in grass carp

The fertilized eggs (Pl.1,a) developed very slowly at standard temperature (Fig. 1,a), the cytoplasm streamed to the animal pole, The first cleavage occurred 1½ -2½ h, (Pl 1,b). The cleavage started as equal and vertical at the first and third cleavages being in the same plane and perpendicular to the second (Fig. 1, a,b). The furrow arised near the animal pole and progressed rapidly toward the vegetal pole, passing through only the blastodisc and not the yolky region. The cleavage cycles continue until the blastodisc became ellipsoidal in shape (Pl. 1,c). The cleavages occurred in quick succession until the blastula stage was reached 3½ -5½ h, forming a cap of cells at one pole of the yolk (Pl. 1,e; Fig. 1,c). The term blastula was to refer the period when the blastodisc began to look ball like. By 5½ hours, the big celled morula became rounded and more regular in shape..It consists of a multicellular blastoderem in which three cell types can be distinguished: An outer layer of enveloping cells (evl), formly attached to each other, a yolk syncytial layer (ysl) or periblast, covering the yolk and between these two layers, the inner or deep cells (dc) which solely contribute to the developing embryo (Fig. 1,d). Shortly afterwards, the epiboly began (Pl. 3,a,b) and the cell mass gradually surrounded the yolk (Fig. 1,e). Deep to the blastodisc, the (ysl) surface began to dome toward the animal pole (dome stage).During epiboly, rearrangement of the deep cells occurred

leading to the formation of the embryonic axis. At about 50% epiboly (PL. 3,b), gastrulation started. During gastrulation period (7½-10h) epiboly continued and in addition, the morphogenetic cell movements of involution, convergence and extension occurred, producing the primary germ layers and embryonic axis. Involution was responsible for the formation of the hypoblast below the epiblast cell layer. At 12-14 h. after fertilization, epiboly was completed (Fig. 1,f). After completion of epiboly the embryonic axis has elongated and the body form became gradually visible. Epiboly was immediately followed by the formation of the first somite (Pl. 3C, Fig. 1g). The process of somite (S) formation continued in caudal direction until 14-24 h., when 33 somite were present. Anterior somites develop first and posterior ones last. At 31 hours, the head and tail region could be distinguished and the eye bulbs were discernible. At 34 hours, the ear vesicles were formed, the tail had freed itself from the egg (Pl. 4C, Fig. 1,h)., and the first movements were observed. The embryo began to grow and elongate,and the tail was straightened at 37 hours , pharyngula stage (Fig. 1,i). Moreover, the eye lense has been formed, and otoliths were present in the auditory vesicle (Pl. 4d). From then onward, the head gradually curved back from the egg . The heart began to beat just at the onset of the pharyngula period . Hatching occurred between 48 and 70 hours after fertilization and shortly before that stage the blood began to circulate. At 48 hours, the head was detached fully from the yolk, the eyes were pigmented, and the larva had grown in length considerably. During the hatching period, the embryo continued to grow at about the same rate as earlier. Morphogenesis of many organ rudiments was rather complete and slows down considerably, with some notable exceptions including the gut and its associated organs. However, these endodermal structures were difficult to visualize in the living embryo because their deep positions, though they are not considered completed here. The time of hatching was not useful as staging index, because individuals within a single developing clutch hatch sporadically during the whole 3rd day of development at standard temperature. In the following 3 days, the yolk gradually disappeared and the larvae started to feed.

Treated eggs

The obtained results indicate that the embryonic development of grass carp was possible within the pH range 5.5-9.5. At pH 4.5, 5.0 and 5.5, the lower the concentration of water, the higher the percentage of dead embryos. Moreover, at pH (9.5, 10.0 and 10.5), the higher the pH of water, the higher the percentage of dead embryo. The highest percentages of eyed embryos were recorded at pH 5.5 and 10.0, as they constitute 15% and 30%, respectively (Fig. 2). The highest percentage of normal hatched larvae were noticed at pH 5.5 and 9.5 as they constitute 26% and 23%, respectively. The results revealed that pH 5.5 and 9.5 did not affect the duration of development up to the beginning of hatching compared to the control. A slight delay of development occurred in the eggs incubated at pH 5.0 and 10.0.

The results of the present study indicate that water pH affects cleavage and blastula formation. The early stages of cleavage, gastrulation and organogenesis were most sensitive to abnormal pH and revealed many morphological changes. The changes and anomalies during development might have caused embryonic mortality. Most interesting of abnormalities in embryo formation concerned cleavage. In strongly acid (pH 4.5) and strongly alkaline (pH 10.0 and pH 10.5) water ,some changes in cleavage process were observed. At the cleavage period, (32-64 cell stage), blastomer cells were uneven and were placed irregularly on the top of the yolk (Pl. 1,d). Eggs incubated at pH 9.5 revealed disturbances at blastula stage. In addition at pH 5.0 cells tended to separate from the yolk (Pl. 1,f)). At pH 7.5, (neutral) the blastula consisted of numerous small cells with larger ones placed on them (pl. 1,e). At the pH 9.0, the blastomers were uneven (pl. 2,a) and not fixed on the yolk . In pH 5.5, the cells of the blastula become fused in an irregular way (Pl. 2, b,c,d). At pH 4.5, most of the eggs stopped to continue their division (Pl. 2,e). Observations of epiboly stages revealed some abnormalities in embryo formation. In weakly acidic (pH 5.5) water, some changes in the embryo and yolk mass were observed (Pl. 3,d) and a considerable frequency of body malformations were also observed. At pH 9.5, the eye bulbs were not discernible (Pl. 4, a) and the tail region could not be distinguished (Pl. 4,b),. At pH 5.5, the head had freed itself from yolk (Pl. 4,c) , while the tail failed to separate from yolk. At pH 7.5 (control). optic cups were clearly visible, the number of somites has increased (Pl, 4,d). In the following hours the tail

gradually became separated from the yolk-sac until was straightened at 48-70 h. after fertilization, (Pl. 5, a). At this stage, the eye lenses, optic vesicles, heart, blood vessels and all the myotomes were present and the gut was formed. Soon the head starts to lift from the yolk-sac, the growing eye became hatched and started its larval life. At pH 5.5 and pH 9.5, the embryo failed to hatch and revealed many morphological changes (Pl. 5, b,c,d)..At hatchery period, the larvae were very sensitive to the environmental impacts. It was possible that the sensitivity of the larvae to acid pH levels (not to alkaline) was higher. Some of these exposed larvae died just after hatching. It was probable that the difference in relative sensitivity occurred because the newly hatched larvae were exposed directly to water, whereas the embryos were shielded by egg envelope and perivitelline fluid. The second reason for death was the changes and anomalities during development that caused larva mortality during hatching. Some encapsulated embryos failed to hatch. It was possible that the quantity and activity of chorionase were considerably reduced in relation to the control.

