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WATER UPTAKE BY TROUT (*SALMO TRUTTA* L.) EGGS EXPOSED AFTER ACTIVATION TO MAGNETIC FIELD*

WCHŁANIANIE WODY PRZEZ JAJA TROCI (*SALMO TRUTTA* L.) POZOSTAJĄCE PO AKTYWACJI POD DZIAŁANIEM POLA MAGNETYCZNEGO

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Study was made of the effect of steady magnetic field (200–250 mT) on water absorption of eggs of trout (*Salmo trutta* L.). It was found that eggs exposed to magnetic field absorbed water in the initial period of activation (until 30 min) much slower than control eggs. Subsequently water absorption was accelerated to such an extent that after complete formation of the perivitelline space the volumes of the treated and control eggs were the same. The authors interpret the change in water absorption dynamics in terms of a reduction of the permeability of egg membrane, resulting from the effect of magnetic field on liquid crystals making part of its structure.

INTRODUCTION

Mature fish eggs are covered by a thick protective layer referred to by different terms: zona radiata, egg membrane, chorion, egg shell. Apart from its protective functions, this layer plays an essential role in gas exchange between the interior of egg and the environment (Winnicki, 1967a; Winnicki, 1968; Grierson and Naville, 1981), and allows for selective transport of various indispensable substances and for the possibility of excretion from the perivitelline space (Winnicki, 1968; Toshimori i Yasuzumi, 1976).

Initially the egg membrane is permeable and thus the egg hatched in water (irrespective whether it is fertilized or not) absorbs water. As a result, the membrane rises

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and between the egg cell and the membrane the perivitelline space is formed; it is filled with water, with an admixture of the contents of cortical alveoles (which burst at this time) comprising hydrophilic substances (Bogucki, 1930). This space, confined by the hardening and semipermeable membrane, serves for ensuring optimal conditions for the development of the embryo and for protecting it against adverse environmental factors (Winnicki, 1968).

According to Winnicki and Cykowska (1973), water uptake by the egg proceeds via two stages, and there are two factors (osmotic and molecular, respectively) decisive of this process. The egg hatched in water contains a small amount of osmotically active substances in the perivitelline space. Osmotic pressure produced by these substances is not high enough to cause the uptake of water molecules from an environment with a high molecular concentration (interior of egg), whereas it is sufficiently high to allow for penetration of a small amount of water from the exterior under the egg membrane. Only then the cortical alveoles burst, liberating hydrophilic colloids, and the real action of the "pump" aspirating water into the perivitelline space begins.

It is well known that some environmental factors (temperature, salinity, pH) may influence the velocity and quantity of water penetration through egg membranes of salmonids. Among these factors, salinity is decisive of the fate of the future embryo; this salinity, expressed as ion concentration, cannot exceed a 0.1 M concentration, in order to permit egg activation (Eddy, 1974); otherwise, no activation takes place. This fact additionally illustrates the role of the egg membrane which – by selective action on the substances penetrating from the outside – ensures an appropriate environment (in the form of perivitelline liquid) for the future embryo.

It was of interest to verify experimentally whether and to what an extent magnetic field influences water penetration through the limit membrane (i.e. the egg membrane), the more so as this membrane is known to exert an effect on many biological processes in animal organisms, including fish (Kalmijn, 1978; Kirschvink et al., 1985; Lindauer and Martin, 1985; Schulten and Windemuth, 1986; Carstensen, 1987).

In earlier studies of the motility of spermatozoa we have observed that magnetic field influences the velocity of water penetration through the membranes of the spermatozoon of Danube salmon (*Hucho hucho* L.) (Formicki et al., 1990a). In this connection, bearing in mind the similarity and, at the same time, the different nature of the biological material forming the membranes of eggs and spermatozoa, we resolved to investigate the dynamics of water penetration through the memranes of Baltic trout (*Salmo trutta* L.) eggs exposed to magnetic field.

MATERIAL AND METHODS

Eggs collected from females of *Salmo trutta* L. were divided into two samples (n = 50). The experimental sample was activated in water, in magnetic field (200 or

250 mT) generated by an electromagnet, and it was exposed to this field; the control sample was activated beyond the action of magnetic field. Both samples were incubated at the same temperature (9°C). During incubation, the volume of eggs in both samples was determined with measuring pipettes allowing for readings exact to 0.01 cm^3, and the volume of one egg was calculated.

