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Fish embryology

SPATIAL ORIENTATION OF TROUT (*SALMO TRUTTA* L.) AND RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALB.) EMBRYOS IN NATURAL AND ARTIFICIAL MAGNETIC FIELDS

ORIENTACJA PRZESTRZENNA ZARODKÓW TROCI (*SALMO TRUTTA* L.) I PSTRĄGA TĘCZOWEGO (*ONCORHYNCHUS MYKISS* WALB.) W NATURALNYM I SZTUCZNYM POLU MAGNETYCZNYM

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Trout (*Salmo trutta* L.) and rainbow trout (*Oncorhynchus mykiss* Walb.) embryos developing in eggs incubated in the geomagnetic field are oriented NW–SE. Artificial horizontal magnetic fields of 0.5 and 1.0 mT, i.e., higher than that of the natural one but of the same direction resulted in a changed alignment of the embryos examined. The embryos placed in those artificial fields were aligned N–S and NE–SW. A 90° clockwise rotation of the artificial horizontal magnetic field poles, relative to the geomagnetic one, resulted in embryos' orientation being changed to N–S and NE–SW.

INTRODUCTION

The Earth's magnetic field affect orientation of numerous living organisms, from bacteria (Blakemore 1975; Blakemore et al. 1980) to mammals (Mather and Baker 1981). The responses observed may be elicited by the magnetic field direction, and even by intensity of the field. Compass reactions involve choice of flight direction by homing pigeons (Walcott and Green 1974) and directional preferences in orientation areas of migratory species such as the European robin (*Erithacus rubecula*) (Wiltschko 1972). Reactions to the geomagnetic field intensity, the so-called map responses, include accurate positioning by pigeons (Gould 1980, 1982; Moore 1980; Walcott 1980).

It has been demonstrated so far that the fish, too, have a magnetic compass they use for navigation and that they can be conditioned to learn to respond to magnetic field. The geomagnetic field can be used for orientation and navigation by the eels *Anguilla anguilla* (Tesch 1974) and *A. rostrata* (Rommel and McCleave 1973; McCleave and Power 1978); yellowfin tuna *(Thunnus albacares)* (Walker 1984); and by sharks and rays (Kalmijn 1982). Conditioned reactions to the magnetic field direction were observed in the ray *Ulorophus helleri* (Kalmijn 1978) and in the yellowfin tuna *(Thunnus albacares)* (Walker 1984).

Salmonids, too, utilise the geomagnetic field in their migrations, as found in the sockeye salmon *(Oncorhynchus nerka)* (Quinn 1980; Quinn et al. 1981; Quinn and Brannon 1982); chum salmon *(O. keta)* (Quinn and Grott 1983); Atlantic salmon *(Salmo salar)* (Rommel and McCleave 1973; Vanrelli and McCleave 1974); and rainbow trout *(S. gairdneri)* (Chew and Brown 1989). The ability to use the Earth's magnetic field was also demonstrated by fry and smolts of the sockeye salmon (Quinn 1980; Quinn et al. 1981; Quinn and Grott 1983) as well as the 6-mo-old juveniles of the rainbow trout (Chew and Brown 1989). On the other hand, in spite of a wide array of species studied, no directional preferences were investigated in fish embryos developing in eggs.

Thus the present study was aimed at finding out whether embryos of trout (*Salmo trutta* L.) and rainbow trout (*Oncorhynchus mykiss* Walb.), developing inside eggs, do orient themselves in the natural and artificial magnetic fields. Additionally, an attempt was made to check if an artificial magnetic field of intensity higher than that of the Earth field, superimposed on the latter, would affect the spatial alignment of a developing embryo, i.e., its spatial orientation.

MATERIAL AND METHODS

The experiments were run in 1996–1997 at the Department of Fish Anatomy and Embryology's Aquarium Laboratory. Eggs and sperm of trout (*S. trutta* L.) and rainbow trout (*O. mykiss* Walb.) spawners were collected at a Polish Anglers' Association capture site on River Wieprza in Darłowo and at the Fish Farm at Żelkowo, respectively. The eggs were transported in 0.75 thermos bottles placed in insulated coolers to ensure an appropriate constant temperature. The sperm was transported under identical conditions, in glass vials. Once in the laboratory, eggs from several females were mixed together and fertilised with sperm of 4–5 males.

