e-ISSN 1734-9168
http://www.isez.pan.krakow.pl/en/folia-biologica.html

Folia Biologica (Kraków), vol. 66 (2018), No 4
https://doi.org/10.3409/fb_66-4.16

Effects of Geomagnetic Field Deprivation on Embryonic Development and Hatching of Prussian Carp (*Carassius gibelio*)

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Accepted December 13, 2018 *Published online December 20, 2018* *Issue online December 31, 2018*

Original article

TOMBARKIEWICZ B., BOJARSKI B.✉, OLESEK A., PAWLAK K., KANIK W., WOJNAR T., LIS M., SOCHA M. 2018. Effects of geomagnetic field deprivation on embryonic development and hatching of Prussian carp (*Carassius gibelio*). Folia Biologica (Kraków) **66**: 151-158.

Fish are sensitive to environmental conditions, including electromagnetic factors. The aim of the present study was to evaluate the influence of geomagnetic field (GMF) deprivation on embryo mortality, percentage of deformed larvae, and hatching rate of the freshwater Prussian carp (*Carassius gibelio*). The results indicate that hypogeomagnetic conditions can affect embryogenesis in Prussian carp. GMF deprivation led to an increase of embryo mortality after 24 and 72 hours of incubation, acceleration of the hatching process and increase of hatched egg percentage till 129 hours. The shielding of the geomagnetic field did not change the percentage of deformed larvae.

Key words: Hypogeomagnetic conditions, freshwater fish, embryogenesis.

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The geomagnetic field (GMF) is a natural field that constitutes an integral part of the geosphere that surrounds the Earth. It reaches a distance substantially exceeding the Earth's radius as well as protects the planet against cosmic radiation and solar wind. The average strength of the Earth's magnetic field depends on the region. On the equator magnetic flux density amounts to approximately 35 μT (0.35 Oe) and the field lines extend parallel to the Earth's surface. Magnetic poles are the points where the field lines run perpendicularly to the Earth's surface and the magnetic flux density reaches values between 60 and 70 μT (0.6-0.7 Oe) (ZHADIN 2001). Multiple environmental observations and laboratory experiments that have been carried out so far indicate that development and subsequent evolution of life on the Earth occurred in the presence of the geomagnetic field (BROWN 1963). Organisms living on the Earth adapted to surrounding environmental conditions, including the presence of the geomagnetic field (RAUSCHER & BISE 1999).

Animals are naturally capable of detecting Earth's magnetic field. Spatial orientation based on sensing GMF is a well-known phenomenon that has been described in amphibians, reptiles, fish, birds and mammals (WILTSCHKO & WILTSCHKO 2005; WANG *et al*. 2006; CLOSE 2012). It has been suggested that GMF present in the seas and oceans is a key navigation marker for fish. Fish can detect electric charges generated as a result of intersecting geomagnetic field lines and ocean currents can also be produced by magnetic disturbances caused by solar activity (ZHADIN 2001). Some fish migrate over large distances and are capable of returning to their birthplace without depending on astrological or hydrological indicators. Experiments show that after transporting fish to another

location, similarly to pigeons, animals swim in circles and after a while choose the proper direction. Sea turtles, like migratory birds, determine position and select an appropriate migratory direction based on slight changes in the magnetic field (PAPI *et al*. 2000). Whales also take advantage of alterations to the magnetic field during migration. It has been hypothesized that cetacean stranding may be associated with losing orientation due to encountering a strong artificial magnetic field (WALKER *et al*. 1992). Under uniform conditions in terms of electrical conductivity, temperature and water depth, fish use mainly GMF for navigation (KRYLOV *et al*. 2016).

