ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XXVII, Fasc. 2 Szczecin 1997

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Fish embryology

EFFECTS OF CONSTANT MAGNETIC FIELDS ON RESPIRATION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALB.) EMBRYOS

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Oxygen uptake and carbon dioxide release by rainbow trout (*Oncorhynchus mykiss* Walb.) embryos exposed to constant magnetic fields of 5 and 10 mT (oxygen uptake and carbon dioxide release) and 50, 150, and 300 mT (oxygen uptake) were measured. The data were compared with those recorded in embryos developing under natural magnetic field (control).

The magnetic fields tested were found to stimulate respiratory processes in the rainbow trout embryos as shown by a significantly increased oxygen consumption, particularly during periods of intensified morphogenesis.

Exposure to 5 and 10 mT magnetic fields resulted in a slightly higher carbon dioxide release, the oxygen consumption being observed to increase as well. The respiratory quotient of the embryos exposed to magnetic fields was slightly higher than that in the control.

INTRODUCTION

The last three decades have supplied a growing evidence that magnetic fields different from those of the natural Earth's background affect life processes of organisms, including fish, exposed to those fields (Tenforde 1979; Blank 1993). The effects are manifested as, i.a., acceleration or slow-down of some functions and are particularly pronounced in some locomotory functions. Our earlier studies demonstrated, i.a., significant effects of magnetic field changes on the cardiac mu-

scle performance in fish embryos and larvae, and on the performance of skeletal muscles operating the juvenile fish pectoral fins (Winnicki and Formicki 1990; Formicki 1992; Winnicki et al. 1993; Formicki and Winnicki 1996). Acting on the premises that all the locomotory responses rely on energy expenditure and that the energy can be mobilised by oxygen supplied from without (as evidenced by acceleration of heart beat rate resulting in intensified circulation and acceleration of pectoral fin respiratory beat rate), it was decided to measure the amount of oxygen taken up (and, in some cases, of carbon dioxide released) by rainbow trout (*Oncorhynchus mykiss* Walb.) embryos exposed to artificial constant magnetic fields and to compare the results with corresponding data obtained under the natural geomagnetic conditions. The question was addressed whether, and how, the respiration could be altered by application of a magnetic field. The tests were run in different fields in an attempt to determine relationships between field parameters and responses of a developing organism.

MATERIAL AND METHODS

The tests were carried out in 1994–1996 at the Department of Fish Anatomy and Embryology, Agricultural University of Szczecin on developing embryos of rainbow trout (*Oncorhynchus mykiss* Walb.)

Materials (i.e., rainbow trout eggs and sperm), obtained from spawners kept at fish farms in Miastko and Żelkowo (District of Słupsk), were transported to the laboratory where insemination (dry) and fertilisation were effected. Subsequently, the hydrated eggs were distributed for incubation on Petri dishes filled with water to 6 mm height. Those "mini-incubators" were kept throughout the period of the development either in an insulated room or in a special water mantle (laboratory hatchery), whereby the constant temperature of 10°C ± 1°C was maintained.

Respiration of the embryos was measured at 6 stages of the embryonic development, a particular attention being paid to the following stages of intensified morphogenesis:

- early gastrulation (development of blastoderm and periblast, formation of the gastrula, division into three embryonic layers, beginning of blastopore closure): 60–80 D°;
- prior to blastopore closure (formation of the primordial neural disc and notochord, termination of epiboly, blastopore closure): 90–100 D°;
- post-blastopore closure (separation of optical cups, formation of 5 neuromeres, appearance of gill arches): 110–130 D°;
- neural tube development into brain and vertebral cord, kidney, and a part of gonads; onset of regular heart beat: 140–150 D°;
- appearance of eyes and further development of circulatory system (appearance of pigment in eyes, development of circulatory system, further development of gonads): 160–200 D°;

– active embryo phase (pre-hatch embryo, onset of opercular movements): 210–290 D° (Vernier 1967; Opuszyński 1979; Klinkhardt et al. 1987; Depeche and Billard 1994).

The O_2 uptake and CO_2 release by the developing embryos were measured either with the Winkler technique (O_2 uptake) in flow-through respirometers, or with the modified Warburg manometric technique (O_2 uptake and CO_2 release).

In the first case, the eggs were transferred to 13.5 mm diameter, 140 mm long cylindrical flow-through plexiglass chambers, one placed between the poles of the magnetic field source and the other away from it. The water oxygen content was determined with the Winkler technique as described in "Physical and chemical methods for water and wastewater analyses" (Hermanowicz et al. 1976). The O_2 uptake was determined as a difference between oxygen contents in the chamber's inlet and outlet. A single test took 240 min. during which time 6 measurements were taken. Water temperature was maintained constant by keeping the test chambers in additional buffering containers to make sure that identical thermal conditions were prevailing in the magnetic field, control, and in the Mariott bottle. Although allowing to remove metabolic products from the test chamber and ensuring stability of gas components in the incoming water, the flow-through technique is of low sensitivity. It was used to determine the differences in oxygen consumption between the control and treatment embryos exposed to magnetic fields of a relatively high inductances (50, 150, and 300 mT) as more conspicuous responses in those fields were anticipated.

