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Embryology

EMBRYONIC DEVELOPMENT OF THE BALTIC SEA-TROUT  
*SALMO TRUTTA* L. IN BUFFER SOLUTIONS

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The present study of the effect of different hydrogen-ion concentrations (pH 5.5-8.3) on the embryonic development of the sea-trout *Salmo trutta* L. from the Baltic Sea has shown that incubation of eggs in buffers, irrespective of the pH of these solutions and the time when the eggs were transferred to them, is lethal to the embryos. The death of the embryos is preceded by the disturbance of the organogenetic process, the derangement of the physiological functions of the organism, and the inhibition of the growth rate.

INTRODUCTION

The literature on the embryonic development of salmonid fishes is voluminous, but the data concerning the effect of the pH of environment on the development of the embryo are rather scanty, and the statements made by different authors in this respect are more often based on speculation than on factual material, which may be due to technical difficulties in establishing and maintaining suitable pH gradients and excluding the direct action of the pH-adjusting substance on the embryo. This is so because the use of chemically active substances to produce a pH gradient does not warrant the maintenance of this gradient and, what is worse, dims the picture of the action of a definite hydrogen-ion concentration on the embryo.

The authors were prompted to embark upon this study by the conclusions presented in the paper on the effect of CO<sub>2</sub> on the absorption of water, the turgor of eggs, and the strength of the egg membrane (Winnicki et al., 1969), showing that a weak acid (H<sub>2</sub>CO<sub>3</sub>) both considerably modulates the process of water absorption and exerts an influence on the strength of the egg membrane.

Studies on the influence of pH on the embryonic development of fishes seem to be of essential importance, especially in our times, when owing to the elimination of natural spawning-grounds and the shrinkage of the area of habitation of salmonids caused by increasing industrialization, the search for and utilization of any accessible source of unpolluted water for rearing these valuable fishes are burning problems. The present study is a modest contribution to their solution.

#### MATERIAL AND METHODS

The study was carried out in the Institute of Ichthyology, Academy of Agriculture, in Szczecin from November 1969 to January 1970.

Eggs of the sea-trout Salmo trutta L. collected at the estuary of the Vistula near Swibno were used as study material.

After spawning, both hard and soft roe were transported "dryly" (without water) in separate thermos flasks to Szczecin, where the spawn was fertilized under laboratory conditions and used for study. Part of the spawn was fertilized on the spot and also transported in thermos flasks to the laboratory.

Investigation consisted in observing the development and behaviour of embryos in the eggs placed in different environments and in different periods of embryonic development, and in analysing the movements of water through the egg membrane over the period of development, special attention being given to the initial period, i.e., that immediately following the fertilization of eggs and their placement in water. At the same time the changes in the strength of the egg membrane were examined.

Absorption of water at the time of egg turgor and its movements through the membrane in the later periods of embryonic development were examined indirectly by the method for the determination of the egg turgor (Winnicki, 1967).

The strength of egg membranes was measured according to modified Scharperclaus's (1940) method (Winnicki et al., 1970).

Immediately after fertilization the trout eggs were placed in phosphate buffers, made by Michaelis's method, at pH ranging from 5.5 to 8.3. Another batch of eggs were milted right in the buffers.

In addition, the eggs developing in tap water in control vessels were transferred to buffers at 5-7-day intervals at later stages of embryonic development, care being taken to do that at the time of intensified sensitivity of embryos (the so-called critical periods; Trifonova et al., 1939; Privolnev, 1941, 1953) or at turning-points in respect of morphophysiology, e.g., the outset of circulation or the period preceding hatching.

Visual observation of the embryos was also conducted using a magnifying glass, and several embryos of each experimental batch were fixed in Bouin's fluid for histological examination.

All the spawn was incubated in stagnant water in vessels placed in a water bath at a temperature of 11-12°C.

## RESULTS

The fertilization of the eggs placed in buffers after spawning is impossible, whereas the eggs fertilized in water and swollen before being transferred to buffers can develop, although the development of the embryos proceeds much worse than it does in the water control and, as a result, it does not lead to the hatching of embryos from these eggs.

