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THE INFLUENCE OF SELECTED ENVIRONMENTAL FACTORS ON COMMON CARP (*Cyprinus carpio*) EMBRYONIC DEVELOPMENT AND HATCHING

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Abstract

Changes in the electromagnetic environment and increasing pollution of the environment are significant threats to animals including fish. In this study we made an attempt of determining some effects of geomagnetic field disturbances (hypogeomagnetic conditions), 1800 MHz electromagnetic field, and a commonly used herbicide (Roundup - the concentrations applied in the experiment corresponded to 0.1; 0.5 or 5 mg/l of the active ingredient) on common carp (Cyprinus carpio) embryonic development and hatching. In the periods of 24 and 48 hours after the start of incubation and then in every 6 hours till the end of the experiment the percentage of dead eggs, the percentage of hatched larvae, and the percentage of deformed larvae were determined. The research conducted showed that the deprivation of the geomagnetic field accelerated the hatching of larvae; a reduction in hatchability was also observed, while exposure to the electromagnetic field resulted in an increase in the percentage of finally hatched larvae (statistically insignificant changes). Roundup exposure resulted in increased mortality, reduced hatchability, and an increased percentage of deformed larvae (statistically significant changes).

Key words: *fish, embryogenesis, EMF, GMF, herbicide.*

INTRODUCTION

The Earth electromagnetic environment including the geomagnetic field (GMF) is a permanent element of the environment, which was present during the phylogenetic development of all living organisms (Rochalska, 2009; Hulot et al., 2019). Then it seems possible that disturbances in this environment could have an impact on the course of life processes of organisms living on Earth. The industrialization of the environment causes increasing threats from artificial magnetic and electromagnetic fields generated by various devices (Fey et al., 2019; Formicki et al., 2021). Disturbances of natural electromagnetic environment may affect some physiological processes related to directional reactions taking place in embryos and larvae of fish (Tański et al., 2005; Formicki et al., 2019). The study conducted by Formicki and Winnicki (1998) showed that additional magnetic field (MF) significantly lengthened fish embryonic development. Moreover, artificial magnetic fields can affect water management processes in fish eggs (Sadowski et al., 2007).

In modern societies the environmental electromagnetic fields (EMF) are increasingly supplemented by anthropogenic EMF. Sakuli et al. (2000) studied the hatching process in zebrafish (*Danio rerio*) embryos treated with MF (50 Hz AC, 1000 μT). They showed that the EMF exposure did not affect the number of deformed larvae and the small hatching delays observed were not detrimental to the embryos.

On the other hand metal elements which are parts of buildings (construction and interior) could weaken the Earth natural magnetic field, which may not be neutral for living organisms (Tsunomura and Tokumoto, 2005). Tombarkiewicz et al. (2018) showed that weakened geomagnetic field influences embryogenesis and dynamics of hatching in Prussian carp (*Carassius gibelio*). The results obtained in the study conducted by Kantserova et al. (2017), who investigated the effect of hypogeomagnetic conditions on Ca^{2+}-dependent proteinases of fish, indicate that these enzymes are capable of direct perceiving of MF, however the effect of GMF on biochemical processes in living organisms has not been sufficiently studied so far.

Growth of civilization causes not only changes to the electromagnetic environment, but also chemical contamination. One of the most serious ecological problems is pesticide pollution of water environment. As shown in an extensive review by Bojarski and Witeska (2020), pesticides can get into groundwater and may affect fish. Alterations in haematological indices or blood biochemical parameters indicate that pesticides may cause pathophysiological changes in these animals (Bojarski & Witeska, 2020). Exposure to herbicides may also result in histopathological changes including hyperplasia and hypertrophy of gill epithelium and changes in liver microstructure, such as vacuolization of hepatocytes (Bojarski et al., 2018). Herbicides are the most commonly used pesticides (Cooper and Dobson, 2007; De et al., 2014). Despite the common use of such agrochemicals, the existing data regarding the effects of herbicide pollution of water ecosystems on fish early life stages is not sufficient.

The goal of the present research was to determine the effects of 1) hypogeomagnetic conditions 2) electromagnetic field (1800 MHz) exposure 3) exposure to herbicide Roundup on common carp (*Cyprinus carpio* Linnaeus, 1758) developing eggs. Embryo mortality, hatching dynamics, and percentage of deformed larvae were analysed.