To ensure a high sensitivity, the Warburg apparatus manometers were used in weaker fields (5 and 10 mT). The duration of the test was shortened to reduce effects of changes in gas ratio in the measuring cell which occur during the test and distort the results. A single test took 90 min. during which the manometer column height was read at 15-min. intervals. As the eggs of salmonids (including those of the rainbow trout) are at some stages of their development particularly prone to mechanical damage and sensitive to vibrations (Privolnev and Razumovskij 1939; Vernier 1967; Winnicki 1967; Opuszyński 1979), it was decided against using a shaker in order to prevent additional stress to or deaths of embryos, which would lead to distortion of data.

Constant magnetic fields were produced by a computer-controlled magnetic field generator for embryo-physiological tests, manufactured at the Institute of Telecommunications and Acoustics, Wrocław Technical University (Duchiewicz and Formicki 1994) and by an electromagnet.

A total of 5 series of tests were run. In 1995, O_2 uptake by the rainbow trout embryos exposed to 50, 150, and 300 mT magnetic fields was measured at various stages of embryonic development, from 60 D° to hatch. The fields were generated with

the electromagnet in order to produce stronger fields. In 1996, the embryos' responses to 5 and 10 mT fields at the same developmental stages were recorded. The fields were generated with the computer-controlled apparatus mentioned above, allowing a precise control and fine tuning of the weak field. An HTM hallotronic teslameter (manufactured by the Institute of Telecommunications and Acoustics, Wrocław Technical University) was used to measure and control field inductance.

Differences between treatments and the control were tested for significance with Student's t test applied with the use of Statgraphics® v. 6.0 Manugistics™ statistical package.

RESULTS

O_2 consumption in 50, 150, and 300 mT magnetic fields

Table 1 summarises data on O_2 consumption by the rainbow trout embryos exposed to 50, 150, and 300 mT fields and in the control. As shown by the data, the mean O_2 uptakes in 50 and 150 mT fields differed significantly from the control throughout the period of observations. Significant differences were found also to exist between all the treatments combined (pooled results in 50, 150, and 300 mT fields) and the control. Differences in O_2 uptake, resulting from magnetic field effects, were observed in some stages of the embryonic development. In the 50 and 150 mT fields, the O_2 uptake clearly increased, relative to the control, within 160–200 D° (appearance of eyes), an increase being also observed within 90–100 D° period (formation of primordial neural disc) in the 150 and 300 mT fields. Additionally, differences in O_2 uptake between the treatment and the control were also recorded within 60–80 D° (gastrulation) in the 50 mT field, while in the 150 mT field the differences occurred within 90–200 D° (from blastopore closure to circulatory system formation). In none of the three field inductances tested were statistically significant treatment vs. control differences recorded for the 210–290 D° stage (active embryo phase). A reversed trend (reduced treatment O_2 uptake relative to the control) was observed in the 50 and 300 mT fields within 140–150 D° (onset of cardiac activity).

O_2 consumption in 5 and 10 mT fields

Data on O_2 consumption by the rainbow trout embryos exposed to 5 and 10 mT constant magnetic fields and in the control are shown in Table 2. As those treatments involved the use of the Warburg apparatus manometers, differences in O_2 consumption at various developmental stages, shown in Tables 1 and 2, may have resulted from different methodologies. This finding supports observations reported by Pattee (1962) who compared results obtained with different techniques and concluded that results obtained under identical conditions may differ considerably. Statistically significant differences between O_2 consumption in magnetic field and in the control were recorded both for the mean uptake in both the 5 mT (90 records, $p = 0.001$) and 10 mT (85 records, $p = 0.036$) fields and for all the records combined (175 records, $p = 0.001$). Application of 5 and 10 mT magnetic

fields resulted in increased O_2 uptake by the embryos within 60–80 D° (onset of gastrulation), 110–130 D° (after blastopore closure), and 140–150 D° (organogenesis), the differences relative to the control being highly significant (significance levels of 0.005 to 0.014). No reversal of the trend, i.e., reduced O_2 uptake in a magnetic field, was recorded. Significant differences relative to the control were noted also in the 5 mT field within 160–200 D° (appearance of eyes). No significant differences compared to the control occurred within 90–100 D° (formation of primordial neural disc) and within 210–290 D° (active embryo phase). In the 10 mT field, no statistically significant differences with the control were recorded within 90–100, 160–200, and 210–290 D°.