Preliminary experiments showed that in both samples the volue of eggs, i.e. the amount of water absorbed during their swelling is after completion of this process closely similar, with a slight downward tendency in eggs exposed to magnetic field. The test for turgor (Winnicki, 1967b), performed in the course of swelling, seemed to indicate that in both samples the water uptake dynamics may differ.

To elucidate this problem, much more numerous spawn samples (each containing several hundred eggs) taken from individual females were activated in magnetic field (250 mT) and were exposed to this field at 9°C for 180 min, i.e. until completion of swelling. Quantitatively equal control samples were activated in water beyond the action of magnetic field and were incubated at 9°C. At short intervals (5–20 min, and in the final phase 40 min), from each sample several tens of eggs were taken and their volume was measured.

In statistical treatment of the results, the significance of the differences between the exposed samples and respective controls was evaluated using the t test of Student; moreover, the analysis of curvilinear regression was applied (Elandt, 1964).

RESULTS

In all cases, eggs exposed to magnetic field absorbed water significantly slower than control eggs; this resulted from a decrease in the amount of water penetrating through membranes of the exposed eggs (Tab. 1).

It was found, moreover, that there is a difference between the exposed and control eggs in the dynamics of water uptake. In the initial period (until 20 min), water uptake by eggs exposed to magnetic field, as compared with control, was inhibited. This slowing down, being very distinct in the initial period, was attenuated over time; there is a tendency for equalizing the water uptake velocity in both samples, so that by the end of the experiment (180 min) the volumes of the exposed and control eggs were closely similar (Tab. 2; Figs. 1).

In the case of eggs exposed to magnetic field prior to activation, there were no significant differencess between the exposed and control eggs in water penetration through membranes.

DISCUSSION AND CONCLUSIONS

The results of the present experiments showed univocally that magnetic field influences water absorption by trout eggs immediately after their activation. This

Table 1

Volume of trout eggs in control and in magnetic field;
SE — standard error, α — significance level

No.	n	Eggs exposed to magnetic field prior to activation					
		Control		Magnetic field 200 mT			α
		x	S.E.	x	S.E.	t [h]	
1	8	77.00	2.05	76.20	1.67	3	0.1
2	9	73.78	5.11	73.76	5.63	3	0.4
		Eggs activated in magnetic field					
		Control		Magnetic field 250 mT			
3	8	73.85	4.15	72.28	3.99	0.5	0.03
4	8	82.52	5.73	74.98	4.02	0.5	0.007
5	9	74.73	5.11	72.45	5.42	0.5	0.039
		Control		Magnetic field 200 mT			
6	9	74.78	5.21	72.40	5.52	2	0.04

* — volume of one egg (mm^3)

Table 2

Water uptake by trout (*Salmo trutta* L.) eggs sample exposed to magnetic field and control;
α— significance level

time [min]	5	10	20	30	40	60	80	100	120	180
α	0.06	0.02	0.07	0.01	0.01	0.05	0.1	0.1	0.1	0.3

* — significance level of the difference in eggs volume between control and sample exposed to magnetic field.

effect does not concern the total balance of the absorbed water, because finally its amount is the same in the exposed and control eggs (Fig. 1), in accordance with the genetic program for the given species. This confirms the statement earlier formulated by Winnicki and Bartel (1967), claiming that despite the "sealing" of the egg membrane water penetrates through it, although this process is slowed down.

Thus, the mechanism of the evident slowing down of water uptake by eggs during the first minutes after their activation calls for elucidation. The causes of this alowing down may be different.