MATERIALS AND METHODS

Artificial spawning

Artificial spawning was performed using common carp (*C. carpio*) R3R8 laboratory line, which were kept in recirculating aquaculture system (RAS). The fish were stimulated using Ovopel (Interfish, Hungary). Each pellet (50-55 mg) contains 25 μg of GnRH oligopeptide analogue and 20 mg of metoclopramide. In the first injection males received 1/2 a pellet per kg body weight, while females received 1/5 a pellet per kg body weight. In the second injection which took place 12 hours after the first injection only females were injected. They received 1 pellet per kg of body weight. After detecting ovulation symptoms, the animals were subjected to artificial spawning.

Incubation

In order to carry out the experiment oocytes taken from 10 females and sperm taken from 3 males were used. Oocytes from all females were mixed. Similarly, sperm from all males were mixed. Next, fertilization was conducted. Fertilized eggs were placed on glass Petri dishes (96 to 362 eggs per every dish in the GMF deprivation part of the experiment, 65 to 144 eggs per every dish in the EMF part of the experiment, and 78 to 141 eggs per every dish in the Roundup part of the experiment). Dechlorinated and aerated tap water was used in the experiment. The water in the dishes was changed every 12 hours. Physicochemical parameters (pH 7.6-7.8, NO_3^- 2-5 mg/L, NO_2^- 0 mg/L, NH_3 0 mg/L, GH 16-18 dGH, KH 8 dKH, PO_4^{3-} 0 mg) were controlled with aquaristical kits produced by Zoolek company (Poland). In the periods of 24 and 48 hours after the start of incubation and then in every 6 hours till the end of the experiment the percentage of dead eggs, the percentage of hatched larvae, and the percentage of deformed larvae were determined.

Geomagnetic field deprivation (hypogeomagnetic conditions)

The control dishes were maintained in an ambient GMF (about 37 μT), while the experimental dishes were kept in special cages with GMF weakened below 12 μT. Hypogeomagnetic conditions were achieved by using cages made of steel (the S235JRG2 type; CMC, Zawiercie, Poland). The construction of the cages was previously described by Roman and Tombarkiewicz (2009). To provide similar lighting conditions, Petri dishes of the control group were placed in cages which does not disturb GMF. Each group (control and experimental) contained 15 dishes.

Electromagnetic field exposure

The control group (15 dishes) contained eggs kept in standard conditions, while experimental eggs (15 dishes) were exposed daily to electromagnetic field. The exposure time was 26 min per day (13 times per day between 6:00 and

23:00 at equal time intervals, 2-minute emissions). The EMF field source was a generator of radio frequency electromagnetic waves. The output power was delivered to a Yagi GSM ceiling antenna with omnidirectional characteristics. The petri dishes were placed in an area where the average electric field intensity was 5.73 V/m (±0.84 V/m), the average magnetic field intensity was 0.016 A/m (±0.010 A/m), the average power density was 0.014 W/m^2 (±0.009 W/m^2) and frequency was 1800 MHz.

Roundup exposure

The eggs kept in the control Petri dishes were not exposed to herbicides or other chemicals. The experimental ones were treated with Roundup 360 Plus (Monsanto), a widely applied pesticide, which contains glyphosate (in the form of potassium salt) as an active substance. The concentrations used in the current study corresponded to 0.1, 0.5 or 5.0 mg/l of the active ingredient (respectively: group R1, R2 and R3). Each group included 20 dishes.