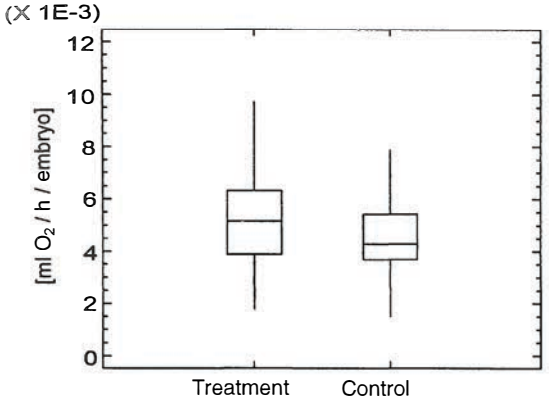

Fig. 1. Oxygen consumption by rainbow trout (*Oncorhynchus mykiss* Walb.) embryos exposed to 50, 150, and 300 mT magnetic fields and inthe control; all treatment observations pooled

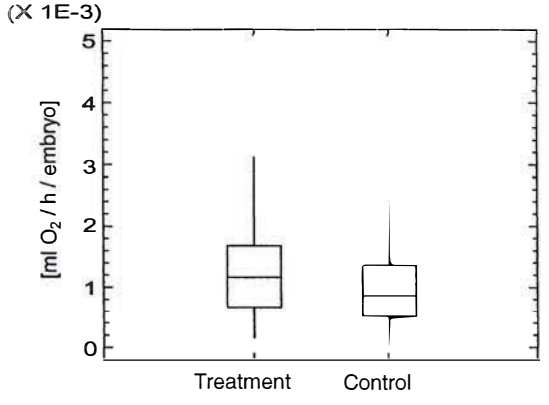

Fig. 2. Oxygen consumption by rainbow trout (*Oncorhynchus mykiss* Walb.) embryos exposed to 5 and 10 mT magnetic fields and in the control; all treatment observations pooled

Noteworthy is the fact that none of the treatment magnetic field inductances (5, 10, 50, 150, and 300 mT) produced statistically significant treatment vs. control differences prior to hatch, i.e., within 210–290 D° (Tables 1 and 2).

In order to visualise differences in rainbow trout embryos' O_2 uptake between the treatments and the control, box-and-whisker plots were prepared with data obtained in different field inductances and with combined data for the three high-inductance treatment (50, 150, and 300 mT). The plots allow to analyse the distribution of data in the groups and to conclude that, generally, exposure to a magnetic field produces larger differences between O_2 uptakes than does the control (as evidenced by larger areas covered by the horizontal lines reflecting the upper and lower quartiles). Both in high (50, 150, and 300 mT; Fig. 1) and in low (5 and 10 mT; Fig. 2) inductance fields, the median O_2 consumption lie above the medians for the control, the quartile ranges being clearly wider.

Table 1

Oxygen consumption (ml O_2/h/embryo) by rainbow trout (*Oncorhynchus mykiss* Walb.) embryos exposed to 50, 150, and 300 mT magnetic fields and in the control

Magnetic field [mT]	Day–degree [D°]	No. of observations [n]	Oxygen consumption by treatment embryos (ml O_2/h/embryo)			Oxygen consumption by control embryos (ml O_2/h/embryo)			Significance level of treatment vs. control difference	
			$\bar{x}_P$	Variance	Standard deviation	$\bar{x}_K$	Variance	Standard deviation		
50	60–80	6	6.02E–3	9.49E–7	9.74E–4	4.12E–3	1.91E–7	4.37E–4	0.000	$\bar{x}_P > \bar{x}_K$
	90–100	6	4.29E–3	6.75E–7	8.21E–4	4.08E–3	2.68E–7	5.18E–4	0.302	$\bar{x}_P = \bar{x}_K$
	110–130	6	4.01E–3	1.98E–7	4.45E–4	4.02E–3	2.16E–7	4.65E–4	0.489	$\bar{x}_P = \bar{x}_K$
	140–150	6	3.23E–3	1.14E–7	3.38E–4	5.12E–3	8.21E–7	9.06E–4	0.001	$\bar{x}_P < \bar{x}_K$
	160–200	12	6.23E–3	3.49E–7	5.91E–4	5.11E–3	7.81E–7	8.84E–4	0.001	$\bar{x}_P > \bar{x}_K$
	210–290	6	6.22E–3	3.93E–7	6.27E–4	5.69E–3	2.65E–7	5.14E–4	0.053	$\bar{x}_P = \bar{x}_K$
Mean		42	5.06E–3	1.83E–6	1.35E–3	4.58E–3	7.27E–7	8.52E–4	0.041	$\bar{x}_P > \bar{x}_K$
150	60–80	6	4.00E–3	2.25E–7	4.75E–4	3.92E–3	4.95E–8	2.22E–4	0.352	$\bar{x}_P = \bar{x}_K$
	90–100	6	5.94E–3	1.84E–6	1.35E–3	2.07E–3	4.16E–7	6.45E–4	0.005	$\bar{x}_P > \bar{x}_K$
	110–130	6	6.40E–3	1.48E–7	3.85E–4	5.62E–3	3.83E–7	6.19E–4	0.013	$\bar{x}_P > \bar{x}_K$
	140–150	12	5.98E–3	2.77E–7	5.26E–4	4.92E–3	9.19E–7	9.58E–4	0.001	$\bar{x}_P > \bar{x}_K$
	160–200	12	4.59E–3	7.46E–7	8.64E–4	3.72E–3	6.09E–7	7.80E–4	0.008	$\bar{x}_P > \bar{x}_K$
	210–290	6	4.16E–3	3.61E–6	1.90E–3	5.13E–3	6.02E–6	2.45E–3	0.231	$\bar{x}_P = \bar{x}_K$
Mean		48	5.16E–3	1.64E–6	1.28E–3	4.40E–3	2.01E–6	1.42E–3	0.004	$\bar{x}_P > \bar{x}_K$
300	60–80	6	3.19E–3	7.96E–7	8.92E–4	3.30E–3	6.06E–7	7.78E–4	0.413	$\bar{x}_P = \bar{x}_K$
	90–100	6	4.86E–3	2.21E–7	4.70E–4	3.50E–3	1.72E–7	4.14E–4	0.000	$\bar{x}_P > \bar{x}_K$
	110–130	6	4.64E–3	5.96E–6	7.72E–4	3.74E–3	1.03E–6	1.02E–3	0.055	$\bar{x}_P = \bar{x}_K$
	140–150	6	4.28E–3	1.04E–7	3.22E–4	5.52E–3	5.15E–7	7.18E–4	0.005	$\bar{x}_P < \bar{x}_K$
	160–200	12	6.26E–3	1.03E–6	1.02E–3	5.39E–3	1.66E–7	4.07E–4	0.005	$\bar{x}_P > \bar{x}_K$
	210–290	12	6.25E–3	1.14E–5	3.38E–3	6.32E–3	9.61E–6	3.10E–3	0.493	$\bar{x}_P = \bar{x}_K$
Mean		48	5.29E–3	4.50E–6	2.12E–3	4.91E–3	4.04E–6	2.01E–3	0.189	$\bar{x}_P = \bar{x}_K$
Mean of all observations		138	5.18E–3	2.70E–6	1.64E–3	4.64E–3	2.41E–6	1.55E–3	0.004	$\bar{x}_P > \bar{x}_K$