In the second expirement, the hatched larvae were used for further rearing. The quality of the larvae hatched in abnormal pH was worse, compared to control at all pH levels.The hatched larvae which were exposed to pH 9.5 died gradually from the beginning of rearing. All individuals died after 9 days, while, the larvae exposed to pH 5.5, died between 12^{th} and 14^{th} (Fig. 3).

The normal newly hatched larvae had free neuromasts with well developed cupulae around the eyes (Pl. 6, a,b). The newly hatched larvae possess neuromasts on each side arranged between the eyes and around the nasal cavities . Some of these neuromasts lie in a position which suggests that they will be incorporated into the later development of the head lateral line canals (Kawase, 1996). The free neuromasts has two functions: mechanoreception and chemoreception. It becomes functional as a mechanoreceptor when it has well developed cupula. The normal larvae become able to avoid an obstacle at hatching stage, when they have free neuromasts and non-functional eyes (lens and retina are poorly differentiated). This indicates with no doubt that the free neuromasts are functioning , and the larvae can detect the origin of mechanical stimuli at this early stage. Excessive number of free neuromasts were found on the head region of hatched larvae incubated at pH (10.0), (Pl. 6,c,d). Alkaline pH (10.0) caused also some anomalies in fish eye. Differentiation of retina was inhibited (Pl. 7,b,c). Excessive number of mucous cells

were observed on body surface of hatched embryo incubated at the same condition. Epithelium was mostly damaged at dorsal part of head. Necrosis of mucous cells was also observed. In the control fish mucous and epithelial cells did not show any anomalies. At pH 5.0, a retardation of development of these superficially located neuromasts and damaging of cilia of their cupulae were found on the head region. It is possible that the sensitivity of the newly hatched larvae to abnormal pH levels was also high and some of them died just after hatching.

Histopathologically, it was found that the acid pH (5.0) caused some anomalies in fish eye ball structure. The lens epithelium was partially delaminated. Differentiation of retina was inhibited and the number of chromatophores was reduced. (Pl. 7 b,c). In contrast, control fish did not show any pathological changes in eye ball structure. Lens epithelium was smooth, and retina had correctly developed layers (Pl. 7,a).

DISCUSSION

The results of the present study indicate that the developmental stages between fertilization and the 'eyed egg' stage, cleavages, gastrulation and organogenesis appear to be most sensitive to acid and alkaline water. The morphological changes and anomalies during development might have caused embryonic mortality. Similar results were observed by Kijashko and Volodin (1978), and Jezierska and Witeska (1995). The highest mortality at the beginning of embryonic development, up to the 'eyed egg' stage was also observed by Trojnar (1977), Hulsman and Powles (1983) and Curtis, (1989). There are some data indicating that the highest mortality at the beginning of development occurs only at the lowest pH values, especially in those where no eggs survive to hatch (Runn *et al.* 1977; Daye and Garside, 1979, Rask 1983). The present results showed similar pattern for low pH values (4.5 and 5.0) but no other data were found in the literature, except Kijashko and Volodin (1978), who noted that the highest mortatlity occurred during the first period of embryonic development of ruff *(Acerina cernua)* eggs incubated at pH 9.0. This was also confirmed for rainbow trout that was subjected to ammonia intoxication (Solbe and Shurben,1989). The embryonic development of grass carp was possible within the pH ranged 5.5 and 9.5, but only about 30% of eggs hatched.

PH did not affect the duration of development up to the beginning of hatching. In contrast, the prolonged duration of egg development was observed by Daye and Garside (1979) and Rask (1983). In common carp, Jezierska and Witeska, (1995) revealed that the eggs incubated at the level of pH 4.5 and 10.5, did not develop longer than 24h. In grass carp, at pH 10.5 and 5.5, eggs did not develop longer than 20h but the eggs kept at pH 5.5 and 9.5 from 'eyed eggs' stage developed and even hatched. The present results show also that increasing acidification and alkalization caused proportional increase of mortality of newly hatched larvae. It is known that during 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 the field by Jordahl and Benson (1987) and in the 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 in relative sensitivity occurred because the newly hatched larvae were exposed directly to 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 grass carp development is possible (5.0 and 10.0) the share of deformed hatch was lower. Some authors confirm that deformations may result from various disturbances caused by low pH. These disturbances are due to 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).

In grass carp, the delay of hatching after the stage of eyed eggs and during the hatching stage may be due to the limiting factors or mechanisms involved in the hatching process. PH might have induced some changes in the structure of the chorion making it more difficult to break and larvae remain encapsulated and can't hatch. The second reason was the reduction of the chorionase activity. Runn *et al.* (1977) found out that the same main limiting factors during hatching process are connected with digesting of the egg envelope by the chorionase. Investigation of this enzyme from several species of fish has shown that the activity of the enzyme is pH dependent with maximum activity occurring around pH 8.0. A reduction in pH decreases this activity (Yamagami, 1973, Hagenmaier, 1974; Waiwood and Haya, 1983; Kugel *et al.* 1990). Peterson (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.Examination of the effect of pH on proteolytic action of Coregoninase hatching enzyme showed its maximal activity at alkaline pH around 9. as also observed in most hatching enzymes of other teleost species (Hagenmaier, 1974). A rapid decrease in enzyme activity was observed at pH values lower than 7, and the activity dropped to zero at pH 6. Since acidification of lakes is a major problem, low pH levels may reduce the success of Coregoninase hatching and cause a reduction in their abundance in numerous lakes. Runn *et al.* (1977) indicated that the transfer 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 (1990) found changes in the ultrastructure of the 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 the present experiment may be an explanation for the delay of grass carp hatching.The experiment showed that the level of pH strongly affects the mortality of grass carp embryos. It was increased in acidic as well as in alkaline water.

