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# EFFECT OF MAGNETIC STORMS ON THE TEMPERATURE CHARACTERISTICS OF DIGESTIVE GLYCOSIDASES IN ROACH FINGERLINGS[1]

**Abstract.** The aftereffects of a typical geomagnetic storm (the duration 24 h, in the frequency range 0–5 Hz) on the activity of glycosidase (maltase, amylolytic activity) in the intestine of a 4-month juvenile roach *Rutilus rutilus* (L.) have been studied. Embryos were exposed to geomagnetic storm 72 h later after fertilization. The amylolytic activity was determined by Nelson method and the maltase activity by the glucose-oxidase method. The level of glycosidase activity in juvenile roach under the impact of geomagnetic storm was significantly lower than that in controls. Temperature characteristics of glycosidase in fish of the experimental and the control groups are similar. Temperature optimum of amylolytic activity was observed at 40 ºC, the temperature optimum of maltase was found at 60 ºC. The $E_{act}$ values of maltase at the temperature 0–30 °C are similar in both groups of yearlings. The $E_{act}$ values of amylolytic activity at feeding temperature 10–20 °C were significantly lower (the efficiency of the process is higher) than at lower temperatures in both groups of yearlings.

**Key words**: fish, roach, digestive glycosidases, amylolytic activity, maltase, geomagnetic storm.

## Introduction

The geomagnetic storm (GMS) is a disturbance of the geomagnetic field, associated with an interaction of the perturbed flow of the solar wind and the Earth's magnetosphere. The geomagnetic storm is different in intensity and the overall picture. The intensity of geomagnetic fluctuations during GMS does not often exceed 1 % of the intensity of the geomagnetic field. However, such a weak impact can cause significant biological responses [1]. Aftereffects of a real GMS reproduced in experimental conditions, on the early stages of roach development manifested as an increase in the mitotic activity in prelarvae cells [2], the decrease in size-weight characteristics and in vertebral phenotypes diversity in juveniles [3]. Multidirectional changes in the activity of glycosidase (enzyme hydrolyzing carbohydrate) were previously found in the gut of juvenile roach subjected to the effect of low-frequency magnetic field during its embryogenesis [4, 5]. At the same time, there is no information about the aftereffect of the magnetic storm on the digestive function of fish.

The goal of the present work is to study the prolonged effects of GMS exposure during embryogenesis upon activities and temperature characteristics of intestinal glycosidase in roach fingerlings.

## Materials and methods of research

The fertilized eggs obtained from the parental roach *Rutilus rutilus* (L.) (8 females and 6 males) caught in May 2012 at the Rybinsk Reservoir spawning grounds were used for the experiments. The eggs were fertilized by a dry method and then were placed in 2 crystallizers filled with the river water. The water was renewed twice a day. The geomagnetic storm (happened on 30–31 October 2003 and recorded at the latitude of the experiments) was reproduced in the experimental setup that allows compensating fluctuations of the geomagnetic field in the working volume and creating user-defined three-component magnetic fields. The crystallizer with embryos was placed in the working volume of the experimental unit 72 h later after fertilization. The geomagnetic storm was reproduced in the unit for 24 hours in the frequency range of 0–5 Hz. Fish in the control group were developed under the natural geomagnetic field. Groups of 500 larvae from control and experimental variants were placed in ponds with natural forage for 4 months after resorption of yolk sac. Roach mortality in the ponds was insignificant and did not depend on the experimental exposure.

Twenty fish specimens from both the control and experimental groups were used for the biochemical analysis. Fish were killed by the excision of the spinal cord, slit open immediately and the intestine was removed and placed on ice. The intestinal chime was deleted. The activity of glycosidase (maltase, amylolytic activity) was determined in the total homogenates (including medial intestine of 20 specimens in each group) prepared on Ringer's solution for cold-blooded animals (110 mM NaCl, 1.9 mM KCl, 13 mM $CaCl_2$). The amylolytic activity (a characteristic of the total activity of enzymes