Organisms are particularly sensitive to environmental conditions, including the geomagnetic field, during embryogenesis, organogenesis and early developmental stages. The resonance mechanism, associated with a particular frequency of the magnetic field which affects membrane permeability in cells placed in a given field, represents one of the mechanisms explaining the effects of magnetic fields on living organisms (LIBOFF 1991). By triggering changes in cell membrane permeability, magnetic fields can affect the whole body through a sequence of various neuropsychological processes. There is certain evidence available demonstrating that permanent magnetic fields produce direct effects on single neurons, nervous tissue and animal brain (ZHADIN 2001). Moreover, research carried out so far by different authors indicate that long-term GMF deprivation is detrimental to animals and leads to histological, pathophysiological and morphological alterations (ASASHIMA *et al.* 1991; TOMBARKIEWICZ *et al*. 2004; TOMBARKIEWICZ 2008; PAPAILIOU *et al.* 2011).

The aim of the study was to assess the impact of hypogeomagnetic conditions on embryogenesis, hatching dynamics, malformation severity and mortality rate of larvae of the Prussian carp, *Carassius gibelio* (Bloch, 1782).

Material and Methods

Biological material and experimental design

Biological material consisted of spawn obtained from six healthy and sexually mature females of Prussian carp (*Carassius gibelio*) and two common carp males (C*yprinus carpio*). Eggs from 6 Prussian carp females and sperm from 2 sexually mature common carp males were collected at the initial stage of the experiment. Ovulation was hormonally induced by stimulating fish with an analogue of salmon GnRH (Bachem Feinchemicalien AG, Switzerland) at a dose of 10 μg per kilogram of body weight and pimozide, which is an antidopaminergic agent (5 mg per kilogram of body weight) (SOCHA *et al*. 2012). After detecting ovulation symptoms, animals were subjected to artificial spawning. The spawn from each female was divided into four Petri dishes (about 220 eggs in each dish), and next two of them were activated with sperm of common carp A and the other two with sperm of carp B (20 μl of sperm were added to each Petri dish). The dishes were placed in control or hypogeomagnetic conditions 10 minutes after egg activation. The incubation lasted for about 5 days. Prior to the experiment tap water was aerated and supplemented with an antifungal agent, Fungi Stop Konzentrat (TETRA, Germany). The water in Petri dishes was replaced every 12 hours.

Stimulated fish eggs were divided into two main groups: control (12 Petri dishes – two dishes from each female – one of them activated by sperm of carp A and the second one by sperm of carp B) and experimental (the same scheme as in the control group). The control group was maintained in a natural GMF (about 37 μT), while the experimental group was incubated in a weakened geomagnetic field.

Hypogeomagnetic conditions were created by placing Petri dishes is shielding cages in which GMF was reduced below 12 μT. In addition, the magnetic field in cages was nearly horizontal since the vertical component of the Earth's magnetic field was damped by a factor of 0.1 or less, as measured with Geo-Scanner BPT 3010 (Weiss and Partner, Bio-PhysikTechnologie, Wassenach, Germany). GMF shielding was achieved by using additional cages made of steel (S235JRG2 type; CMC, Zawiercie, Poland) (ROMAN & TOMBARKIEWICZ 2009).

To ensure comparable lighting conditions, Petri dishes of the control group were placed in the cages of the same construction but made of a material that does not disturb the geomagnetic field.

During incubation, fish eggs were classified as live (fertilized eggs with live embryos) or as dead (eggs which were not fertilized or died during embryogenesis) according to SOCHA *et al*. (2012).

After a 24-hour incubation, live and dead eggs were counted to assess embryo mortality rate. Counting, each time in the same order, was repeated after 48 and 72 hours of incubation. Live and dead eggs were distinguished based on their color – dead eggs were white, as opposed to live eggs, which were transparent.

All hatched larvae were counted every three hours from the beginning of hatching and classified within each group into non-deformed and de-

formed (with vertebral curvatures, yolk sac malformation or head deformation). The observations were conducted using a magnifying glass (magnification 10 x). Counting of larvae was performed until the end of hatching in the experimental group. The timing of the end of this experiment was set at 129 hours of incubation, when every live egg of the experimental group reached the larvae stage and further observation revealed that eggs of the control group showed no progress of hatching although they were not dead.