The present results indicate that the embryonic development of grass carp is possible within the pH range of 5.5 and 9.5. At pH 5.5, only about 30% of eggs hatched while 62% of the embryos were dead. At pH 9.5, about 32% of eggs hatched and 60% were dead. Mortality during the development indicates that the most sensitive stages was the first period after fertilization until eyed stage and hatching larvae. More than 60% of the eggs did not hatch when exposed to pH 5.5 and 9.5. Those that did hatch had tail deformities. Similar tail malformations have been observed in Sheephead minnow (*Cyprinodon variegates* , Cyprinodontidae) after exposure to herbicide trifuralin (Couch *et al.* 1979)), in Japanese carp (*Puntius gonionotus*, Bleeker) after exposure to hydrogen sulfide (H_2S)

Yusoff *et al.* 1998) and in other fish, due to chemical contaminants in general (Moore and Hixson, 1977; Muramoto, 1981).

In the normal and newly hatched larvae of grass carp, neuromast cells were observed on the head anterior to the auditory region. Neuromast primordial were first detected just behind the auditory region. The free ends of the sensory cells were directed towards the surrounding environment. In fishes, the neuromasts are either contained in canals or located on the epithelium of the head, trunk and tail. However, superficial position of these sensory organs are characteristics of fish larval stages (Muniz, 1979; Blaxter *et al.*, 1983a,b; Metcalfe *et al.*, 1985 Janssen *et al.*, 1987 Lashein, 1999). Superficial position of the sensory neuromasts in the developing larvae may thus perform an effective function for receiving vibratory responses which may be in the form of food substances or attacking predators. Exposing the newly hatched larvae to low levels of PH involves a retardation of neurological development of the free neuromasts. Lashein, (1999) found that the exposure of the newly hatched larvae to environmental factors can alter the normal structure of neuromasts and consequently may affect its ability to react with the environment.

The present results revealed that changes observed in the body surface of the newly hatched larvae incubated in alkaline water were *a typical reaction to inappropriate pH values, and comprised* excessive secretion of mucous. Similar reaction of epithelium was observed in newly hatched larvae of Atlantic salmon by (Day and Garside, 1976; 1980b). These authors observed extensive necrosis, peeling of epidermis and exposure of inner skin at pH over 10.0.The abnormal levels of pH cause histopathological changes in the developing eyes of hatched larvae of grass carp. Limiting external membrane of retina was lacking and the number of chromatophores was reduced. Similar pathological effects were noticed in Atlantic salmon by Day and Garside (1980a,b). The changes consisted of anaplasia of lens filaments, peeling of eyeball epithelium, and distression of iris chromatophores.

In conclusion, with increased industrialization, contamination of the environment may affect the pH of lakes and reservoirs. Environmental conditions during embryonic development may affect not only embryos, but also hatched larvae, and their further development that may cause a severe destruction of fish economy. However, further studies are needed to investigate the effect of

different levels of pH the different organs of developing embryos and hatched larvae during further rearing.

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LEGEND OF FIGURES.

Plate (1): (a-f), face views of early embryonic stages, (a), fertilized egg; (b), first cleavage, (c), cleavage cycles continue until the blastodisc become ellipsoidal in shape. (d) blastomeres cells were uneven and were placed irregularly on the top (incubated in alkaline water, pH. 10.0). (e), blastula stage, a cape of cells at one pole of the yolk (incubated in neutral water, pH. 7.5). (f) the cap of cells tended to separate from the yolk (incubated in acid water, pH. 5.0). Abbreviations, (bl), blastodisc; (dc), deep cells; .(evl), enveloping layer (y), yolk; (ysl), yolk syncytial layer.

Plate (2): (a-e), face views of blastula, (a), blastomeres were uneven (incubated in alkaline water, pH. 9.5). (b), the cells of blastula become fused (incubated in acide water, pH. 5.5). (c,d,e), disturbances in blastula stages. (c,d), (incubated in pH. 10.0), (e), (incubated in strongly acid water, pH. 4.5). Abbreviations, (bl), blastodisc; (e), eye; (s), somite; (t), tail; (y), yolk.

Plate (3): Different stages of epiboly. (a), cell mass gradually surrounded the yolk. (b) about 50% epiboly, gastrulation

started. (c), epiboly was immediately followed by the formation of the first somite . (d), changes in the progression of epiboly and degeneration of yolk. (incubated in acid water, pH. 5.5). Abbreviations, (bl), blastodisc; (e), eye; (s), somite; (t), tail; (y), yolk.

Plate (4): (a-d): A considerable frequences of body malformations (a), the eye bulbs were not discernible (incubated in strongly alkaline water, pH 10.5). (b), the tail region could not be distinguished (incubated in alkaline water, pH.

e (incubated in alkaline water Ph. 10.0). (b-d), all embryos failed to hatch (incubated in acid water, pH. 5.0). (e), normal newly hatched larvae (incubated in neutral water, pH. 7.5).

Plate (6): Scanning electron microgarphs showing, (a,b), normal newly hatched larvae had free neuromasts (arrows) with well developed cupulae around the eyes. (c,d), Excessive number of non-functional free neuromasts were present on the head region (arrows) and Undifferentiated eyes (e), (incubated in alkaline water, pH. 10.0). .X 1200

Plate (7): Photomicrographs of transverse sections through head region showing (a), the normal structure of eye of the normal newly hatched larva (incubated in neutral water pH. 7.5). (c,d) , differentiation of retina was inhibited and the number of chromatophores was reduced (incubated in acid water pH. 5.0). Abbreviations , (ce), cone ellipsoid; (cn),cone nuclei; (g), ganglion layer; (in), inner nuclear layer ;(ip), inner plexiform; (l), lens ; (os), outer segment of single cone; (pe), pigment epithelial layer; (u.r), undifferential retina. X 240.