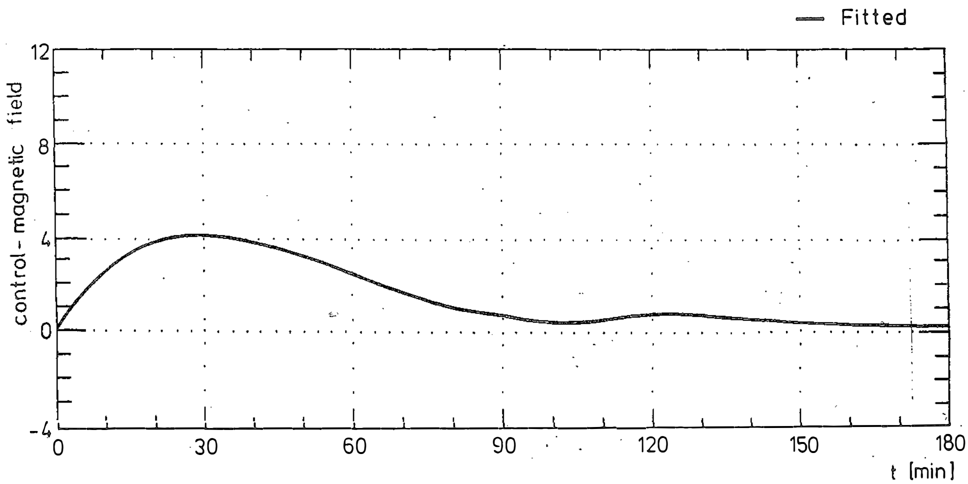

Fig. 1. Water uptake by trout (*Salmo trutta* L.) eggs, difference between control and magnetic field exposure;

$$y = 0.324111\,t - 0.0080377\,t^2 + 0.000066\,t^3 - 1.711849 * 10^{-7} t^4; p < 0.001$$

Firstly, it could be assumed that magnetic field reduces, immediately after egg activation, the osmotic activity of the active substances decisive of the start of the water absorption process (Winnicki and Cykowska, 1973); this, in turn, delays bursting of the cortical alveoles and liberation of hydrophilic colloids from them (Bogucki, 1930). These colloids are known to be responsible for "filling" of the perivitelline space with water to a state of considerable turgor. As an effect, the substantial delay in the water absorption rate during the first minutes after activation is followed by a "hunt" for equalizing the volumes of the exposed and control eggs. This mechanism seems, however, to be hardly possible

Secondly it could be assumed that magnetic field accelerates closing the micropyle, i.e. the main "inlet gate" through which water penetrates into the egg after its being placed in water. The effect of this mechanism would be similar as in the former case. This hypothesis also has to be dispensed with, because – if it were valid – also the fertilization process would be adversely affected. Yet, Formicki et al., 1990b, have shown that in magnetic field fertilization proceeds more effectively, and the percentage of fertilized eggs is higher than in control samples.

It seems likely that slowing down of the water absorption process, i.e. lowering of the dynamics of water penetration into the perivitelline space, is related to the effect of magnetic field on liquid crystals of which all biological membranes are built; the latter have been shown to be very sensitive to the action of steady magnetic field. Within the range of low-strength fields, this effect manifests itself by a drop (but not a complete elimination) in membrane permeability (Johnson and Porter, 1970; Lŏsche, 1973; Rościszewski, 1973; Wadas, 1978; Labes, 1979).

This accounts for the initial (considerable as compared with control) decrease in the dynamics of water absorption, followed by its slow making up at a rate equal to that displayed by control eggs.

In this light, the present results allow for drawing the following conclusions:

1. Exposure of eggs to magnetic field prior to their activation exerts no effect on the later water absorption process.
2. Exposure of eggs at the moment of activation to magnetic field, followed by incubation in magnetic field, causes in the initial period slowing down of their water uptake.
3. Incubation of eggs, from the moment of their activation in water, in weak magnetic field does not rule out complete filling of the perivitelline space, in accordance with the genetic program for the species, but only shifts it in time.

REFERENCES

Bogucki M., 1930, Recherches aur la perméabilité des membranes et sur la pression osmotique des oeufs des oeufs des Salmonides. Protoplasma, 9: 345–367.

Carstensen E.L., 1987, Biological Effects of Transmission Line Fields. Elsevier, Science Publishing Comp., Inc., New York, Amsterdam, London.

Eddy F.B., 1974, Osmotic properties of the perivitelline fluid and some properties of the chorion of Atlantic salmon eggs (Salmo salar L.). J. Zool. London, 174, 237–243.

Elandt R., 1964, Statystyka matematyczna w zastosowaniu do doświadczalnictwa rolniczego. [Mathematical statistics in design of agricultural experiments], PWN, Warszawa.

Formicki K., A. Sobociński, A. Winnicki, 1990a, Motility of spermatozoa of Danube salmon (Hucho hucho L.) exposed to magnetic field prior to activation. Pol. Arch. Biol., 37, 3: 435–443.

Formicki K., A. Sobociński, A. Winnicki, Z. Mongiało, 1990b, The fertilization rate of trout (Salmo trutta L.) eggs by spermatozoa exposed to magnetic field prior to activation. Acta Ichthyol. Piscat., 21, 1: 95–100.