Statistical analysis

The Shapiro-Wilk test was applied for testing the compliance of the analysed data with the normal distribution. The Levene's test was applied for testing the homogeneity of variances. The level of significance was set at $\alpha = 0.05$.
Embryo mortality and hatching dynamics were analysed using two-way mixed ANOVA. The significance level of each test was set at 0.05. If the two-way mixed ANOVA did not show statistical significance for the group factor, no post-hoc analysis followed. If the statistical significance was found for the group factor, a series of one-way ANOVA for each time point was performed. At a given time point, a statistically significant difference was recognized provided that p.adj < 0.05, where p.adj was the product of p.value from the given one-way ANOVA test and the number of comparisons in the series. The number of comparisons was 7 for hatching dynamics in the experiment with Roundup and 22 for embryo mortality in the same experiment. In the other cases (hatching dynamics and embryo mortality in experiments on GMF deprivation and exposure to EMF), performing a post-hoc test was not necessary. Only the time points corresponding to the statistically significant differences found by one-way ANOVA tests described above (p.adj <0.05) were taken into account. Post-hoc analysis was continued using a series of t-tests for independent groups at selected time points. At every such time point only the control group was compared to the groups R1, R2 and R3, respectively. The Bonferroni correction for three comparisons was applied for the interpretation of each test result, that is, the test was statistically significant for p.adj <0.05, where p.adj = 3 * p.value and p.value is the result of a given t-test. The percentage of deformed larvae in each of the three experiments performed was analysed using a one-way ANOVA test. The significance level of each test was set at 0.05. In the case of statistical significance, a series of t-tests for independent groups was performed as a post-hoc analysis, where only the control group was compared with the groups R1, R2 and R3, respectively. The Bonferroni correction for three comparisons was applied for the interpretation of each test result, that is, the test was statistically significant for p.adj < 0.05, where p.adj = 3 * p.value and p.value is the result of a given t-test.

RESULTS AND DISCUSSIONS

Geomagnetic field deprivation (hypogeomagnetic conditions)

Embryo mortality at the time points in the control group and the group exposed to the hypogeomagnetic conditions were similar (Figure 1) - statistical analysis showed that the group factor was not significant (p = 0.0820).

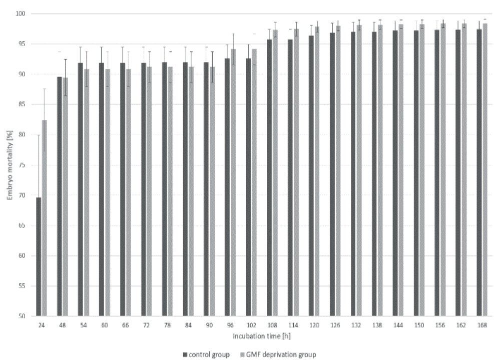

Figure 1. The effect of hypogeomagnetic conditions on embryo mortality rate of common carp (*Cyprinus carpio*)

On the other hand, the studies by Tombarkiewicz et al. (2018) showed that incubation of Prussian carp (*C. gibelio*) eggs under hypogeomagnetic conditions resulted in increased mortality (the difference between the experimental and control groups was statistically significant after 24 and 72 hours).
Even though an acceleration of the hatching process was observed in the GMF deprivation group (the beginning of hatching after 96 hours of incubation in the experimental group and after 108 hours in the control group) (Figure 2), the statistical analysis did not show a significant difference between both groups in terms of hatching dynamics (p = 0.871).

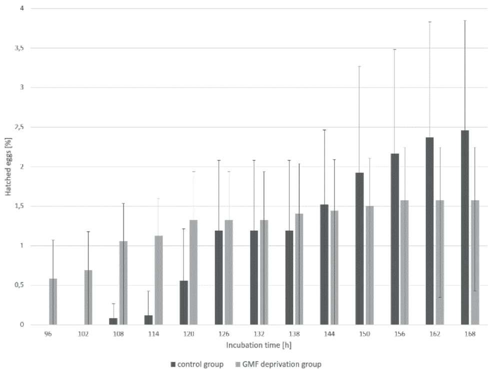

Figure 2. The effect of hypogeomagnetic conditions on hatching dynamics of common carp (*Cyprinus carpio*)

It is to be noted, however, that the statistical analysis did not include the observations after 96 and 102 hours of incubation due to the fact that in the case of the control group all values were zero. Generally, 56.24% more larvae hatched in the control group than in the experimental group. The results obtained in the experiment are partially in line with the results obtained earlier by Tombarkiewicz et al. (2018) who observed that hatching of Prussian carp (*C. gibelio*) larvae started 6 hours earlier in hypogeomagnetic conditions; however, the percentage of hatched larvae in the experimental group at the end of the experiment was higher than in the control group by 13%.
The percentage of deformed larvae in both groups (Fig. 3) were similar (p=0.951). Moreover, Tombarkiewicz et al. (2018) revealed that the ratio of deformed to non-deformed Prussian carp (*C. gibelio*) larvae of the control group and the GMF deprivation group were comparable. On the other hand, Asashima et al. (1991) observed that a shielded environment (5 nT) caused a significantly higher percentage of Japanese fire belly newt (*Cynops pyrrhogaster*) deformed embryos.