$\bar{x}_P$— mean oxygen consumption by treatment embryos; $\bar{x}_K$—mean oxygen consumption by control embryos; Mean number of embryos observed per treatment was 100.

Table 2

Oxygen consumption (ml O_2/h/embryo) by rainbow trout (*Oncorhynchus mykiss* Walb.) embryos exposed to 5 and 10 mT magnetic fields and in the control

Magnetic field [mT]	Day–degree [D°]	No. of observations [n]	Oxygen consumption by treatment embryos (ml O_2/h/embryo)			Oxygen consumption by control embryos (ml O_2/h/embryo)			Significance level of treatment vs. control difference	
			$\bar{x}_P$	Variance	Standard deviation	$\bar{x}_K$	Variance	Standard deviation		
5	60–80	12	0.57E–3	1.73E–7	4.16E–4	0.27E–3	0.25E–7	1.60E–4	0.012	$\bar{x}_P > \bar{x}_K$
	90–100	12	0.83E–3	1.47E–7	3.83E–4	0.93E–3	0.87E–7	2.95E–4	0.228	$\bar{x}_P = \bar{x}_K$
	110–130	18	1.74E–3	3.29E–7	5.74E–4	1.21E–3	3.04E–7	5.51E–4	0.007	$\bar{x}_P > \bar{x}_K$
	140–150	18	1.23E–3	3.58E–7	5.98E–4	0.77E–3	1.53E–7	3.91E–4	0.005	$\bar{x}_P > \bar{x}_K$
	160–200	18	1.92E–3	1.38E–6	1.17E–3	0.90E–3	3.42E–7	5.85E–4	0.001	$\bar{x}_P > \bar{x}_K$
	210–290	12	1.80E–3	1.85E–7	4.30E–4	1.45E–3	4.24E–7	6.51E–4	0.066	$\bar{x}_P = \bar{x}_K$
Mean		90	1.40E–3	7.00E–7	8.36E–4	0.93E–3	3.36E–7	5.80E–2	0.000	$\bar{x}_P > \bar{x}_K$
10	60–80	6	0.62E–3	0.21E–7	1.46E–4	0.40E–3	0.06E–7	0.78E–4	0.005	$\bar{x}_P > \bar{x}_K$
	90–100	12	1.63E–3	8.48E–7	9.20E–4	1.44E–3	8.36E–7	9.14E–4	0.313	$\bar{x}_P = \bar{x}_K$
	110–130	25	1.05E–3	2.74E–7	5.23E–4	0.70E–3	1.53E–7	3.91E–4	0.005	$\bar{x}_P > \bar{x}_K$
	140–150	12	1.09E–3	2.56E–7	5.06E–4	0.74E–3	0.23E–7	1.54E–4	0.014	$\bar{x}_P > \bar{x}_K$
	160–200	12	1.04E–3	3.89E–7	6.23E–4	0.96E–3	1.63E–7	4.04E–4	0.343	$\bar{x}_P = \bar{x}_K$
	210–290	18	1.30E–3	8.53E–7	9.23E–4	1.42E–3	1.66E–7	4.01E–4	0.309	$\bar{x}_P = \bar{x}_K$
Mean		85	1.16E–3	5.10E–7	7.14E–4	0.98E–3	3.38E–7	5.82E–4	0.036	$\bar{x}_P > \bar{x}_K$
Mean of all observations		175	1.29E–3	6.16E–7	7.84E–4	1.00E–3	4.56E–7	6.75E–4	0.000	$\bar{x}_P > \bar{x}_K$

$\bar{x}_P$— mean oxygen consumption by treatment embryos; $\bar{x}_K$—mean oxygen consumption by control embryos; Mean number of embryos observed per treatment was 35.