The data concerning the mortality rate of the embryos developing in water and next transferred to buffers at selected values of pH (5.5, 6.9, 8.3) are presented in Fig. 1. Observations showed that the eggs placed in buffers 1, 2, 5 and 7 days after their fertilization kept developing and no evident disturbances were noted in their development before the closing of the blastopore, when all the embryos died. The transference of eggs at the time when the blastopore was closing (epibole 1/2-3/4) caused their instantaneous death. The analogous handling of the control eggs, which had been developing in water, also affected the development of embryos, but the mortality rate did not exceed 14% and the further development proceeded normally.

The placement of eggs in buffers just after the closing of the blastopore induced various disturbances in the development of the embryos.

A marked decrease in the development rate was noted as early as the 6th day of stay of the eggs in buffers, especially in those with the extreme values of pH (5.5 and 8.3). It was manifested by the lack of pigment in the eyes or one eye in many specimens, the abnormal distortion of about 50% of the embryos, their slower and less dynamic movements, and the lower heart rate than in the embryos developing in water.

The development of embryos in buffers at pH approximating to 7.0, although differentiated, little differed from that of the controls in water.

An abnormal increase in the perivitelline space, accompanied by a corresponding decrease in the volume of the yolk ball itself (approximately by half), was observed in most of the eggs in solutions at pH 7.8 and 8.3.

The inhibition of the growth and development rate and the disproportions in the development of embryos were manifested more and more distinctly with time.

After 10 (115 D<sup>0</sup>) days of stay in buffers, in addition to the irregularity of development, there was an underdevelopment of the blood vessel network on the surface of the yolk sac as compared with that in the control embryos, and single embryos began to die then. Death occurred first in buffers with the extreme values of pH (5.5 and 7.8; 8.3) and the last embryos died at pH 5.5 after 16 days of stay in a buffer (180 D<sup>0</sup>), at pH 8.3 after 17 days (190 D<sup>0</sup>), and at pH 6.9 after 23 days (250 D<sup>0</sup>).

The embryos transferred to a solution after their circulation had stabilized (260 D<sup>0</sup>) behaved in a somewhat different manner. In this case, as in the previous experiment, in buffers at pH 6.9 and 8.3 the last embryos died after a lapse of 16 and 17 days, respectively (at the time of hatching of the embryos from the control eggs), whereas the embryos placed in a solution at pH 5.5 lived considerably longer, and single specimens even as long as 540 D<sup>0</sup>, or 100-120 D<sup>0</sup> longer than the development of the control embryos untransferred to buffers.

The placement of embryos in buffers after the stabilization of their circulation did not cause any evident teratological changes, but only physiologi-