The experiments involved developing eggs of trout and rainbow trout. The fertilised eggs were incubated on a substrate made of a black plastic netting stretched on glass supports forming a base so that the netting was placed 3 cm above the bottom of much larger, flat-bottom water-filled glass containers. The netting mesh size was 4 mm, which ensured that the developing eggs were unable to change their position. The netting with eggs was submerged to the depth of 3 cm. Thus optimal conditions for egg incubation were created as a large amount of water around the eggs ensured good oxygenation. A single container with eggs on a netting, placed in the geomagnetic or an artificial magnetic field formed an experimental unit.

The magnetic fields were generated by ferritic magnets with expanders, arranged at distances appropriate to produce the desired field (0.5 or 1.0 mT). The eggs were continuously exposed to a field, from immediately after fertilisation to the termination of the experiment, i.e., until the embryo orientation was determined. As that period took about 2 weeks, the magnets were used.

The magnetic field intensity was measured with an HTM-12 hallotronic teslameter at a single point.

A total of 15 experimental units were used: 7 units containing trout eggs and 8 units containing rainbow trout eggs (Table 1). Two units per species were placed in the geomagnetic field. Those units served simultaneously as a control for the eggs of the remaining units, placed in the artificial fields superimposed on the geomagnetic field. Six and five units were placed in 0.5 mT and 1.0 mT fields, respectively. The units placed in the natural magnetic field (control) and those in the artificial fields contained several hundred eggs each.

Table 1

Directional preferences of trout and rainbow trout embryos in natural and artificial magnetic fields (Raleigh test's mean vector, r and significance level)

Species	Magnetic field (mT)	Developmental stage (D°)	No. of embryos in units (inds)	Directions preferred	Mean vector, *r*	Significance level
Trout (*Salmo trutta* L.)	geomagnetic field *	135	449	NW–SE	0.2691	$p < 0.001$
		186	666	NW–SSE	0.1279	$p < 0.005$
	0,5	135	446	NE, N	0.2042	$p < 0.001$
		186	825	N–S, NE	0.1698	$p < 0.005$
	1,0	135	656	N–S, NE–SW	0.2569	$p < 0.001$
		186	786	N–S	0.1592	$p < 0.006$
	0,5 **	135	168	N–S, NE–SW	0.1653	$p < 0.005$
Rainbow trout (*Oncorhynchus mykiss* Walb.)	geomagnetic field *	107	750	NW–SE	0.2223	$p < 0.001$
		121	646	NW–SE	0.2093	$p < 0.001$
	0,5	107	549	N–S, NE–SW	0.2261	$p < 0.001$
		121	979	N–S, NE–SW	0.1946	$p < 0.001$
	1,0	107	657	N–S, NE–SW	0.1972	$p < 0.06$
		121	838	N–S, NE–SW	0.1621	$p < 0.006$
	0,5 **	107	297	N–S	0.1237	$p < 0.05$
	1,0 **	107	214	N–S, NE	0.2171	$p < 0.001$

* embryos incubated in geomagnetic field (control).

** magnetic field poles rotated 90° clockwise.

The containers with netting were filled with well-oxygenated water of a suitable temperature and the fertilised eggs were carefully spooned, with a plastic spoon, into the netting meshes.

Eight experimental units were exposed to 0.5 and 1.0 mT magnetic fields co-directional with the Earth's one, the three remaining units being exposed to a field of the same intensity, but of poles rotated clockwise by 90° relative to the natural field, i.e., perpendicular towards it (Table 1). The units were placed in the same darkened room, arranged far apart, and kept under identical conditions and at constant temperature.

Water temperature was measured daily in order to calculate the number of degree-days (D°). Every 3 days, some of the water was removed and new water was added with the use of a 5 mm diameter tubing, the care being taken not to disturb the eggs on the netting.

Orientation of the embryos was determined twice. As the embryos D° not move at the initial stage of their development, the first reading was taken immediately after the blastopore closed, when an outline of the embryo was visible, i.e., after 135 and 107 D° for trout and rainbow trout, respectively. The second reading (on other eggs of the same species) was taken before caudal movements appeared, after 186 and 121 D° for trout and rainbow trout, respectively, as motility of the embryos could have distorted the results.