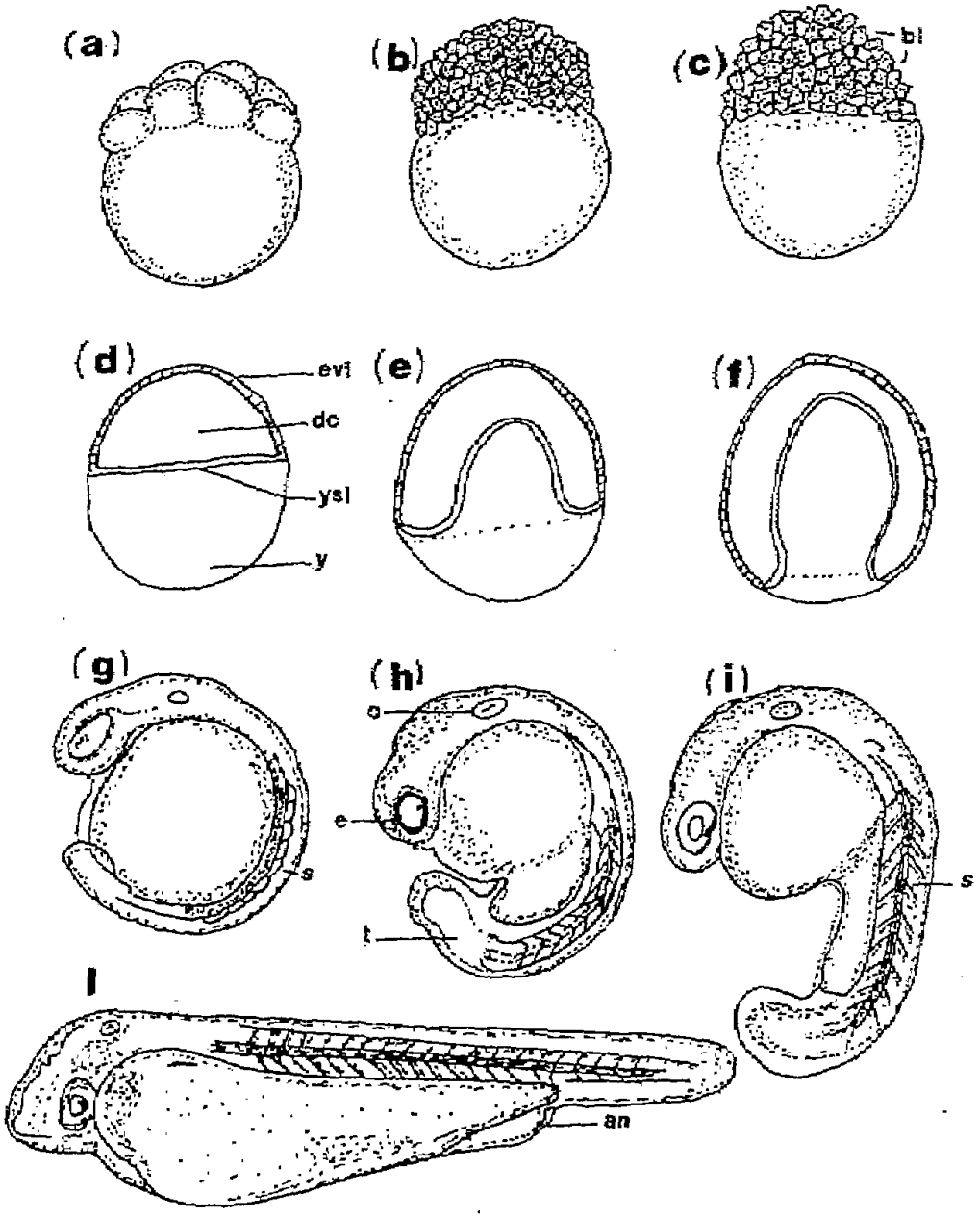


Fig. (1): Schematic diagrams illustrating various stages of the embryonic development of grass carp. (a,b), cleavage stages, (c), blastula; undifferentiated ball of cells perched on top of the non-dividing yolk. (d,e,f), three different stages of epiboly, just after the formation of yolk syncytial layer (ysl). (d), the 4h. stage, shortly before the onset of epiboly. (e), the 50% epiboly stage. (c), the 90% epiboly stage. (g), segmentation stage showing a variety of morphogenetic processes which occure during segmentation period. The notochord differentiated and somites appear sequentially along the axis. (h) tail bud stage, tail extends greatly. (f), pharyngula stage, embryos showing all the character of vertebrate phylotypic stage. (l), the newly hatched larva. Abbreviations, (an), anus; (bl), blastodisc; (dc), deep cells; (e) eye; (evl), enveloping layer; (o), otic vesicle; (s), somite; (t), tail; (y), yolk; (ysl), yolk syncytial layer.

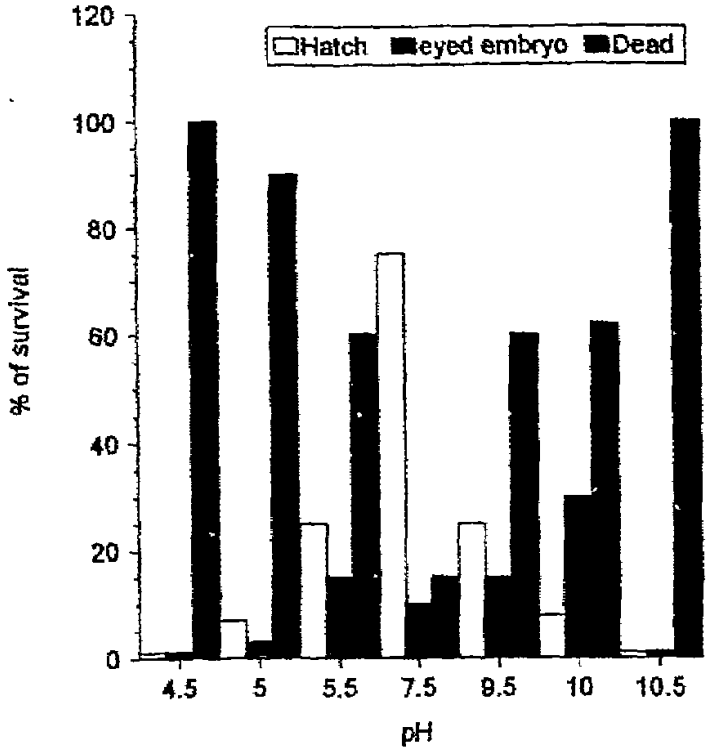


Fig. (2) : The effect of pH on survival (average from 3 replicates).