Table 3

Carbon dioxide release (ml CO2/h/embryo) by rainbow trout (*Oncorhynchus mykiss* Walb.) embryos exposed to 5 and 10 mT magnetic fields and in the control

Magnetic field [mT]	Day–degree [D°]	No. of observations [n]	Carbon dioxide release by treatment embryos (ml O_2/h/embryo)			Carbon dioxide release by control embryos (ml O_2/h/embryo)			Significance level of treatment vs. control difference	
			$\bar{x}_P$	Variance	Standard deviation	$\bar{x}_K$	Variance	Standard deviation		
5	60–80	12	3.7E–4	6.0E–8	2.4E–4	2.5E–4	1.9E–8	1.3E–4	0.076	$\bar{x}_P = \bar{x}_K$
	90–100	12	5.5E–4	7.8E–8	2.7E–4	5.6E–4	3.8E–8	1.9E–4	0.448	$\bar{x}_P = \bar{x}_K$
	110–130	18	1.04E–3	2.39E–7	4.8E–4	9.0E–4	2.05E–7	4.5E–4	0.191	$\bar{x}_P = \bar{x}_K$
	140–150	18	6.3E–4	8.3E–8	2.8E–4	6.0E–4	1.95E–7	4.4E–4	0.405	$\bar{x}_P = \bar{x}_K$
	160–200	18	7.0E–4	1.78E–7	4.2E–4	4.9E–4	7.9E–8	2.8E–4	0.044	$\bar{x}_P > \bar{x}_K$
	210–290	12	9.2E–4	5.2E–8	2.2E–4	9.9E–4	1.64E–7	4.0E–4	0.294	$\bar{x}_P = \bar{x}_K$
Mean		90	7.2E–4	1.67E–7	4.0E–4	6.4E–4	1.75E–7	4.1E–4	0.097	$\bar{x}_P = \bar{x}_K$
10	60–80	6	3.7E–4	4.2E–8	2.0E–4	2.4E–4	1.4E–8	1.2E–4	0.110	$\bar{x}_P = \bar{x}_K$
	90–100	12	7.6E–4	3.65E–7	6.0E–4	7.6E–4	2.28E–7	4.7E–4	0.490	$\bar{x}_P = \bar{x}_K$
	110–130	25	5.8E–4	1.15E–7	3.3E–4	6.7E–4	1.87E–7	4.3E–4	0.186	$\bar{x}_P = \bar{x}_K$
	140–150	12	4.7E–4	6.6E–8	2.5E–4	4.7E–4	5.3E–8	2.3E–4	0.496	$\bar{x}_P = \bar{x}_K$
	160–200	12	1.08E–3	4.17E–7	6.4E–4	8.3E–4	2.20E–7	4.6E–4	0.153	$\bar{x}_P = \bar{x}_K$
	210–290	18	7.5E–4	2.97E–7	5.4E–4	7.6E–4	1.14E–7	3.3E–4	0.481	$\bar{x}_P = \bar{x}_K$
Mean		85	6.8E–4	2.42E–7	4.9E–4	6.3E–4	3.38E–7	4.1E–4	0.446	$\bar{x}_P = \bar{x}_K$
Mean of all observations		175	7.0E–4	2.04E–7	4.5E–4	6.3E–4	4.56E–7	4.1E–4	0.162	$\bar{x}_P = \bar{x}_K$

$\bar{x}_P$— mean carbon dioxide release by treatment embryos; $\bar{x}_K$—mean carbon dioxide release by control embryos; Mean number of embryos observed per treatment was 35.

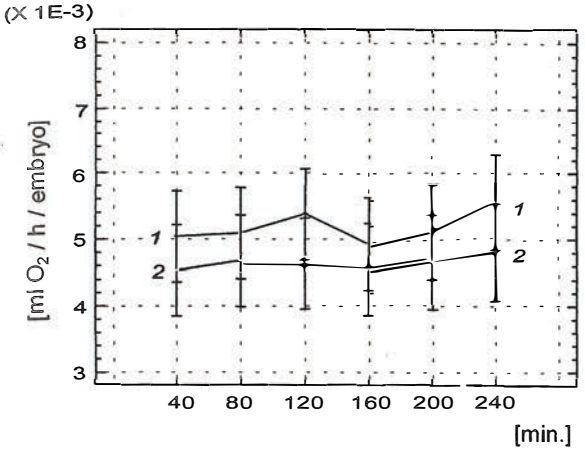

Fig. 3. Changes in oxygen consumption by rainbow trout (*Oncorhynchus mykiss* Walb.) embryos exposed to magnetic fields and in the control throughout the experiment; observations in 50, 150, and 300 mT fields pooled; 1—oxygen consumption in magnetic fields; 2—oxygen consumption in control