Embryo mortality rate (percentage of dead eggs after 24, 48 and 72 hours of incubation compared to all seeded eggs), hatchability (total ratio of hatched eggs to all seeded eggs), hatching dynamics and the ratio of deformed to non-deformed larvae every three hours from the beginning of hatching were determined based on the results of counting.

To check the impact of female, male and status of GMF on the embryo mortality rate and hatchability, calculations were made according to the following linear model:

$$Y_{ijkl} = \mu + female_i + male_j + GMF_k + e_{ijkl}$$

where:

- Y_{ijkl} – value of observation (% embryo mortality at 24, 48 and 72 hour of incubation; % of hatching)
- μ – general average
- $female_i$ – the effect of the i-th female (i = 1,…, 6)
- $male_j$ – the effect of the j-th male (j = A, B)
- GMF_k – the effect of geomagnetic field (control group – non disturbed GMF, experimental group – hypogeomagnetic conditions)
- e_{ijkl} – random error

The effects of male and female proved to be insignificant. The differences between control and experimental (GMF-deprivation) groups regarding the embryo mortality rate and hatchability were analyzed with a post-hoc Tukey's test. The effect of GMF deprivation on the dynamics of hatching at particular incubation hours was estimated using analysis of variance in a hierarchical system (field nested in time) and followed by Tukey's test. The significance level was set at $\alpha = 0.05$. Before each ANOVA, the normality of distribution and homogeneity of variance were examined using Shapiro-Wilk's test and Levene's test, respectively. The PROC GLM in SAS (SAS Institute Inc., ver. 9.2) was used. The obtained data was presented in percentage values ± SD.

Results

Embryo mortality

After 24-hour incubation some dead eggs, cloudy and whitish, detached from the bottom of Petri dishes, while the zona pellucida of live embryos was transparent, enabling further observation of embryo development. Both control and experimental groups revealed the presence of embryos at the stage of gastrulation. Embryos on yolks were clearly visible. At this stage of incubation, embryo mortality rate amounted to 25±7% in the control group and to 33±7% in the experimental group. The difference between mortality rates in both groups was statistically significant (p=0.0001) (Fig. 1).

After 48 hours of incubation the number of dead eggs increased. The eggs were at the late organogenesis stage and it was possible to identify meta-

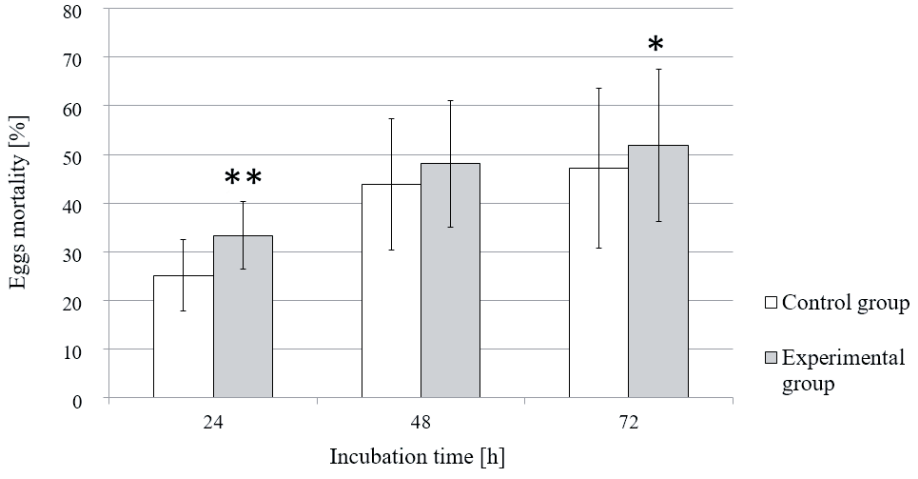

Fig. 1. The effect of geomagnetic field deprivation on egg mortality rate of Prussian carp (*Carassius gibelio*) in the 24th, 48th and 72nd hour of incubation. Statistically significant differences ($p<0.05$) in relation to control marked with *, while highly significant ($p<0.01$) with ** (Tukey's test). Data presented as mean percentage values ± SD.