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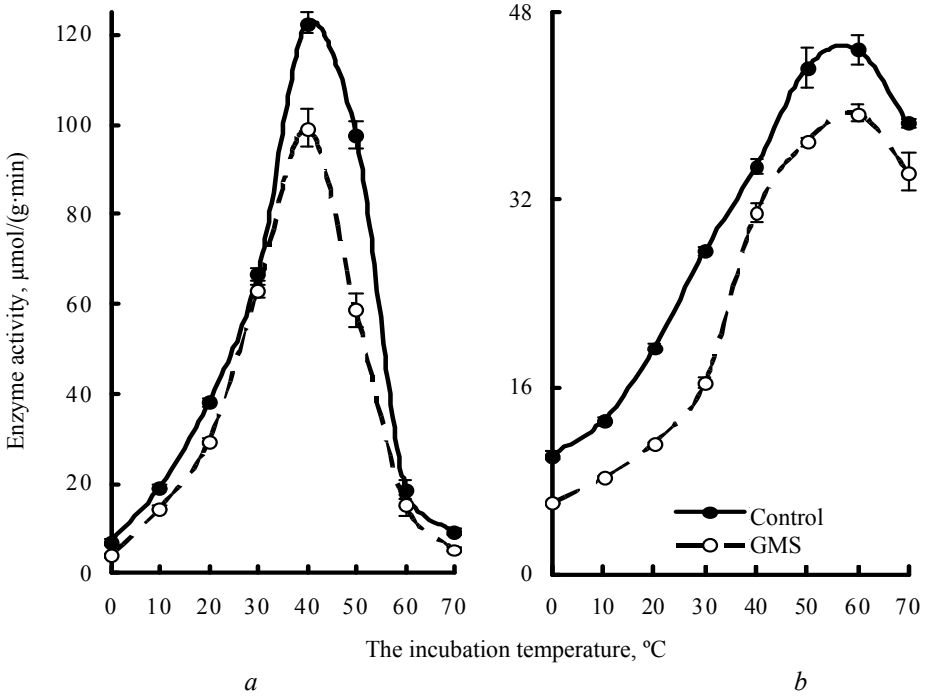
capable hydrolyzing of starch, namely, α-amylase EC 3.2.1.1, glucoamylase EC 3.2.1.3, and maltase EC 3.2.1.20) were assessed by analyzing hexose accumulation according to the modified method of Nelson [6]. The activity of maltase was assayed by the glucoseoxidase method using the Fotoglyukoza (LLC Impakt, Russia) clinical biochemistry kit. The incubation of homogenates and substrates (1.8 % solution of starch or of 50 mM maltose) was carried out at the temperature of 0–70 °C and pH 7.4 during 20–30 min. Enzymatic activity was determined in five replicates at each point with the background (glucose content in the original homogenate) taken into account and expressed in micromoles of the reaction product formed per 1 min of incubation per 1 g wet tissue weight (μmol/(g·min)). The activation energy ($E_{act}$) was calculated using a graphical method of Arrhenius. The results are presented as mean values and errors of the mean ($M \pm m$). The significance of the differences was assessed by one-way analysis of variances (ANOVA, LSD-test) at $p \leq 0.05$.

**Results and discussion**

The body weight of the fingerlings of the control group is 6.0 ± 0.2 g, body length – 7.1 ± 0.1 cm. The fish body weight of the experimental group is 25 %, length – 9 % lower compared to the control ($p < 0.05$) and is 4.5 ± 0.1 g and 6.4 ± 0.1 cm, respectively. In most cases, the glycosidase activity in fish of the control group was significantly higher than in the experimental group in all range of the incubation temperature, $p < 0.05$. At the standard temperature of 20 ºC the amylolytic activity in roach of the experimental group was by 22 % lower than in the fish of the control group and was 29.6 ± 0.7 and 38.1 ± 1.1 μmol/(g·min), respectively. The maltase activity in fish after exposure to GMS was 26 % lower than in control – 10.9 ± 0.1 and 14.8 ± 0.3 μmol/(g·min). The patterns of curves of temperature dependence of glycosidase in roach of the control and the experimental groups were exceptionally close (Fig.). The temperature optimum of starch hydrolysis in yearlings of the both groups was 40 ºC, the maximum level of the amylolytic activity was 122.1 ± 2.4 and 99.2 ± 4.3 μmol/(g·min), respectively. The relative enzyme activity was 4–6 %, 15–16 and 30–31 % of the maximum at 0, 10 and 20 ºC in the both groups of fish. The temperature optimum of α-amylase in the adult roach from the Rybinsk reservoir was observed at 40 ºC, the optimum of the amylolytic activity was found at the temperature of 50 ºC [7]. The discrepancy between our and previous results may be due to differences in the spectrum of nutrition as well as different quantitative ratio of pancreatic (α-amylase) and membrane (glucoamylase and maltase) enzymes hydrolyzing starch in juveniles and adults of the same species.