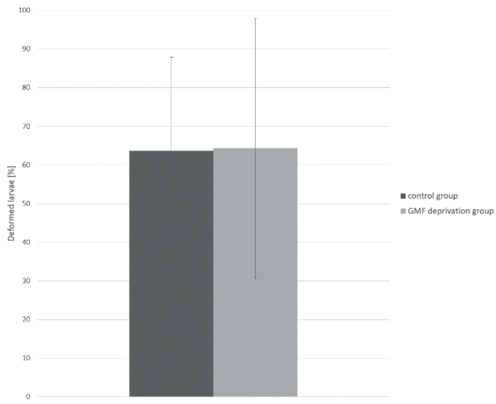

Figure 3. The effect of hypogeomagnetic conditions on the occurrence of deformations in common carp (*Cyprinus carpio*)

Electromagnetic field exposure

Embryo mortality at individual time points in the control group and the group exposed to EMF was similar (Figure 4) - statistical analysis showed that the group factor was not significant (p = 0.0910). Moreover, Li et al. (2014) showed that exposure to extremely low frequency magnetic field (ELF-MF) during incubation of zebrafish (*D. rerio*) embryos did not result in increased mortality.

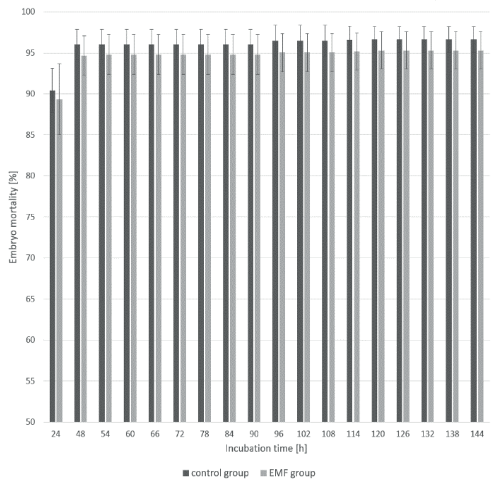

Figure 4. The effect of electromagnetic field exposure on embryo mortality rate of common carp (*Cyprinus carpio*)

In our study, the first hatched larvae were observed after 96 hours of incubation – both in the control and experimental groups. The percentage of hatched larvae at individual time points seemed to be higher in the experimental group (Figure 5), however, the analysis performed showed that the group factor was statistically insignificant ($p = 0.226$). Finally, 38.83% more larvae hatched in the experimental group than in the control group.

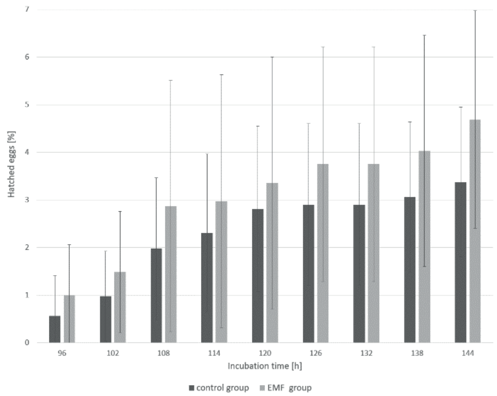

Figure 5. The effect of electromagnetic field exposure on hatching dynamics of common carp (*Cyprinus carpio*)

Lee & Yang (2014) demonstrated that exposure to an electromagnetic field (3.2 kHz; 15-60 µT) significantly accelerated embryonic development of Japanese rice fish (*Oryzias latipes*). Moreover, in the study conducted by Pawlak et al. (2016) a significant acceleration of hatching of chicken (*Gallus gallus domesticus*) exposed to 900 MHz electromagnetic field during incubation was demonstrated. On the other hand, Li et al. (2014) showed that ELF-MF exposure caused significantly decreased hatching rate of zebrafish (*D. rerio*).
In the present study the percentage of deformed larvae among all hatched larvae was higher in the experimental group (Fig. 6), however, these differences were not statistically significant ($p = 0.058$). Similarly, Li et al. (2014) demonstrated that exposure to ELF-MF did not cause increased percentage of malformations in zebrafish (*D. rerio*).