Grierson J.P., A.C. Naville, 1981, Helicolloidal architecture of fish egg-shell. Tissue Cell, 13: 819–830.

Johnson J.J., R.S. Porter, 1970, Liquid Crystals and Ordered Fluids. Plenum Press, New York-London.

Kalmijn A.J., 1978, Experimental evidence of geomagnetic orientation in elasmobranch fishes. In: Animal Migration, Navigation and Homing [Schmidt-Koenig K., Keeton W.T., eds.], Springer Verlag, New York: 354–355.

Kirschvink J.L., M.M. Walker, S.B. Chang, A.E. Dizon, K.A. Peterson, 1985, Chains of single domain magnetite particles in chinook salmon, Oncorhynchus tschawytscha. J. comp. Physiol., 157: 375–381.

Labes M.M., 1979, Magnetic field coupling with liquid crystalline structures. In: Magnetic Field Effects on Biological Systems [Tenforde T.S., ed.], Plenum Press, New York-London: 85–86.

Lindauer M., H. Martin, 1985, The biological significance of the Earth's magnetic field. In: Sensory Physiology, Springer-Verlag, Berlin-Heidelberg, 123–145.

Lösche A., 1973, Rezonans magnetyczny w ciekłych kryształach. [Magnetic resonance in liquid crystals]. Post. Fizyki, 24: 407–422.

Rościszewski K., 1973, Teoria faz ciekłokrystalicznych i związanych z nimi przejść fazowych. Post. Fizyki. [Theory of liquid crystals phases and related phase transitions], 24: 393–406. (in Polish),

Schulten K., A. Windemuth, 1986, Model for a physiological magnetic compass. In: Biophysical Effects of Steady Magnetic Fields [Maret G., Kiepenheuer J., Boccara N., eds.], Springer Verlag, Berlin-Heidelberg-New York: 99–106.

Toshimori K., F. Yasuzumi, 1976, The morphology and the function of the oocyte chorion in the teleost, Plecoglossus altivalis. J. Electron. Microsc., 25: 210.

Wadas M., 1978, Biomagnetyzm. [Biomagnetism], PWN, Warszawa. (in Polish).
Winnicki A., 1967a, Embryonic development and growth of Salmo trutta L. and Salmo gairdneri Rich. in conditions unfavorable to respiration. Zool. Pol., 17: 45–58.
Winnicki A., 1967b, The turgor of the salmonid fish eggs during hatching. Bull. Acad. Pol. Sci., Cl II, 15, 12: 785–787.
Winnicki A., 1968, Rola i właściwości osłonek jajowych ryb łososiowatych. [Role and properties of egg membranes in salmonids. Doctor habilitatus degree dissertation]. Rozprawa habilitacyjna, Wyższa Szkoła Rolnicza w Olsztynie, Olsztyn. (in Polish).
Winnicki A., Bartel R., 1968, The effect of limited water intake on the strength of coverings in the salmonid fishes. Zool. Pol., 17: 351–364.
Winnicki A., C. Cykowska, 1973, New data on the mechanism of water uptake in salmonid eggs. Acta Ichthyol. Pisc., 3, 1: 3–9.

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STRESZCZENIE

Badano wchłanianie wody przez jaja troci (*Salmo trutta* L.) poddane wpływowi stałego pola magnetycznego (200–250 mT). Okazało się, że objętość jaj poddanych działaniu pola magnetycznego, w początkowym okresie aktywacji (do 30 min), jest mniejsza niż w próbach kontrolnych, czyli wchłaniają one wodę znacznie wolniej [Tab. 1, 2; Rys. 1]. W miarę wypełniania przestrzeni okołożółtkowej proces ten ulega przyśpieszeniu, tak że po całkowitym jej utworzeniu, objętość jaj poddanych działaniu pola magnetycznego i jaj w próbach kontrolnych jest taka sama [Tab. 2; Rys. 1]. W przypadku jaj poddanych działaniu pola magnetycznego występuje zmniejszenie dynamiki wchłaniania wody, przesuwające w czasie zaprogramowanie biologicznie wypełnienie przestrzeni okołożółtkowej. Zmianę dynamiki wchłaniania wody, autorzy tłumaczą zmniejszeniem przepuszczalności osłonki jajowej spowodowanym oddziaływaniem pola magnetycznego na ciekłe kryształy współtworzące jej strukturę.

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