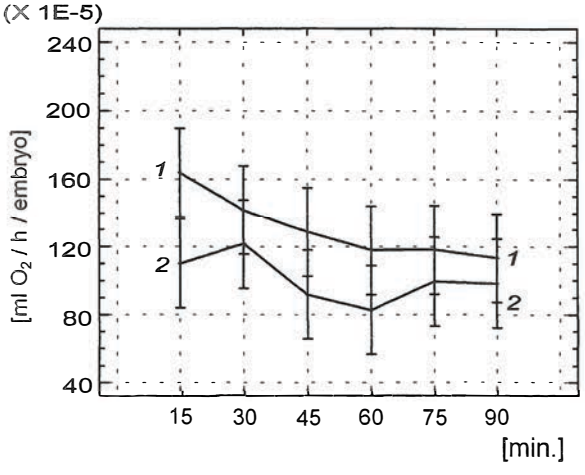

Fig. 4. Changes in oxygen consumption by rainbow trout (*Oncorhynchus mykiss* Walb.) embryos exposed to magnetic fields and in the control throughout the experiment; observations in 5 and 10 mT fields pooled; 1—oxygen consumption in magnetic fields; 2—oxygen consumption in control

No statistically significant differences in O_2 consumption relative to the time of exposure to magnetic field were detected (Figs 3 and 4). However, one can notice a certain similarity between a decrease in O_2 uptake rate and time of exposure in 5 and 10 mT fields. On the other hand, for longer periods of time in 50, 150, and 300 mT treatments both pooled and analysed separately, a slightly accelerated O_2 uptake with time of exposure could be observed. All the differences were, however, slight and not significant.

CO_2 release

No significant differences in CO_2 release could be detected both with respect to embryonic developmental stages and field inductances (Table 3). An exception is provided by the difference between mean releases within 160–200 D° for those embryos exposed to 5 mT field (0.00070 and 0.00049 ml CO_2/h/embryo in treatment and control, respectively; $p = 0.044$). A slight (statistically non-significant) increase in the amount of CO_2 released in the magnetic field relative to the control was recorded within 60–80 D° in the 5mT field and within 60–80

and 160–200 D° in the 10 mT one. The amount of CO_2 released did not changed significantly throughout a single test. Changes in the amount of CO_2 released in the magnetic field during the embryonic development were similar to the changes in the amount of O_2 taken up by an embryo exposed to a magnetic field. This is particularly well visible within 160–200 D° in the 5 mT field when the amount of O_2 consumed increased relative to the control and the amount of CO_2 released increased significantly as well. Such similarities were observed to occur also, to some extent, in the 5 mT field within 60–80 and 110–130 D° and in the 10 mT field within 60–80 D°.

Respiratory quotient

Oxygen uptake and carbon dioxide release was measured simultaneously in the 5 and 10 mT treatments. The results allowed to calculate the respiratory quotient (RQ). Fig. 5 shows pooled RQ values obtained for embryos exposed to both magnetic fields and the control. Generally, RQ values were higher in the control, but no statistical significance of the differences could be detected. The lowest and highest treatment RQ values in the magnetic field were 0.47 and 0.7, respectively; the respective control values were 0.56 and 0.8.

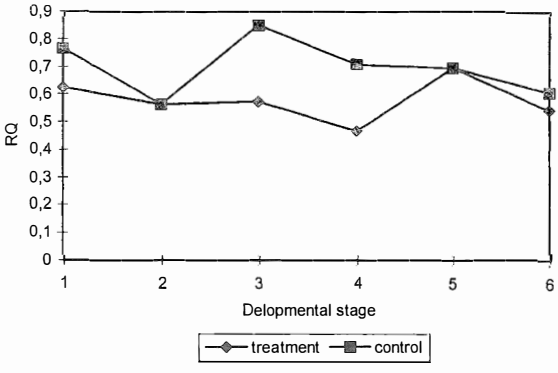

Fig. 5. Respiratory quotients of embryos exposed to 5 and 10 mT fields (pooled observations) and in the control

DISCUSSION

The experiment described in this paper was aimed at addressing a question if, and to what extent, a magnetic field affected embryonic respiration. The answer was sought by comparing the amounts of O_2 consumed and CO_2 released by embryos exposed to artificial magnetic fields and the natural one. The physiological background of magnetic field effects on respiration was investigated as well.

To elucidate the questions addressed, it was necessary to review a number of phenomena, observed in experimental studies involving exposure to magnetic fields, which could affect O_2 uptake of embryos. With respect to constant magnetic field effects on early embryonic development of salmonids, it could be assumed that magnetic field particularly affects those embryos the development of

which undergoes some critical phases. The critical phases during which the embryo is particularly sensitive to external stimuli involve intensive metabolism, related to different stages of cellular division and organogenesis. Studies reported by Trifonova and Popov (1937) on O_2 deficiency effects and those by Privolnev and Razumovskij (1939) involving temperature changes allowed to distinguish three critical phases: the onset of cleavage; beginning of gastrulation; and differentiation of an embryo.