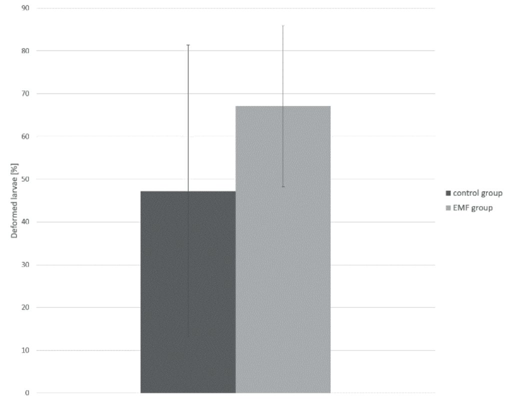

Figure 6. The effect of electromagnetic field exposure on the occurrence of deformations in common carp (*Cyprinus carpio*)

Roundup exposure

There was a statistically significant increase in the percentage of dead eggs (Figure 7) in group R1 after 144, 150 and 168 h of incubation compared to the control value. The percentage of dead eggs was also statistically significantly higher than in the control in group R2 after 24, 114, 120, 126, 132, 138, 144, 150, 156, 162 and 168 h. The proportion of dead eggs was statistically significantly increased in group R3 compared to the control group after 24, 54, 60, 66, 114, 120, 126, 132, 138, 144, 150, 156, 162 and 168 h. There was also a statistically significant reduction in the percentage of dead eggs in group R1 compared to the control value after 78 h of incubation.

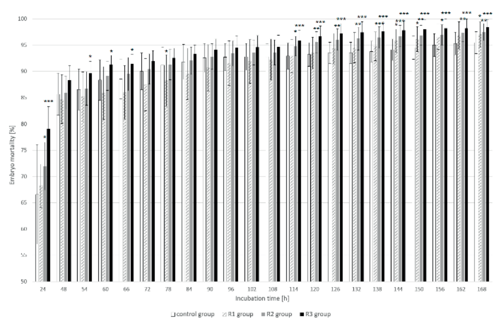

Figure 7. The effect of Roundup exposure on embryo mortality rate of common carp (*Cyprinus carpio*)

Fiorino et al. (2018) observed significantly higher mortality of common carp (*C. carpio*) eggs after 48 hours post fertilization (hpf) at 10 mg/l and after 96 hours at 5, 10, and 50 mg/l; after 120 hpf at 0.005, 5, 10, and 50 mg/l compared to the control. In the case of zebrafish (*D. rerio*) the same authors also noted increased embryo mortality. Significant differences were found at 0.05, 5, 10, and 50 mg/l after 48, 72, and 96 hpf and at 5 and 50 mg/l after 120 hpf. Moreover, Zhang et al. (2021) who studied the effects of glyphosate exposure (72 hours) on zebrafish (*D. rerio*) embryos demonstrated that the survival rate was significantly reduced in comparison to the control in groups exposed to 10, 100, and 700 ng/ml of the herbicide. Similarly, Socha et al. (2021) observed significantly increased mortality rate of common carp (*C. carpio*) embryos exposed to 1 and 10 µl/l of Roundup.

The first hatched larvae were observed after 108 hours of incubation in group R3 (Figure 8).

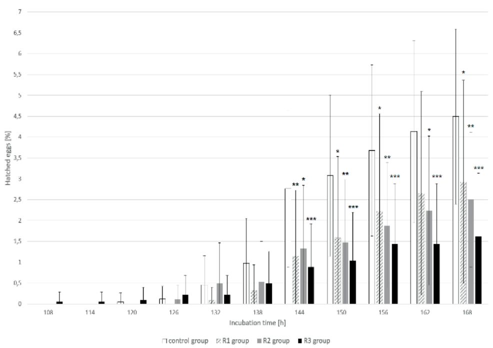

Figure 8. The effect of Roundup exposure on hatching dynamics of common carp (*Cyprinus carpio*)