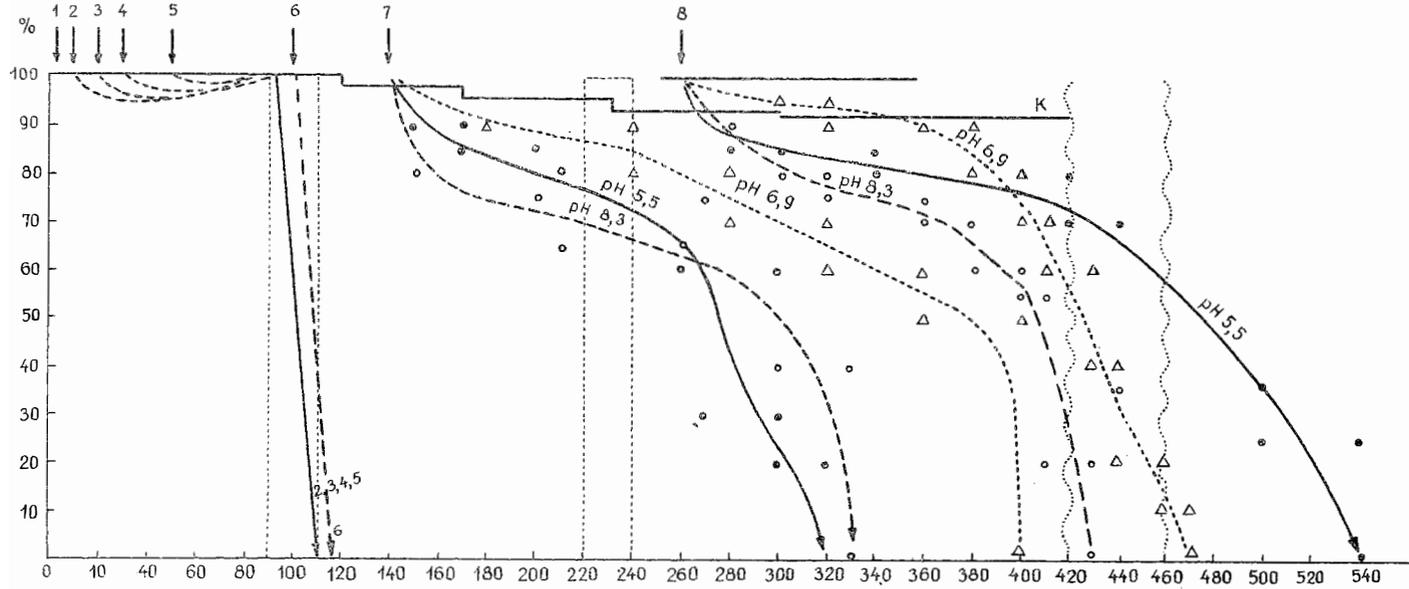


Fig.1. Embryos mortality after transferring from the water into the buffer solutions. (1,2,...,8 - samples followed numbers; ↓ - the instant of eggs transfer from water into the buffer solutions; ●—● the solution on pH 5.5; ○—○ the solution on pH 8.3; △—△ the solution on pH 6.9; K - control sample; [ ] the blastoporus closing period; [ ] the blood stabilization period; [ ] the larvae hatching period)