Effect of the incubation temperature on the activity of glycosidases:
*a* – amylolytic activity, μmol/(g·min); *b* – maltase activity, μmol/(g·min), in the intestine of the roach of the control and the experimental (under a magnetic storm) groups

The maximum of maltase activity recorded at 60 ºC is 122.1 ± 2.4 and 99.2 ± 4.3 μmol/(g·min) in fish of the control and the experimental groups, respectively. The zone of the maximal activity of maltase (40–70 ºC) is much larger than that of the amylolytic activity. The relative activity of maltase in fish of the control group (23, 29 and 43 %) was slightly higher than that of the experimental group (15, 21 and 28 % of maximum) at 0, 10 and 20 ºC, respectively. It should be noted that the temperature stability of maltase at low and especially postmaximal temperatures is higher. So, at 70 ºC it was amounted to 86–87 % of the maximum, while the relative activity of enzymes hydrolyzing starch was only 5–8 %. These data agree well with those obtained previously for carp fish species [7–9]. The comparison of the values of $E_{act}$ of the amylolytic activity in roach fingerlings in the vital temperature range from 0 to 30 °C showed no significant differences between fish of the control and the experimental groups (Tabl.).

**The activation energy of intestinal glycosidases in roach fingerlings of the control and the experimental (under a magnetic storm) groups**

| Fish groups | The activation energy, kcal/mol | | Breakpoint, °C |
|---|---|---|---|
| | before breakpoint | after breakpoint | |
| **Amylolitic activity** | | | |
| Control | 15.9 | 10.6 | 10 |
| GMS | 20.1 | 12.4 | 10 |
| **Maltase activity** | | | |
| Control | 5.49 | | no |
| GMS | 5.42 | | no |

The breakpoint of the Arrhenius plot was identified at 10 ºC, while the values $E_{act}$ in the temperature range 10–30 °C were 1.5–1.6 times lower (higher process efficiency) than at lower temperatures. In the adult roach the breakpoint of the Arrhenius plot was recorded at 20 ºC and the values $E_{act}$ were, 12 and 7.9 kcal/mol, respectively [7]. The values $E_{act}$ of maltase in the temperature range 0–30 °C in fish of the experimental and the control groups were equal, the breakpoint of the Arrhenius plot was absent. These results corresponded to the data, which were previously obtained in the study of the thermal characteristics of maltase in roach [9].

## Conclusion

The results demonstrate a low level of the glycosidase activities (amylolytic activity and maltase) in the intestine of the juvenile roach exposed to GMS (24 h duration, in the frequency range 0–5 Hz) 72 h later after fertilization. Temperature characteristics of glycosidase in fish of the experimental and the control groups are similar. The temperature optimum of amylolytic activity is recorded at 40 ºC, and the temperature optimum of maltase is observed at 60 ºC. The $E_{act}$ values of amylolytic activity at the temperature of feeding 10–30 ºC are lower (the efficiency of process is higher) than at lower temperatures. The $E_{act}$ values of maltase in the temperature range 0–30 ºC in fish of the experimental and the control groups are equal.

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# ВЛИЯНИЕ МАГНИТНОЙ БУРИ НА ТЕМПЕРАТУРНЫЕ ХАРАКТЕРИСТИКИ ПИЩЕВАРИТЕЛЬНЫХ ГЛИКОЗИДАЗ У СЕГОЛЕТОК ПЛОТВЫ

Изучены отдаленные последствия действия типичной магнитной бури (продолжительностью 24 часа, в диапазоне частот 0–5 Гц) на активность гликозидаз в кишечнике 4-месячной молоди плотвы *Rutilus rutilus* (L.). Экспозиции в магнитной буре подвергались эмбрионы спустя 72 часа после оплодотворения. Для определения активности мальтазы и амилолитической активности использованы глюкозооксидазный метод и модифицированный метод Нельсона. Уровень активности гликозидаз (мальтаза и амилолитическая активность) у сеголеток плотвы, подвергнутых действию магнитной бури, достоверно ниже, чем в контроле. Температурные характеристики гликозидаз у рыб опытной и контрольной групп близки. Температурный оптимум амилолитической активности отмечен при температуре 40 ºС, температурный оптимум мальтазы – при температуре 60 ºС. Значения $E_{акт}$ мальтазы в диапазоне значений температуры 0–30 °С у рыб опытной и контрольной групп равны. Значения $E_{акт}$ амилолитической активности при температуре питания 10–20 °С достоверно ниже (эффективность процесса выше), чем при более низкой температуре у сеголеток обеих групп.

**Ключевые слова**: рыбы, плотва, пищеварительные гликозидазы, амилолитическая активность, мальтаза, магнитная буря.

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