meric structures. The embryo surrounding the yolk had a slightly developed head with a visible brain and optic vesicles and a clearly marked tail. Development in the experimental group was accelerated and embryos were characterized by higher mobility. The number of dead eggs in the control group was lower, 44±13%, as compared to the experimental group, 48±13%. The difference was statistically insignificant (Fig. 1).

In the 72nd hour of incubation embryos were moving inside the zona pellucida, had developed eyes, unconstrained tails, a cardiovascular system with a beating heart and some of them displayed signs of pigmentation changes. At this stage of incubation the average mortality rate in the control group reached 47±16%, while in the experimental group 53±16%. The difference was statistically significant (p=0.0329) (Fig. 1).

Hatchability

Towards assessing the effects of hypogeomagnetic conditions on the hatchability of Prussian carp, after finishing the experiment, the percentage of hatched eggs in the experimental group was compared with the percentage of hatched eggs in the control group. The results are presented in Fig. 2. At the end of the experiment the percentage of eggs hatched into larvae in the experimental group amounted to 47±15% and was significantly higher (p=0.0035) than in the control group, in which at the end of the experiment (129th hour of incubation) approximately 34±10% eggs reached the larval stage.

Hatching dynamics

Hatching into larvae commenced 6 hours earlier in the experimental group and the percentage of hatched larvae in this group at the end of the experiment was greater by 13% than in the control group (Fig. 2). Observations of hatching dynamics were repeated every three hours until larvae stopped hatching, when the majority of Prussian carp embryos remaining in Petri dishes were already dead. The first larvae in the experimental group hatched in the 93rd hour of incubation, while in the control group in the 99th hour. The hatching dynamics of Prussian carp are presented in Fig. 3 taking into account the average percentage of hatched larvae in both control and experimental groups. Hatching dynamics were significantly higher in the experimental group at 105, 108, 114, 117, 123-129 ($p \leq 0.0089$) and the 120th hour of incubation (p=0.0357). After leaving the zona pellucida, larvae were motile and their length reached approximately 5 mm. They had a yolk sac and underdeveloped mouth.

Ratio of deformed to non-deformed larvae

The ratio of deformed to non-deformed larvae in particular incubation hours in both groups was comparable. The percentage of deformed larvae between the 99th and 129th hour of incubation in the experimental group ranged between 5% and 31%, while in the control group between 13% and 34% (Fig. 4). The most frequently occurring deformations included: scoliosis, kyphosis, lordosis, yolk sac malformation with scoliosis, shortened and upward-directed spine, malformed yolk sack with edema, C-shaped body with deformed tail and spine as well as yolk sack edema.

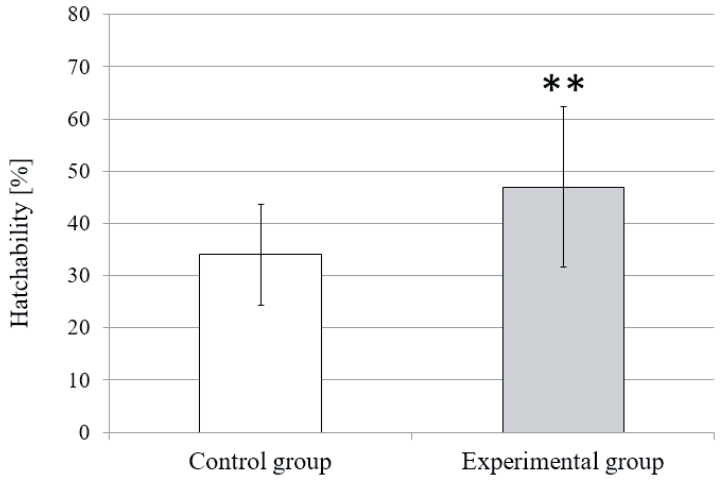

Fig. 2. The effect of geomagnetic field deprivation on the hatchability of Prussian carp (*Carassius gibelio*). Statistically highly significant differences ($p<0.01$) in relation to control marked with ** (Tukey's test). Data presented as mean percentage values ± SD.