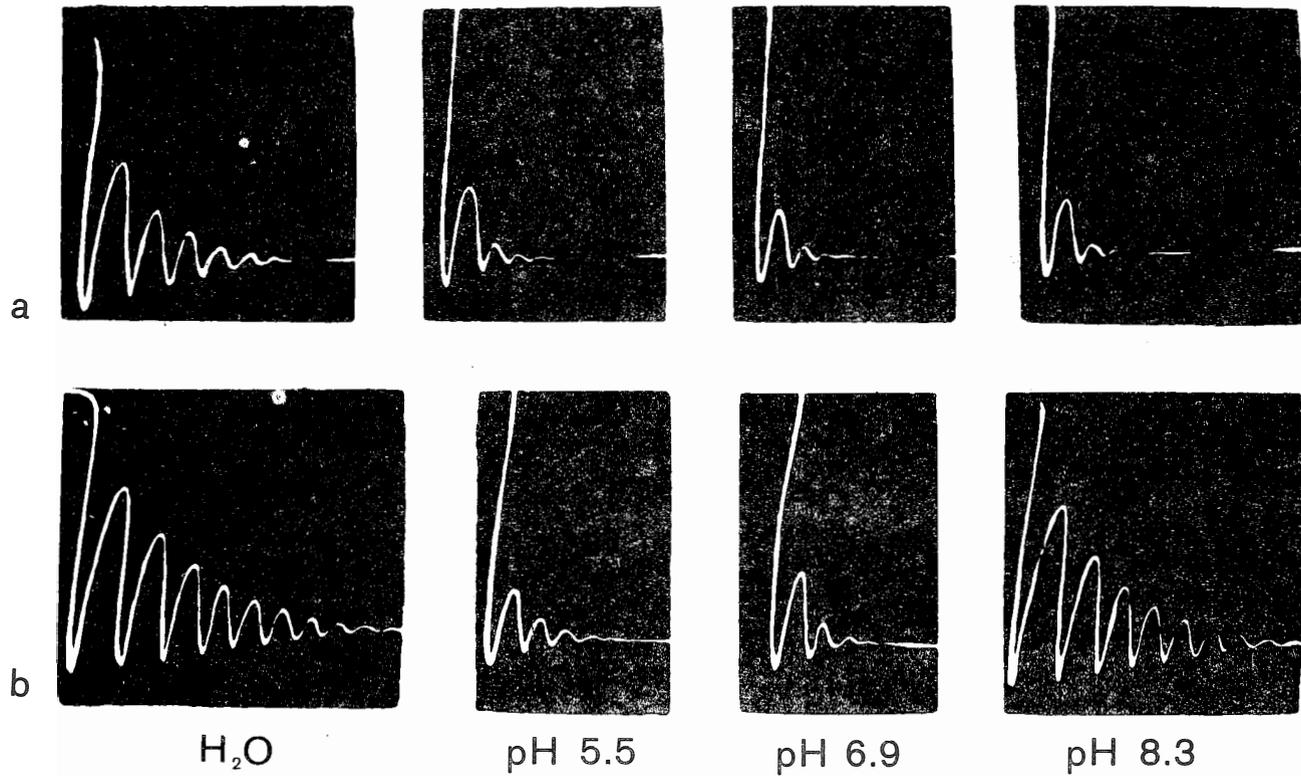


Fig.2. Turgor of the eggs developing into the buffer solutions (a - 35 minutes after; b - 48 hours after the instant of fertilization)

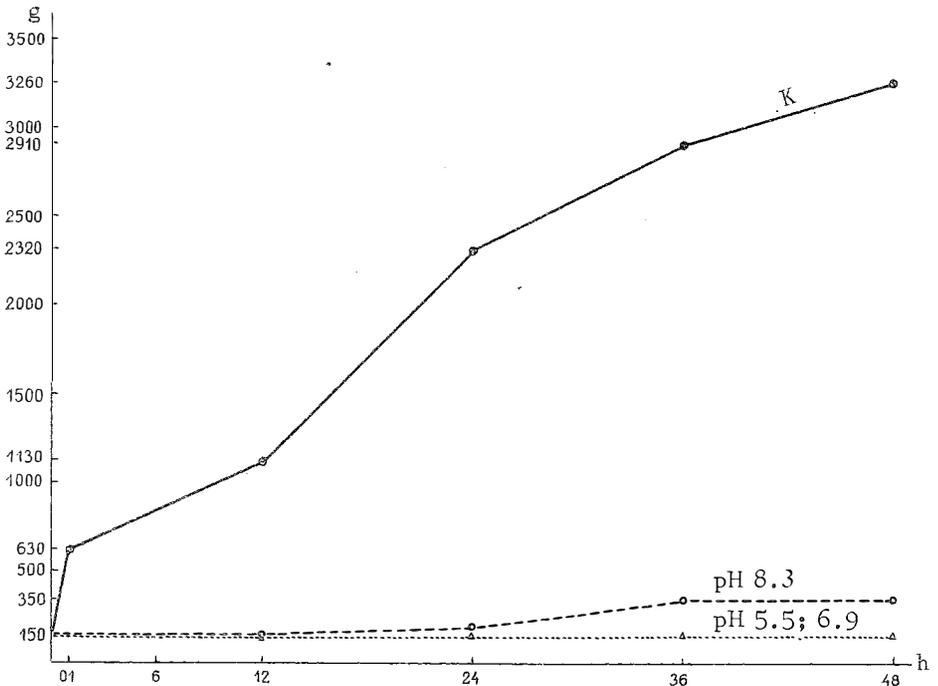


Fig. 3. The strength of egg membranes just after the fertilization in water (K) and in the buffer solutions

cal ones, above all, the slowing of the heart action and, in consequence, blood circulation, the remarkable slowing of movements performed by the embryo in that period and, what is the most important, the entire inhibition of the quantitative increment of melanophores in the skin.

It has been demonstrated that the buffers thoroughly inhibit the absorption of water by eggs in the initial period of embryonic development, which is manifested by the fact that there is no increase in turgor (Fig. 2a). The turgor of these eggs does not attain a value approximating to that for the eggs that develop in water, until the former have remained in buffers at pH 7.9 and 8.3 for 24 hours (Fig. 2b).