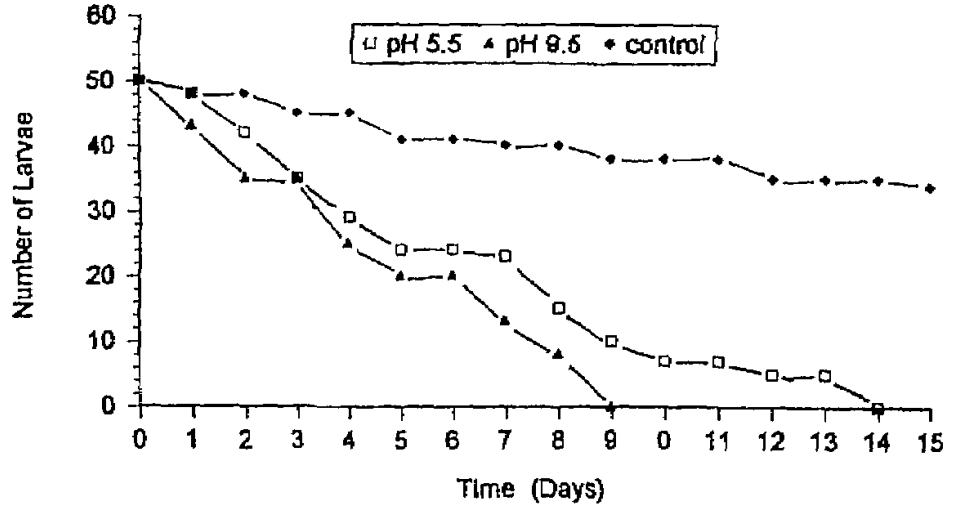
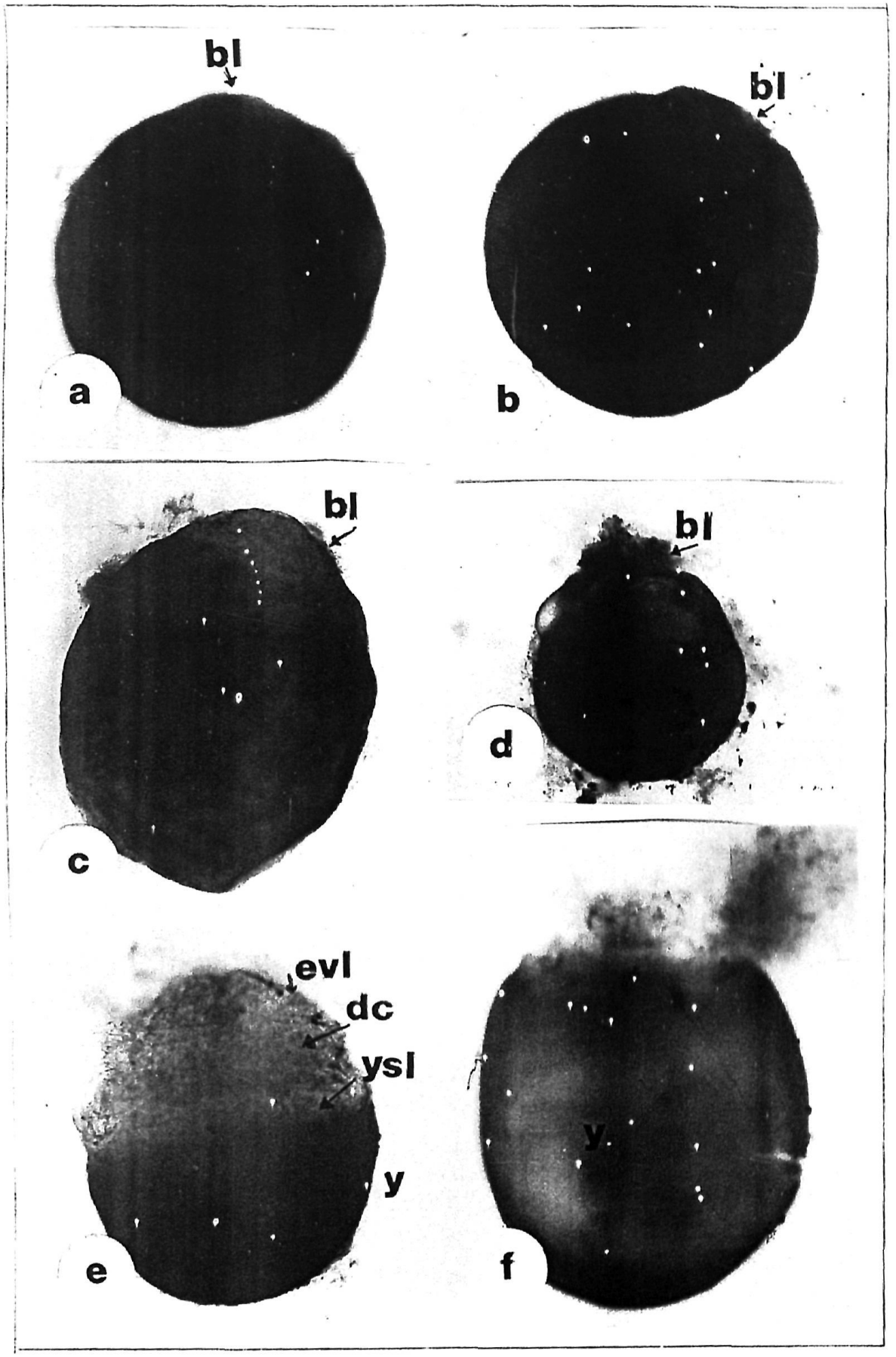
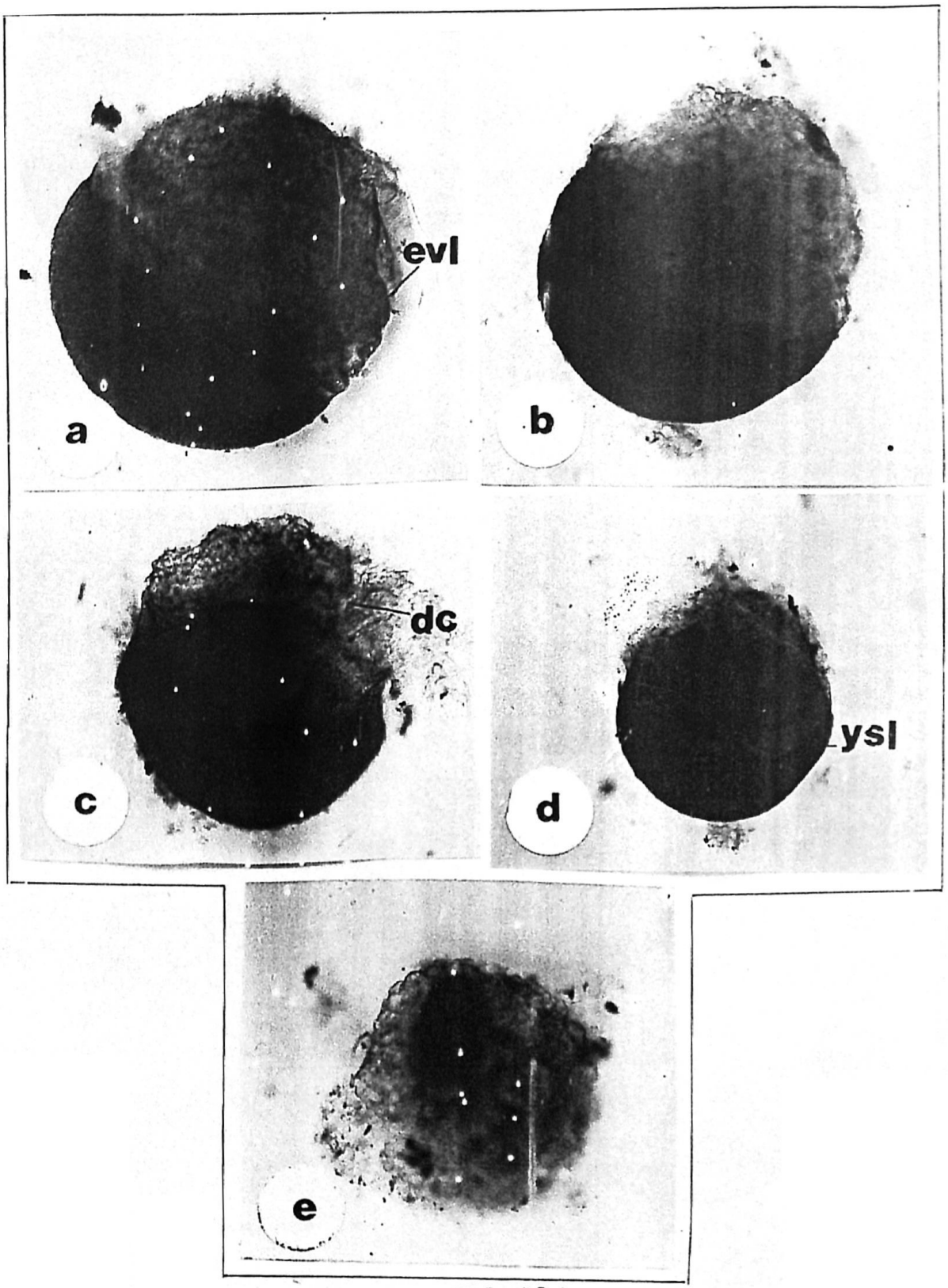