The determinations were made after the water filling the containers was first very slowly and carefully removed through a 5 mm diameter plastic tube, following which the eggs were saturated with diluted acetic acid. Application of the acid resulted in the embryo's outline becoming more conspicuous, well-visible behind the egg membranes. In addition, before each reading, the embryos placed on Petri dishes were photographed. The alignment (orientation) of each embryo was determined using a 16-rhumb circular scale (direction rose), with a hole in the centre to allow viewing the embryo. The alignment of the frontal part of the embryo was determined. Once the orientation of an embryo was read, the egg was removed from the dish to avoid repeated readings. The results thus obtained were double checked by comparing them with photographs taken before readings.

The results were treated statistically with Rayleigh's test (Batschelet 1981) and presented as circular (radar) graphs.

RESULTS

As shown by the readings of embryonic orientation, the trout embryos incubated in the natural magnetic field showed, both after 135 and 186 D°, a preference towards NW–SE and towards NW and SSE on 186 D° (Fig. 1).

On the other hand, the embryos exposed to the experimental 0.5 mT magnetic field were found to choose, both after 135 and 186 D°, primarily a NE orientation as well as N after 135 D° and N–S and NE after 186 D° (Fig. 2).

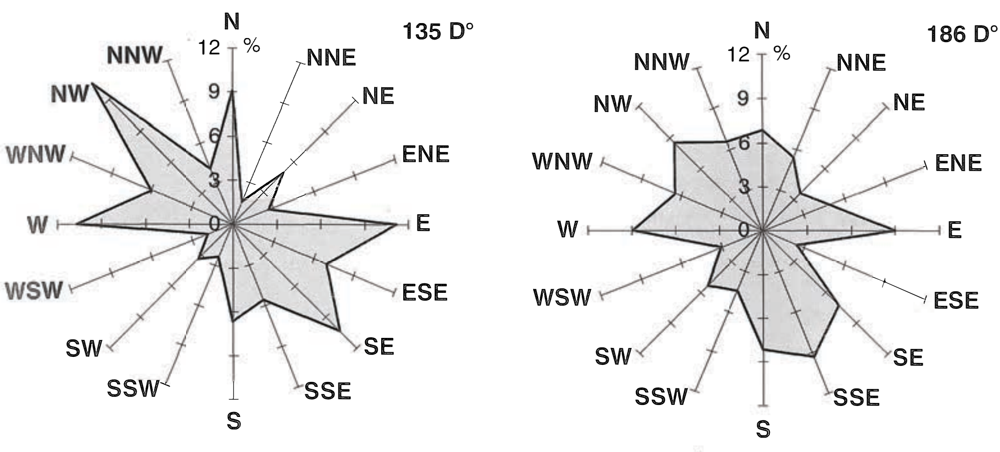

Fig. 1. Directional preferences of trout (*Salmo trutta* L.) embryos in the geomagnetic field (135 and 186 D°)

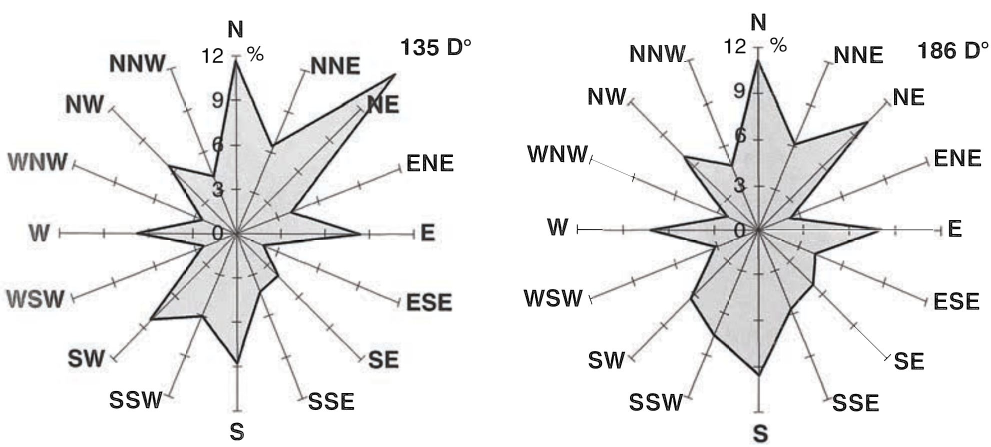

Fig. 2. Directional preferences of trout (*Salmo trutta* L.) embryos in 0.5 mT horizontal artificial magnetic field (135 and 186 D°)

Those embryos incubated in the 1.0 mT field, chose—both after 135 and 186 D°—to align themselves N–W and NE–SW (Fig. 3).