Studies on incubation of fertilised eggs of trout (*Salmo trutta* L.), rainbow trout (*O. mykiss* Walb.), and Danube salmon (*Hucho hucho* L.) exposed to magnetic field demonstrated, too, the presence of periods of increased embryo's sensitivity to magnetic field. The embryos proved particularly sensitive to magnetic field effects immediately after fertilisation (cleavage and gastrulation) (Formicki and Winnicki 1982). The periods of increased sensitivity of the embryo to magnetic field effects seem to partially overlap the so-called critical phases distinguished by other workers.

Experiments involving magnetic field effects on embryonic respiration were started at 60 D°, i.e., at the morula stage. Differences in O_2 consumption between embryos exposed to a field and the control were found to involve eggs from the autumn spawning (exposure to 5 and 10 mT fields). In the 50 mT field, too (the spring spawning eggs), the period of increased sensitivity coincided with the morula stage. An increased sensitivity to magnetic field effects in the 150 and 300 mT fields, manifested as an increased O_2 uptake was recorded within 90–100 D°. The evident similarity of responses to magnetic field effects was observed also during organogenesis and during the circulatory system formation in the embryos (140–200 D°). The intensified sensitivity was in that case manifested not only as an increase, but also—in two treatments (50 and 150 mT fields)—as a reduced O_2 consumption in the magnetic field, relative to the control.

Earlier experiments demonstrated clear effects of magnetic fields on motoric activity of the cardiac muscle and pectoral fins. Application of a magnetic field was shown to produce a change in cardiac contraction rhythm (Winnicki and Formicki 1990) and to affect movements of the pectoral fins (Winnicki et al. 1993). An increase in oxygen demand could be explained by the increased heart beat rate. It should be borne in mind, however, that the increased O_2 consumption at the beginning of an experiment results most probably from increased response to magnetic stimulation at the moment the field is being applied. After this initial period, the oxygen consumption decreases somewhat to stabilise at a level higher than that of the control. Thus an increase in the cardiac muscle activity rate (reverting, after several minutes, to the initial state, the one before the field was applied) may be one, but not the only, factor resulting in the increased O_2 uptake.

Magnetic field affects various levels of organisation and structure of systems studied, which makes it difficult to single out a mechanism responsible for the increased oxygen consumption. At the cellular level, magnetic field effects on biological membranes may provide an explanation for the increased oxygen uptake in the magnetic field and for different responses of an organism at various developmental stages. Some layers of the cellular membrane display liquid crystal properties. Application of a magnetic field results in a change in the state of liquid crystals, which alters their permeability. Moreover, particles penetrating the membranes are electrically charged. Thus the magnetic field affects not only the membrane permeability, but also the electric charges actively transported across the membranes (Wadas 1978). Changes in transport of particles or atoms across biological membranes may result in acceleration or slow-down of cellular or organismic metabolism (Rosen 1993; Tenforde 1993). Such effects would be particularly pronounced during periods of intensified metabolic activity, that is during the critical phases.

On the other hand, Nossol et al. (1993) focused on low density magnetic field interacting with enzymes which catalyse cellular biochemical reactions. Operation and efficiency of intracellular metabolic pathways depend on numerous consecutive enzymatic processes. For this reason, even a weak stimulation affecting one of the initial links may substantially affect subsequent biochemical reactions (Karlson 1971). A 300 μT constant magnetic field activates cytochrome oxydase. The enzyme is responsible for electron transport to particulate oxygen. An increased activity of the enzyme can be explained by magnetic field effects on either the enzyme's structure or on protons or electrons participating in the reaction (Nossol et al. 1993). On the other hand, magnetic field effects on enzymes seem to be selective, which means that not all the enzymes respond to the field and the response in those that do respond is not uniform (Haberditzl 1967). The magnetic field effects on enzymatic activity may explain the absence of significant differences between CO_2 release by treatment and control embryos.

It seems, however, that the following mechanism would be most consistent with the results obtained. Application of a magnetic field reduces permeability of membranes in capillaries and respiratory epithelium of fish larvae. Consequently, at the moment of field application and for some time after it, oxygen diffusion rate slows down and the amount of oxygen taken up decreases. During this time, the larval body passes through an oxygen debt phase during which the cells use up a normal amount of oxygen, but oxygen content in the arterial blood decreases (anactic hypoxia). The larva obtains the necessary energy anaerobically from chemical substances in the body (Szabuniewicz 1964; Depeche and Billard 1994). As in other aquatic organisms, such a state cannot be prolonged in a fish larva because it can lead to asphyxia. Hence the observed compensatory reaction observed,

involving acceleration of the heart beat rate to increase blood circulation. After a moment, another compensatory mechanism is turned on and visible as intensified fin motility, whereby oxygenated water is fed into the larval breathing system (Winnicki and Formicki 1990; Formicki and Winnicki 1996).