Fig. (3). Survival of hatched larvae incubated in different levels of alkaline and acid water.



Pl.(1)



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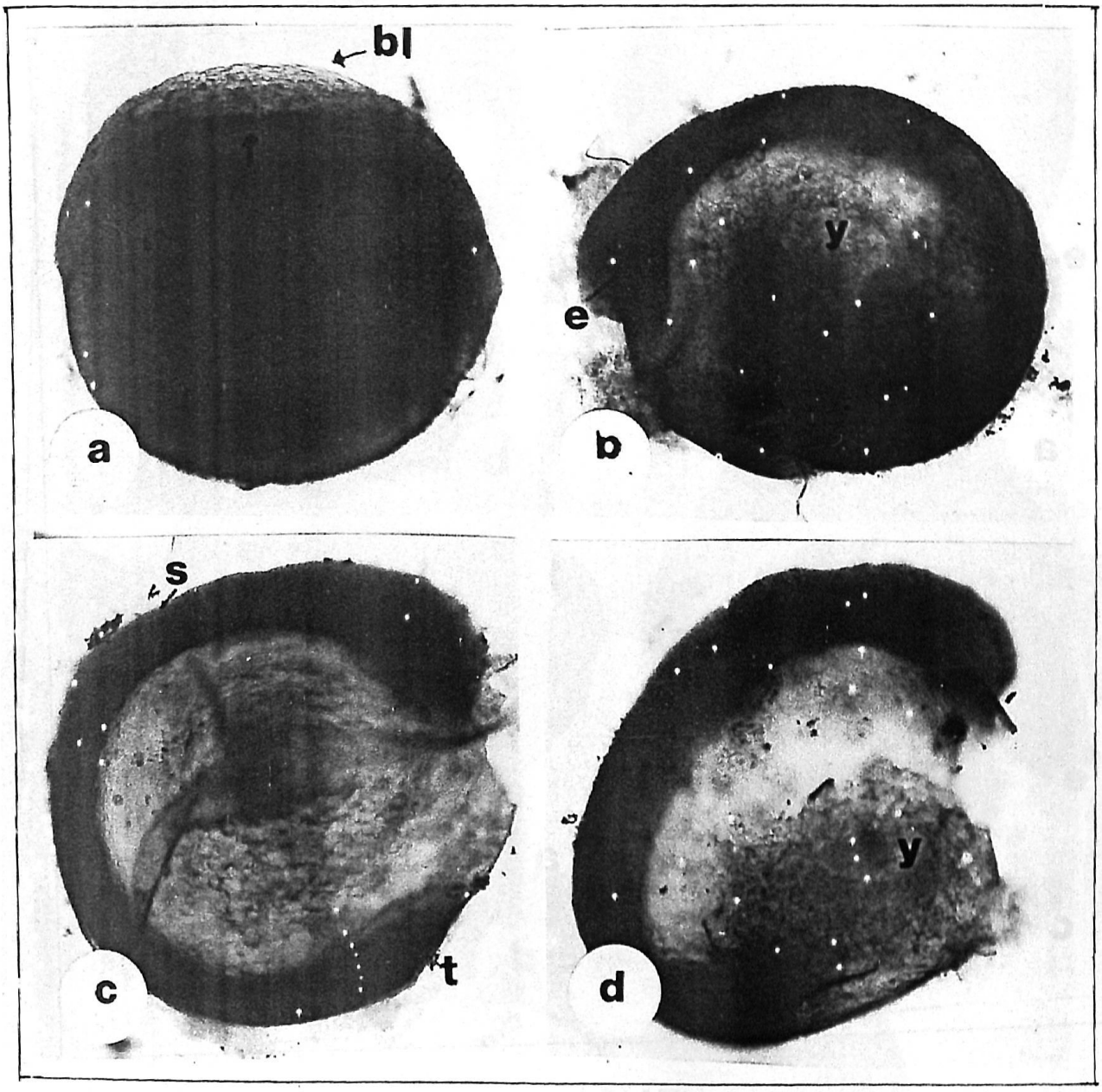
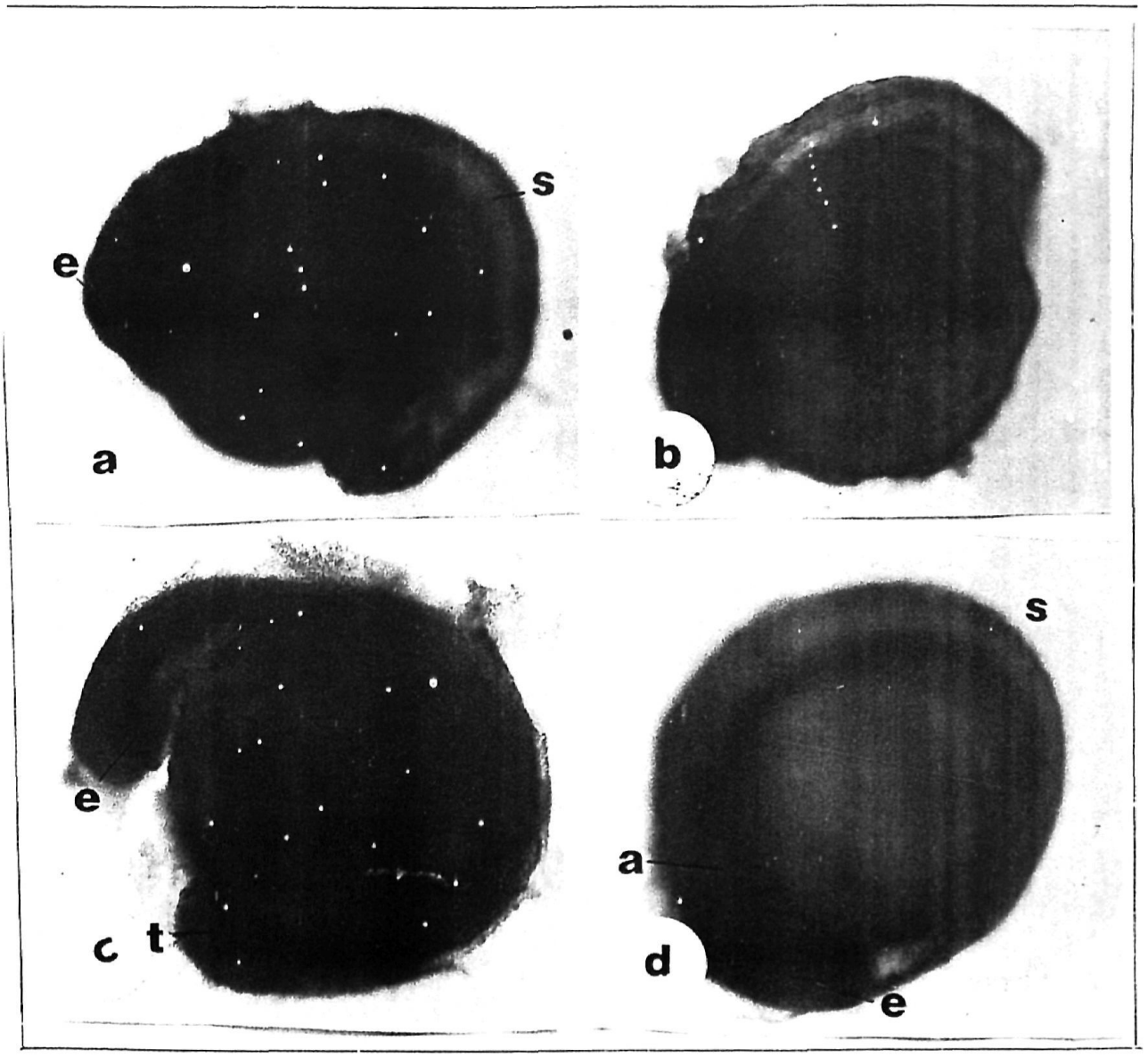
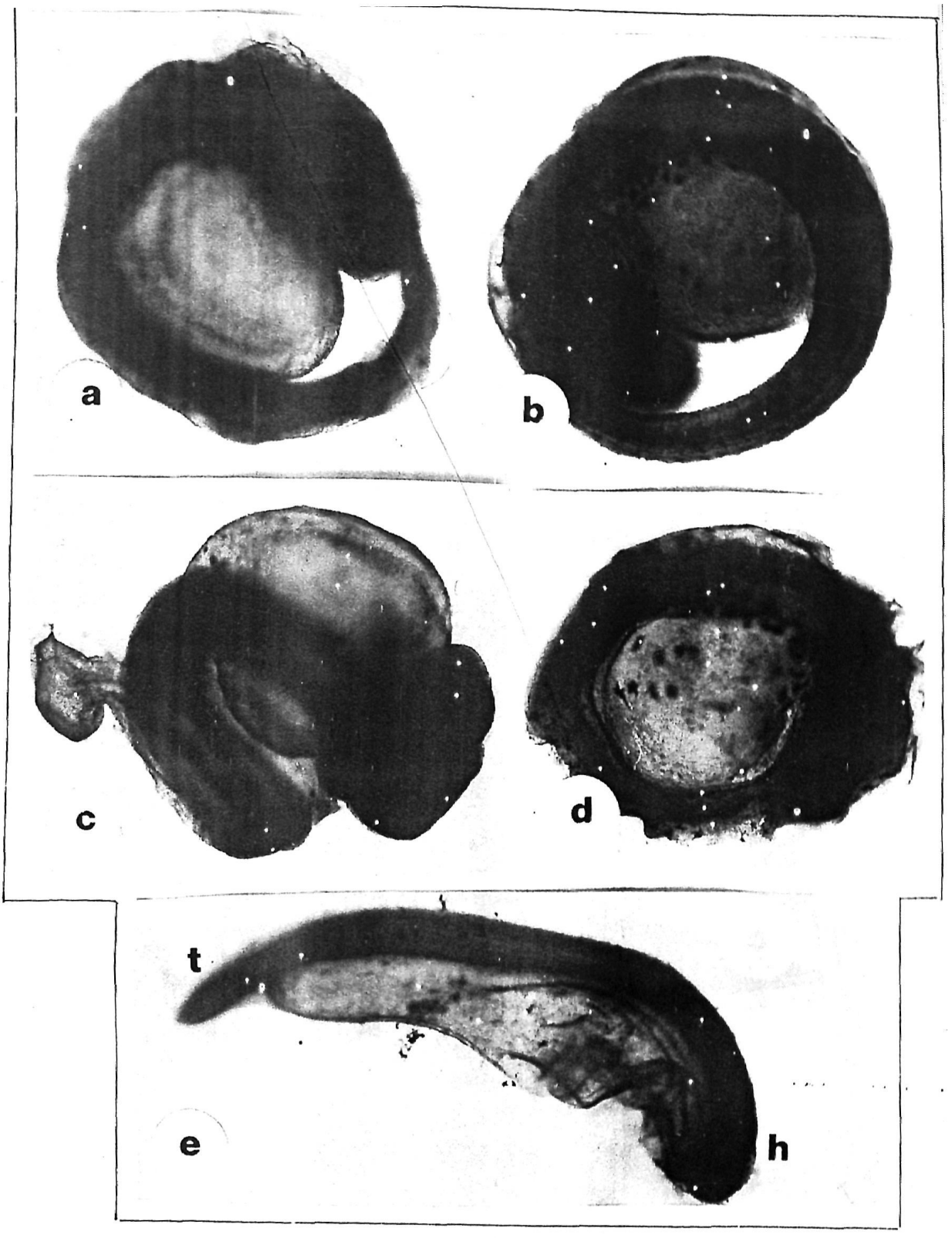