Magnetic field poles applied to one of the samples placed in the artificial 0.5 mT field were rotated clockwise by 90° relative to the natural field; the embryonic alignment showed a preference mainly towards N–S, a weaker preference toward NE–SW being recorded as well. The orientation was determined after the blastopore closure (135 D°) (Fig. 4).

Results as to spatial orientation of the rainbow trout embryos were similar. After the blastopore closure (107 D°), the embryos placed in the natural magnetic field aligned themselves NW–SE; after 121 D°, i.e., before mobility began, the orientation was identical (Fig. 5).

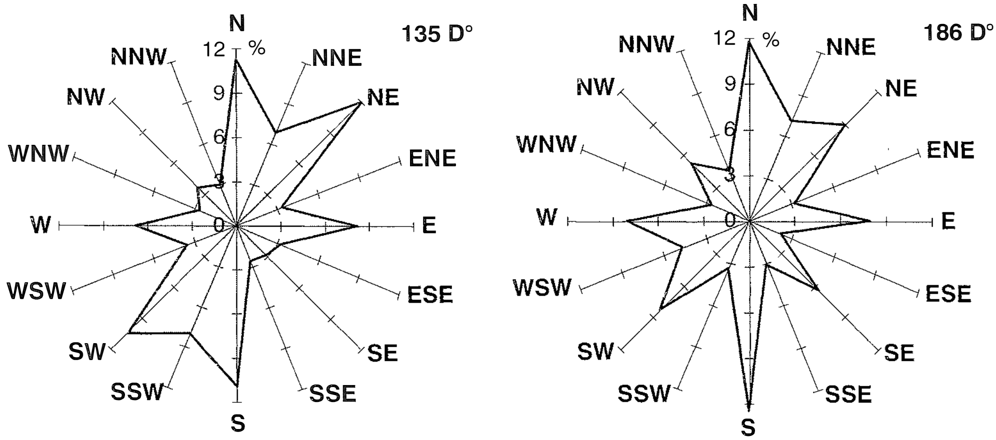

Fig. 3. Directional preferences of trout (*Salmo trutta* L.) embryos in 1.0 mT horizontal artificial magnetic field (135 and 186 D°)

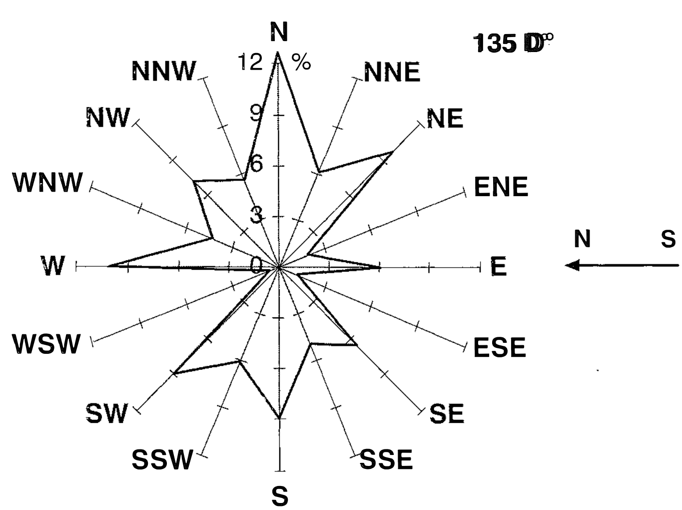

Fig. 4. Directional preferences of trout (*Salmo trutta* L.) embryos in 90°-rotated 0.5 mT horizontal artificial magnetic field (135 D°)

The preferred orientation in the 0.5 mT artificial magnetic field superimposed on the natural one was N–S and to some extent NE–SE on both occasions (after 107 and 121 D°) (Fig. 6).