The next group with hatched larvae were observed was the control group (the first larvae were found after 120 hours of incubation). Hatching dynamics differed significantly depending on the group (p = 0.0007). After 144 hours of incubation, a statistically significant reduction in the percentage of hatched larvae was observed in groups R1, R2 and R3 compared to the control value. A similar situation took place after 150 and 156 hours of incubation. After 162 hours of incubation, the percentage of hatched larvae was significantly lower in group R2 and in group R3, while the value recorded in the case of group R1 did not differ significantly from the control value. After 168 hours of incubation a statistically significant reduction in the percentage of hatched larvae was found in groups R1, R2, and R3 compared to the control group.

It should be emphasised that the statistical analysis did not include the observations made after 108, 114, 120 and 126 hours of incubation due to the lack of sufficient variability within the analysed groups. In the study conducted by Fiorino et al. (2018) common carp (*C. carpio*) eggs exposed to glyphosate exhibited significantly lower hatching rate at 72 (10, and 50 mg/l), 96 (50 mg/l), and 120 hpf (5, 10, and 50 mg/l). On the other hand, low concentration (0.05 mg/l) resulted in significantly higher hatching rate. In the study performed by the same authors, hatching of zebrafish (*D. rerio*) eggs exposed to glyphosate started at 96 hpf in the control group and in the experimental groups treated with the lowest concentrations of glyphosate (0.005 and 0.05 mg/l), while embryos exposed to the same herbicide applied at concentrations of 5, 10, and 50 mg/l began to hatch at 72 hpf. A significantly higher hatching rate was noted at some groups (i.e., 5, 10 and 50 mg/l) at 72 hpf and in all treated groups at 96 hpf. Moreover, Zhang et al. (2021) observed significantly lower hatching rate of zebrafish (*D. rerio*) eggs exposed to 10, 100, and 700 ng/ml of the same chemical. The study conducted by Socha et al. (2021) showed a significant retardation of the hatching rate of common carp (*C. carpio*) in the group treated with 10 µl/l of Roundup.

The percentage of deformed larvae (Figure 9) among all hatched larvae depended on the group factor (p = 0.0000).

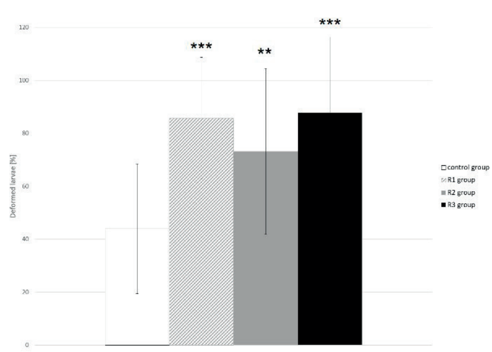

Figure 9. The effect of Roundup exposure on the occurrence of deformations in common carp (*Cyprinus carpio*)

This parameter was statistically significantly higher in groups R1, R2 and R3 compared to the control value.

In the study performed by Fiorino et al. (2018) malformations such as pericardial edema, yolk sac edema, hematoma, and late development were observed in glyphosate-exposed individuals (0.005, 0.05, 5, and 10 mg/l). In case of zebrafish (*D. rerio*) larvae hatched from eggs exposed to the same herbicide exhibited lower percentages of malformations in comparison to common carp (*C. carpio*). According to Zhang et al. (2021) exposure to glyphosate (10, 100, and 700 ng/ml) resulted in significantly increased percentages of deformed zebrafish (*D. rerio*) larvae. Similarly, Socha et al. (2021) revealed that Roundup treatment resulted in significantly increased percentage of deformed common carp (*C. carpio*) larvae.

CONCLUSIONS

The research conducted showed that the deprivation of the geomagnetic field caused hatching acceleration by 12 hours. At the same time in this group a decrease in the percentage of finally hatched larvae was observed, while exposure to the 1800 MHz electromagnetic field resulted in an increase in this parameter (statistically insignificant changes). Exposure of embryos to Roundup caused significantly increased mortality, marked reduction in hatchability, and clear increase in the percentage of deformed larvae. Due to the high embryo mortality detected in this study in all groups (including the control groups), the results should be considered preliminary. Due to high mortality further research is necessary to confirm the observed phenomena.

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