pl.(3)

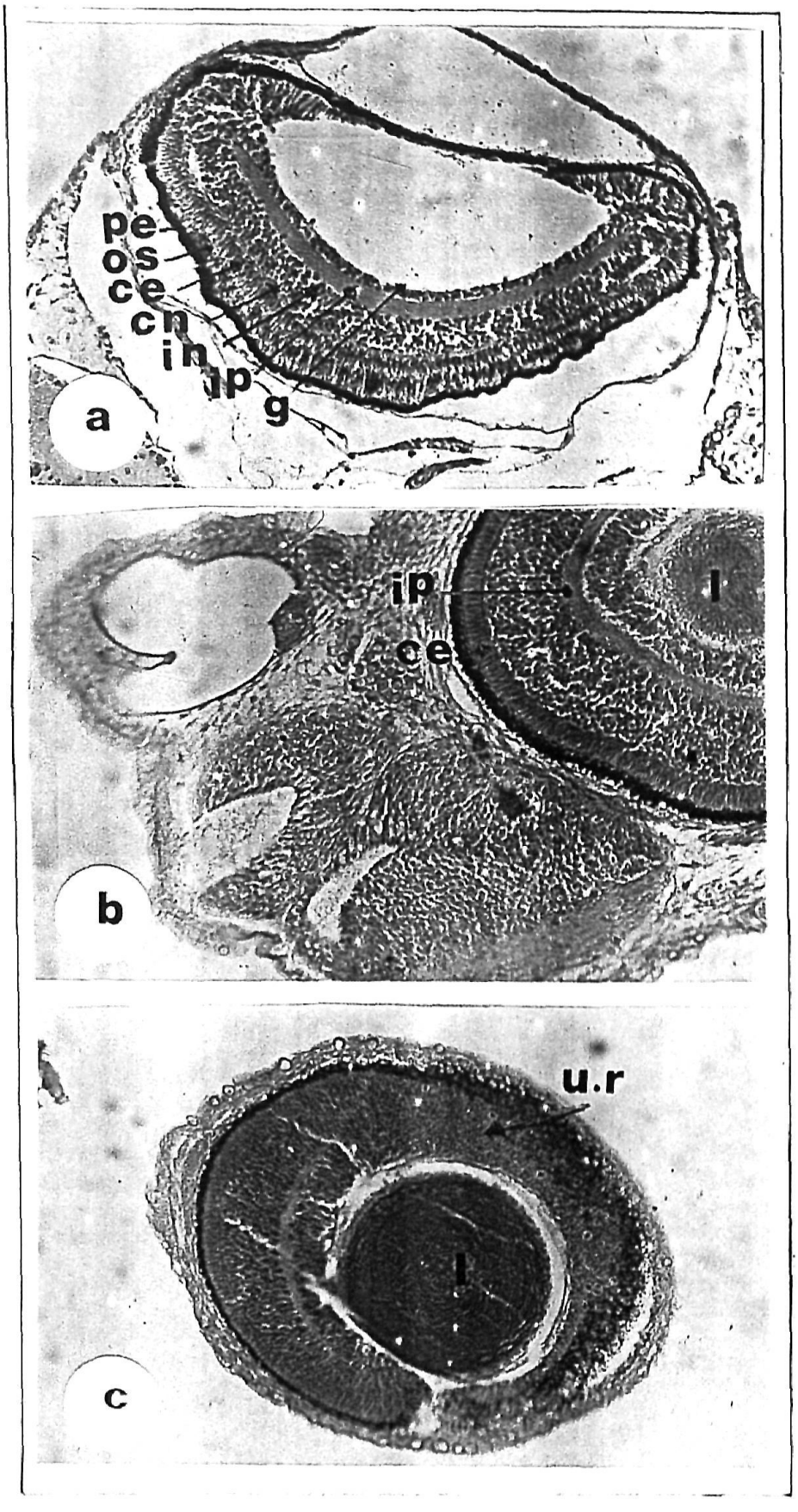


pl.(4)



pl.(5)

pl.(9)



pl.(7)