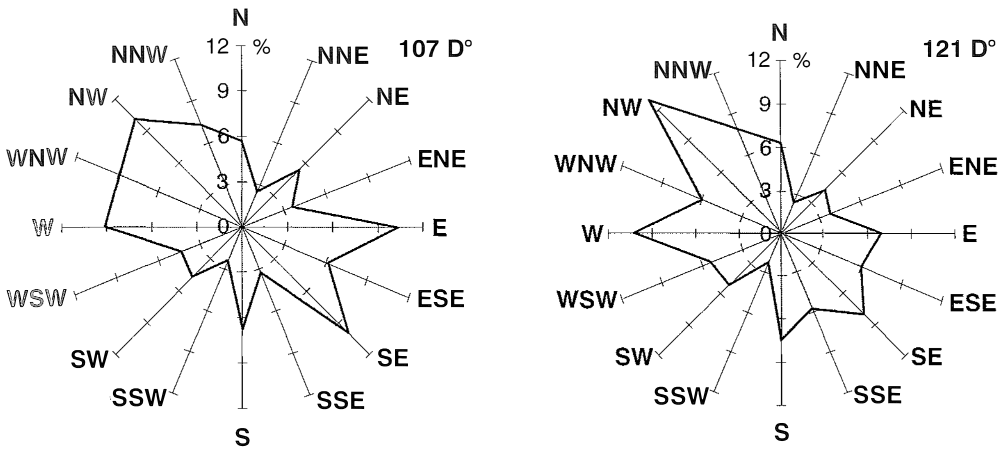

Fig. 5. Directional preferences of rainbow trout (*Oncorhynchus mykiss* Walb.) embryos in the geomagnetic field (107 and 121 D°)

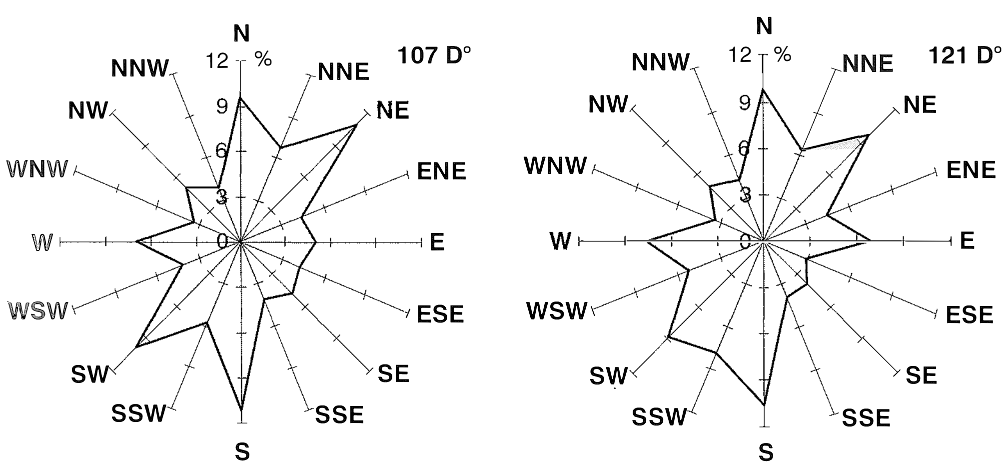

Fig. 6. Directional preferences of rainbow trout (*Oncorhynchus mykiss* Walb.) embryos in 0.5 mT horizontal artificial magnetic field (107 and 121 D°)

In the 1.0 mT magnetic field, the preferred orientation was generally N–S as well, with some deviation towards NE–SW on both occasions (Fig. 7).

Similarly to the trout embryos, the rainbow trout ones were exposed to 90°-rotated artificial magnetic fields of 0.5 and 1.0 mT inductance. The preferred directions were N–S and to some extent NE in the 0.5 mT field, while in the 1.0 mT field, the embryos preferentially aligned themselves N–S, with a NE–SW deviation. The orientation was determined after the blastopore closure (Fig. 8).