The placement of the eggs in buffers (including all the variants under study) in later phases of development did not bring about a decrease in their turgor except for a slight fall in it observed 1-3 days before hatching.

Buffers make it impossible to demonstrate the activity of the ferment causing the hardening of the egg shell, which is indicated by the results of measurements of the egg membrane strength summarized in Fig. 3. Whereas in the controls the membrane attained a mean strength of over 3.0 kg after 2 days, the membrane of the eggs incubated in buffers at pH 5.5, 5.9, 6.3, 6.9, 7.2 and 7.9 retained a strength equal to that found in the eggs directly after spawning. The buffer at pH 8.3 was the only exception, in which the strength of membranes grew regularly though slightly towards the end of the second day reached a value of 450 g, which is three times as high as the strength

of the egg membranes in the remaining buffers but for all that somewhat smaller than a seventh of the strength attained by the membranes of the control eggs.

## DISCUSSION AND CONCLUSIONS

The complete and relatively rapid (2 days) mortality of eggs mixed with sperm and immediately after that placed in buffers, demonstrated in this study, is easy to explain, especially if this fact has been juxtaposed with the results obtained in studies on the turgor of eggs and the strength of their membrane, for the placement of these eggs in buffers, owing to the notable osmotic pressure created by them, inhibited, the permeation of water through the membrane to the perivitelline space. This, in consequence, prevented the formation of the perivitelline space, for neither hydrophilous colloids (Bogucki, 1930) nor osmotically active substances (Yamamoto, 1939, 1956) contained in the cortical alveoli had been released, and their presence in the perivitelline fluid is directly responsible for the process of water uptake by the egg.

Neither was it possible for the membrane to increase in strength, because the ferment which causes its hardening also occurs in the cortical alveoli (Zotin, 1958, 1961). The lack of the perivitelline fluid, naturally, stopped the spermatozoon from entering the egg and thus the fertilization of the egg became impossible (Rotschild, 1958).

On the margin of the foregoing interpretation one might put forward the supposition that the eggs which after spawning are placed for 3-8 minutes in water prior to their transfer to buffers and thus long before the completion of the process of water absorption but after the outpour of the contents of the cortical alveoli into the perivitelline space, will continue to take up water and their turgor will increase, for the sum of the osmotic and hydrophilous forces of the perivitelline fluid will exceed the osmotic pressure of the buffers. This is so, because, according to the results obtained by Svetlov (1928, 1929) and confirmed by Klekowski and Domurat (1967), the value of  $\Delta$  of the perivitelline fluid in the salmon or seatrout eggs is  $0.02^{\circ}\text{C}$ , which converted into pressure units gives  $0.24 \text{ atm.}$ , whereas the  $1/15\text{M}$  solution (used in the study) exerts an osmotic pressure of  $1.49 \text{ atm.}$

The lack of changes in turgor in the eggs transferred to buffers after their complete saturation with water (Fig. 1 - 2nd, 3rd, 4th and 5th consecutive tests) may be explained by the fact that the shells at that time were quite impermeable (Krogh and Using, 1937; Zotin, 1961; Fischer, 1963), however, pH also exerts an influence, which manifests itself in the period of closing of the blastopore, when all the embryos die. It is characteristic that the eggs also die in the analogous manner and in the same phase of development, if they are transferred from water to buffers in this very period of closing of the blastopore. This is not to be wondered at, because the closing of the blastopore is a critical period in the development of the embryo, which in this period is most sensitive to all, even small, changes in the external environment, and such disturbances usually have a fatal end (Privolnev, 1953).

The action of buffers on the embryo is different before and after the stabilization of circulation. After the closing of the blastopore but before the stabilization of circulation they, as a rule, induce developmental disturbances and immediately after this period nearly completely inhibit growth; in both these cases they cause an evident slackening of physiological functions and lead, although not instantaneously, to death.

It might be supposed that the action of these solutions is purely physical (higher osmotic pressure of the external environment than under natural conditions). However, the less steep mortality curve and the smaller disturbances of development in the solution at pH 6.9 in the 7th test (Fig. 1 - after the closing of the blastopore) show that the exclusiveness of this factor is out of the question and suggest that in this case the main, although not only, influence is exerted by pH.