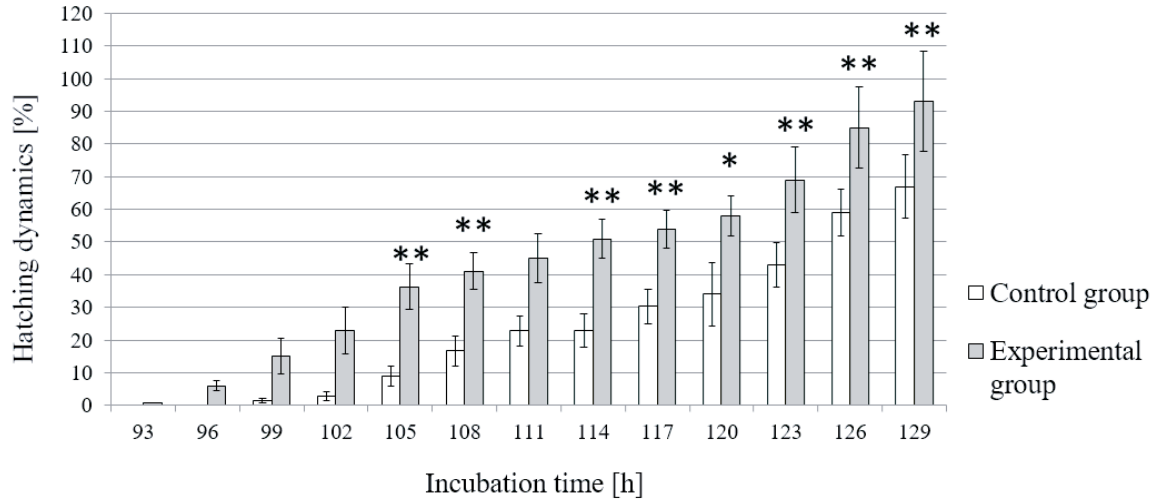

Fig. 3. The effect of geomagnetic field deprivation on the hatching dynamics of Prussian carp (*Carassius gibelio*) larvae. Statistically significant differences ($p<0.05$) in relation to control were marked with *, while highly significant ($p<0.01$) with ** (Tukey's test). Data presented as mean percentage values ± SD.