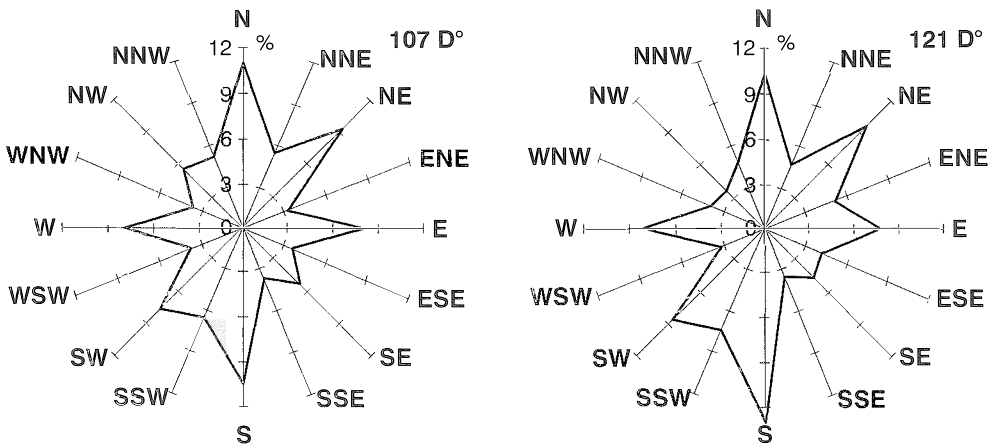

Fig. 7. Directional preferences of rainbow trout (*Oncorhynchus mykiss* Walb.) embryos in 1.0 mT horizontal artificial magnetic field (107 and 121 D°)

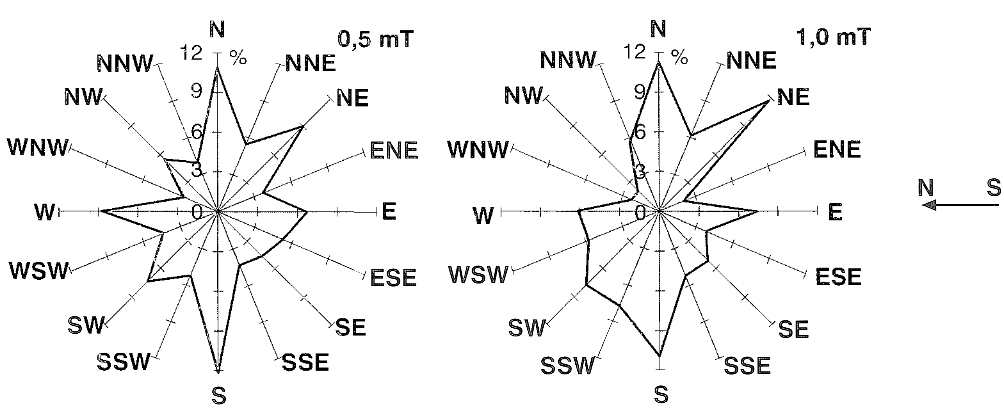

Fig. 8. Directional preferences of rainbow trout (*Oncorhynchus mykiss* Walb.) embryos in 90°-rotated 0.5 and 1.0 mT horizontal artificial magnetic fields (107 D°)

DISCUSSION

As already pointed out in the Introduction, studies on fish orientation in a magnetic field, carried out mainly on adults, but also on smolt and fry of salmonids, showed a clear effect of the Earth's magnetic field on the direction of fish movement. A substantial reduction of the natural field intensity (screening) resulted in a loss of orientation (Chew and Brown 1989).

The trout and rainbow trout embryos exposed to the natural and artificial magnetic fields were clearly affected by them and preferred certain directions in their alignment in the egg. In the natural field, after the blastopore closed and before motility began, the embryonic symmetry axis was most frequently aligned NW–SE.

The artificial magnetic field, superimposed on the natural one, resulted in a change in preferred direction of alignment by several degrees clockwise, relative to the original orientation in the natural magnetic field, towards N–S and NE–SW.

The data obtained in samples exposed to 90°-rotated fields confirmed a stronger effect of the artificial field on the direction of alignment as the embryos oriented themselves to the new field applied. The preferred directions (N–S and NE–SW) were very similar to those selected in the artificial fields of direction identical with the natural one.

As demonstrated by the results, under stable thermal conditions and in the darkness, that is under conditions similar to those prevailing in the nests of the salmonids tested, the embryos displayed an ability to orient themselves in a magnetic field. A possibility that the sight and olfaction, utilised by migrating fish juveniles and adults, could be used in this orientation has to be rejected. The sight and olfaction in trout and rainbow trout embryos at the developmental stages used in the experiment have not formed yet; the nervous system has not been formed yet either.