A magnetic field can produce stress; an embryo faces then a necessity to adapt to changed environmental conditions. Undergoing an adaptation, the embryo has to make up for its oxygen demand which is higher than that in the control. Those phenomena occur, however, during minute-long periods of time and do not explain the fact that the embryos placed in a magnetic field for prolonged periods of time, at different developmental stages, take up more oxygen than the control ones, while the magnetic field applied throughout the embryonic period extends incubation, which most likely slows down biochemical processes (Formicki, 1991). It would thus seem that the oxygen demand in a magnetic field should be lower, while the reverse is in fact true. It seems that the paradox can be explained by progressive, hyperbiotic processes involving growth and differentiation of cells. Studies on *in vitro* tissue cultures showed that embryonic cells grew faster than adult cells due to cellular growth activators. One can assume that the factors stimulating cellular growth are substantially activated in a magnetic field. This is evidenced by a a higher weight of hatching embryos (Formicki and Winnicki 1982; Formicki 1991) and by the higher oxygen demand of growing cells and tissues. Kholodov et al. (1996) found increased biological oxidation in the brain as a response to application of a magnetic field. The *in vitro* studies of McDonald (1993) demonstrated constant magnetic field to have stimulated growth of cells isolated from neonatal rats. Zhang et al. (1993), when studying domestic hen embryos, found a relationship between alternated magnetic field effects, increased O_2 consumption, and faster growth.

In the present study, apart from the O_2 uptake, the CO_2 release was measured in the treatments involving 5 and 10 mT fields. The results showed a slight increase in the amount of CO_2 released in the magnetic field, relative to the control. The oxygen consumption by developing embryos has been rather extensively treated in the literature, while much fewer studies dealt with CO_2 release (Winnicki 1967). In her experiments with carp embryos, Kamler (1976) found proteins and lipids to be the major sources of the energy expended, the contributions of both types of substances being similar and amounting to 50 and 49%, respectively. The RQ values around 0.7, arrived at by Winnicki (1963) for the rainbow trout, point to lipids as the major energy source for the developing embryos of the species. In the present work, too, the RQ values were close to 0.7, while the magnetic field-affected embryos showed lower RQ values.

The data obtained during the experiment reported here suggest that magnetic field affects oxygen consumption much stronger than it does the CO_2 release. No-

teworthy is a reference to the RQ values mentioned. Should the increase in the O_2 uptake be identical with the amount of CO_2 release, the treatment and control RQ values would be identical. As seen in Fig. 5, the treatment value was in fact lower. Further studies are needed to explain if the mechanism responsible involves selective effects of magnetic field on intracellular metabolic pathways, on substrates of katabolic processes, or altered CO_2 evacuation across shell membranes.

CONCLUSION

1. A constant magnetic field stimulates respiration of rainbow trout embryos, as evidenced by the statistically significant increase in the oxygen uptake.
2. Respiratory stimulation in magnetic field is not uniform throughout the embryonic development and is particularly pronounced during periods of intensified morphogenesis: gastrulation and blastopore closure, organogenesis, and formation and development of circulatory system.
3. Rainbow trout embryos respond to magnetic field by increasing their oxygen uptake at the moment of magnetic field application. The altered level of oxygen demand does not change significantly with time throughout the experiment.
4. At the final stage of incubation (prior to hatching), the magnetic field of inductance values used (5, 10, 50, 150, and 300 mT) did not produce any significant increase in the embryonic oxygen consumption.
5. Application of 5 and 10 mT magnetic fields resulted in a slightly higher carbon dioxide release (at an increased amount of oxygen consumed in the magnetic field), so that the respiratory quotient in those embryos exposed to the fields was slightly lower than that in the control.

ACKNOWLEDGEMENT

The study was financially supported by the Agicultural University's grant No. BW/DRY/067/95 and partly through the State Committee for Scientific Research grant No. PO6 D 002 09.

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WPŁYW STAŁYCH PÓL MAGNETYCZNYCH NA ODDYCHANIE ZARODKÓW PSTRĄGA TĘCZOWEGO (*ONCORHYNCHUS MYKISS* WALB.)

STRESZCZENIE

Badano ilość pobieranego tlenu i wydalanego dwutlenku węgla przez zarodki pstrąga tęczowego (*Oncorhynchus mykiss* Walb.) poddane działaniu stałego pola magnetycznego o wartości 5 i 10 mT (tlen i dwutlenek węgla) oraz 50, 150 i 300 mT (tlen) i porównywano wyniki uzyskane z wynikami zarejestrowanymi u zarodków rozwijających się w warunkach naturalnego pola geomagnetycznego (kontrola).

Stwierdzono, że pole magnetyczne stymuluje procesy oddechowe u zarodków pstrąga tęczowego, czego wyrazem jest istotny wzrost zużycia tlenu, szczególnie większy w okresach wzmożonej morfogenezy.

Pole magnetyczne (5 i 10 mT) powoduje nieznacznie większe wydalanie dwutlenku węgla, przy wzroście ilości zużywanego tlenu. Współczynnik oddechowy zarodków poddanych działaniu pola jest nieco wyższy od współczynnika oddechowego zarodków w kontroli.

Received: 20 December 1997

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