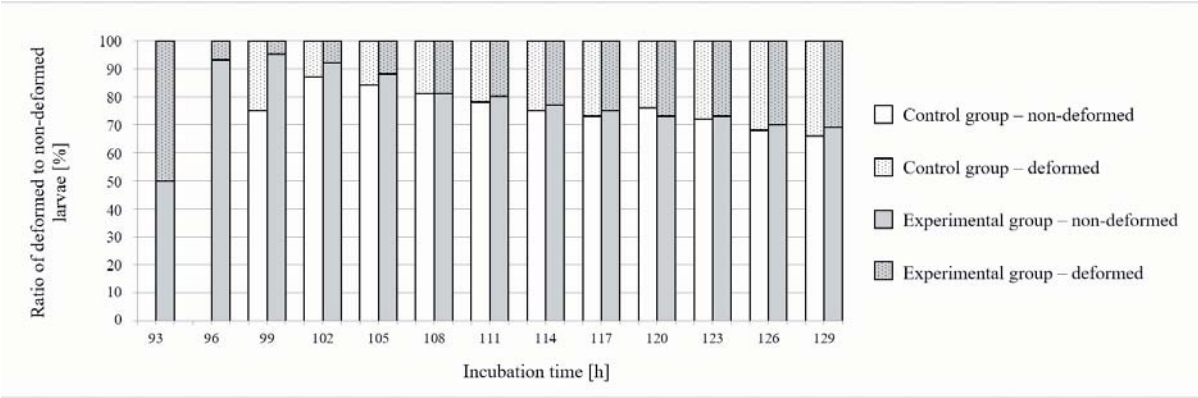

Fig. 4. The effect of geomagnetic field deprivation on the occurrence of deformations in Prussian carp (*Carassius gibelio*) larvae hatched at particular incubation hours. Data ae presented as mean percentage values, SD values were omitted.

Discussion

The animal body is particularly susceptible to environmental stimuli, including the geomagnetic field, during embryogenesis, organogenesis and early developmental stages. The geomagnetic field exerts effects on living organisms through affecting water, constituting a basic component of all organisms and providing medium for multiple chemical reactions (ZHADIN 2001; PAPAILIOU *et al*. 2011). Water particles display enhanced reactivity in the presence of magnetic and electric fields. Moreover, a magnetic field can affect living organisms by changing properties of magnetic elements that commonly occur in the body, altering properties of liquid crystals that (similarly to magnetic elements) can be found in each living cell (cell membranes, nucleic acids, myelin sheath covering nerve fibers, etc. are liquid crystals) (COWIN 2004; BOULIGAND 2008) as well as generating ion currents in various systems, such as nervous, muscular, cardiovascular and the brain (MICEK & MICEK-ILNICKA 2001; MITSUTAKE *et al.* 2005).

The present study indicates that deprivation (weakening) of the geomagnetic field affects embryogenesis and the process of larvae hatching in Prussian carp. It can be hypothesized that hypogeomagnetic conditions changed the permeability of cell membranes resulting in electrolyte imbalance and an acute condition that led to embryo death. Observations made in the 24th and72nd hours

of incubation show that deprivation of GMF caused an increase in the mortality rate. Earlier studies conducted by FORMICKI & WINNICKI (1998) were aimed at assessing the effect of a permanent magnetic field generated by permanent magnets (experimental MF ranging between 2.5 mT and 13 mT) on embryonic development of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*). An additional permanent magnetic field slowed down embryonic development and prolonged the incubation period as compared with the control group. A magnetic field of lower values (1-5 mT) exerted more evident positive influence on the embryonic and larvae development of the fish than the field of higher values. Larvae hatched from eggs which remained under the influence of magnetic fields during the entire incubation were longer and heavier at the moment of completed yolk resorption (FORMICKI & WINNICKI 1998). Our experiment demonstrated that the electromagnetic environment affects the percentage of hatched eggs and hatching dynamics in Prussian carp. Deprivation of the geomagnetic field accelerated hatching into larvae by 6 hours. It is likely that this could be the reason why the hatching rate of Prussian carp at the end of the experiment was significantly higher in the experimental group.