The spatial alignment preferences, demonstrated by the developing trout and rainbow trout embryos, may be related to effects of magnetic fields on magnetite particles contained in the ethmoidal and posterior parts of the skull of many fish species, i.a., *Thunnus albacares, Th. alalunga, Th. obesus, Sarda sarda, Scomber* sp., *Engraulis mordax, Oncorhynchus tschawytscha* (Walker et al. 1985). A possibility of the nervous system using the forces affecting magnetite particles had been pointed out to earlier (Ising 1945; Lowenstam 1962; Keeton 1972), but it was only after magnetite crystals were discovered in magnetotactic bacteria (Frankel et al. 1979; Frankel and Blackemore 1980), bees (Gould et al. 1978), birds (Walcott et al. 1979), and mammals (Zoeger 1981) that the theory received a good backing. If magnetite, indispensable for orientation, is synthesised metabolically throughout an individual's life and accumulated in the skull's ethmoidal part, the adults contain higher amounts of the substance and have a better orientation by being able to utilise the ambient magnetic field than juveniles (Kirschvink and Gould 1981; Kirschvink et al. 1985; Chew and Brown 1989). It would be difficult, though, to suspect the presence of large quantities of magnetite in embryos; even if, however, magnetite is present, the nervous system has not been formed yet and the active selection of alignment by the embryos in the ambient magnetic field is rather doubtful. It seems that the directional preferences observed in the embryos we tested could be related to spontaneous motoricity of the blastodisc, or the ectoplasm covering the entire surface of the egg cell, and manifest as spin and circular movements of the embryonic disc, as observed at much earlier developmental stages in pike (Rezničenko 1956). The motoricity could play a corrective role in other fish species (including those we tested). This line of reasoning is supported by observations made by Korzelecka and Winnicki (pers. comm.) who detected a wave of contractions in a form of a streak moving slowly along the surface of a developing embryo.

CONCLUSION

Salmonid embryos are sensitive to constant magnetic fields and display certain directional preferences, as evidenced by changes in their symmetry axis alignment relative to the geomagnetic field lines. The sensitivity is displayed also in artificial magnetic fields. There is a relationship between the responses and the magnetic field value as shown by responses to the natural and stronger artificial fields.

ACKNOWLEDGEMENT

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ORIENTACJA PRZESTRZENNA ZARODKÓW TROCI (*SALMO TRUTTA* L.) I PSTRĄGA TĘCZOWEGO (*ONCORHYNCHUS MYKISS* WALB.) W NATURALNYM I SZTUCZNYM POLU MAGNETYCZNYM

STRESZCZENIE

Jaja troci (*Salmo trutta* L.) i pstrąga tęczowego (*Oncorhynchus mykiss* Walb.) inkubowano w warunkach uniemożliwiających zmianę ich położenia, bez wpływu światła i w stałej temperaturze. Określano położenie przestrzenne rozwijających się zarodków, w okresach po zamknięciu blastoporu i poprzedzającym ich pierwsze ruchy, w polu geomagnetycznym oraz w horyzontalnym sztucznie wytworzonym polu magnetycznym, nałożonym na ziemskie (0,5 i 1,0 mT).

Stwierdzono, że zarodki rozwijające się w ziemskim polu magnetycznym układają się w kierunkach północny zachód – południowy wschód (NW–SE). Sztucznie wytworzone horyzontalne pole magnetyczne, o tym samym kierunku co naturalne pole geomagnetyczne i na nie nałożone, powodowało zmianę preferencji kierunkowych u zarodków w porównaniu z zarodkami umieszczonymi w polu ziemskim.

Zarodki układały się w kierunkach północ – południe (N–S) i północny wschód – południowy zachód (NE–SW). Obrócenie biegunów (zmiana położenia) sztucznie wytworzonego horyzontalnego pola o 90° zgodnie z ruchem wskazówek zegara, w odniesieniu do naturalnego pola geomagnetycznego, powodowało, że rozwijające się zarodki troci i pstrąga tęczowego preferowały kierunki północ – południe (N–S) oraz północny wschód – południowy zachód (NE–SW) tego pola.

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