Different developmental disturbances, the slackening of physiological functions, and the inhibition of growth indicate that although the egg has a very stable barrier in the form of the egg membrane (Zotin, 1953; Winnicki, 1963), a change in the pH of the environment causes marked shifts in the pH of the internal environment of the egg and that of the embryo itself, which in a fundamental manner modifies or stops the activity of the development and growth controlling enzymes.

The situation seems somewhat different, if the eggs are transferred from water to buffers after the stabilization of circulation.

To be sure, the slackening of physiological functions and the inhibition of growth due to presumably the same causes are also observed here, but in this period the membrane is already permeable to small molecules and partly to water (Ykeda, 1934; Krogh and Using, 1937; Kao et al., 1954; Zotin, 1961), which makes a partial escape of salt from the egg possible. In addition, the embryo itself is already furnished with the skin, which acts fairly efficiently as regards physiology and is capable, among other things, of secreting alkaline mucus in the period preceding hatching (Hayes, 1942). This last fact might explain the relatively high resistance of the embryos to the solution at pH 5.5.

The course of mortality curves and the variety of developmental disturbances indicate that the homogeneity of eggs derived from one female is only apparent, since, in reality, they are very much differentiated regarding their physiology. Differences in size of the egg itself (Bartel, 1971) and in structure of the egg membrane between individual eggs (Winnicki et al., 1970) may in a way lie at the basis of this differentiation.

To sum up the foregoing it may be stated as follows:

- a) The osmotic pressure created by a 1/15 M buffer solution prevents the egg from taking up water and thus makes its fertilization impossible.
- b) Incubation of eggs in buffers, irrespective of the pH of the buffers and the time of transfer of the eggs to them, always ends in death of the embryos, which is preceded by disturbances in the process of organogenesis, impairment to the physiological functions of the organism and the inhibition of the growth rate.

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## ROZWÓJ ZARODKOWY TROCI BAŁTYCKIEJ *SALMO TRUTTA* L. W PŁYNACH BUFOROWYCH

### S t r e s z c z e n i e

Badano wpływ pH środowiska na rozwój zarodkowy troci bałtyckiej *Salmo trutta* L. ze szczególnym uwzględnieniem okresów krytycznych.

Stwierdzono, że zapłodnienie jaj w płynach buforowych jest niemożliwe. Natomiast jaja zapłodnione w wodzie, a następnie umieszczone w roztworach buforowych rozwijają się lecz znacznie gorzej niż w warunkach normalnych i w konsekwencji do wylęgu z tych jaj nigdy nie dochodzi.

Zależnie od okresu rozwoju, w którym poddano działaniu buforu zarodki, obserwowano takie odchylenia od normy, jak: zwolnienie tempa rozwoju, nie-naturalne zwiększenie przestrzeni periwitelarnej, niedorozwój sieci naczyń krwionośnych na powierzchni woreczka żółtkowego, zwolnienie akcji serca oraz brak przrostu melanoforów w skórze.

ЗАРОДЫШЕВОЕ РАЗВИТИЕ МОРСКОЙ КУМЖИ *SALMO TRUTTA* L.  
В БУФОРНЫХ ЖИДКОСТЯХ

Р е з ю м е

Изучали влияние pH окружающей среды на зародышевое развитие морской (балтийской) кумжи *Salmo trutta* L. обращая специальное внимание на критические периоды.

Нашли, что оплодотворение яиц в применяемых буферных жидкостях является невозможным. Яйца оплодотворены в воде и затем помещены в буферных жидкостях развиваются, но медленнее и хуже чем в нормальных условиях и в итоге выклёв личинок из этих яиц невозможен.

Влияние буферной жидкости на зародышей зависит от периода развития и проявляется в таких изменениях, как: замедление темпов развития, сверхестественное увеличение перивителлинового пространства, недоразвитие сети кровеносных сосудов на поверхности желточного мешка, замедление сердцебиений и заторможение прироста меланофоров в коже.

Received 10.IV.1972

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