LEE & YANG (2014) conducted research on medaka (*Oryzias latipes*) and showed that exposure to an electromagnetic field (15-60 μT; 3.2 kHz) 5 hours after fertilization accelerates embryonic development. Conflicting results were obtained by CAMERON *et al*. (1985), who reported that development of fertilized eggs of medaka after 48-hour exposure was delayed (60 Hz, 0.1 mT). SKAULI *et al*. (2000), who carried out experiments on zebrafish (*Danio rerio*), demonstrated that development of embryos subjected to electromagnetic field exposure (50 Hz, 1 mT) that commenced 48 hours after fertilization was delayed but similar findings were not obtained in case of exposing embryos to the same stimuli 2 hours after fertilization. WAN *et al*. (2014) reported that geomagnetic field weakening to a value around zero had strong bioeffects on the growth, development and reproduction of small brown planthopper (*Laodelphax striatellus*) and brown planthopper (*Nilaparvata lugens*). An important part of our research was concerned with identification of developmental defects in larvae. Our results show that deprivation of GMF did not produce a profound effect on the number of pathological deformations in larvae and the slightly higher number of these defects in the experimental group was not statistically significant. It is worth mentioning that embryo mortality on the first and third days of incubation in the experimental group was significantly higher (Fig. 1), which was probably caused by developmental disturbances. ASASHIMA *et al.* (1991) demonstrated that geomagnetic field shielding increases the occurrence of developmental disturbances in fire bellied newts (*Cynops pyrrhogaster*). Early ovulation, fertilization as well as developmental stages from gastrula to neurula are sensitive to low geomagnetic fields. These data and other reports (MO *et al*. 2012; GOLOVANOVA *et al*. 2017) show that a weakened geomagnetic field has a detrimental effect on egg cells that may be indicative of evolutionary adaptation to the strength of the geomagnetic field on the surface of the Earth. Studies on the effects of an electromagnetic field (900 MHz) on the chicken embryo carried out by PAWLAK *et al*. (2018) revealed that an electromagnetic field accelerates hatching but has no influence on the embryo mortality, number of developmental defects and hatching rate; *in vitro* studies indicate that magnetic conditions have an impact on enzymatic reactions and cell division.

FORMICKI *et al*. (2004) studied the behavior of brown trout embryos and larvae (*Salmo trutta*) exposed to an additional permanent magnetic field. They demonstrated that during early ontogenesis, when the organism is no longer an embryo but not yet an adult capable of independent life in water, it is especially sensitive to an additional magnetic field. This stage of development involves formation of certain structures, mechanisms and organs that are responsible for providing information about the external environment, including electromagnetic conditions. Exposing juvenile fish to a magnetic field triggers a specific response, that is moving towards a certain direction. This response is connected to the system of exteroceptors, that are undergoing development at that time. SADOWSKI *et al*. (2003) and TAŃSKI *et al.* (2005), who studied embryo spatial orientation in the magnetic field, examined a few species of fish: salmon (*Salmo salar*), trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), vendace (*Coregonus albula*), pike (*Esox lucius*) and rudd (*Scardinius erythrophthalmus*). Embryos of all studied fish species under artificial permanent magnetic fields displayed directional preference corresponding to their axis of symmetry. Special orientation is possible due to the presence of magnetic material, that is magnetite, which is produced by many fish species.

The results of the present study indicate that geomagnetic field deprivation has an impact on embryonic development and hatching dynamics of Prussian carp (*Carassius gibelio*). Hypogeomagnetic conditions caused an increase of embryo mortality rate and affected hatching dynamics of Prussian carp. Geomagnetic field shielding accel-

erated hatching into larvae by 6 hours. In view of the results obtained in this study, it is worth examining the materials used for manufacturing apparatuses for fish egg incubation as well as the presence of materials in laboratories in which experiments on fish eggs and larvae are conducted, that may disturb GMF.

Acknowledgments

This work has been supported by DS 3263 (Department of Veterinary Science, Animal Reproduction and Welfare, University of Agriculture in Kraków).

The studies were carried out in the University of Agriculture in Kraków, Faculty of Animal Science, Institute of Veterinary Sciences, Department of Veterinary Science, Animal Reproduction and Welfare; Al. Mickiewicza 24/28, 30-059 Kraków, Poland.

Author Contributions

Research concept and design: B.T., B.B., M.S.; Collection and/or assembly of data: B.T., B.B., A.O., W.K., T.W., M.L., M.S.; Data analysis and interpretation: B.T., A.O., W.K., T.W., M.L., M.S.; Writing the article: B.T., B.B., K.P., M.S.; Critical revision of the article: B.T., B.B., K.P., M.S.; Final approval of article: B.T., B.B., K.P., M.S.

Conflict of Interest

The authors declare no conflict of interest.

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