Scientific Annals of the Polish Society of Animal Production - Vol. 16 (2020), No 2, 59-64

DOI: 10.5604/01.3001.0014.2018

The effect of Roundup on gametes of grass carp *Ctenopharyngodon idella* Val. – preliminary research

Katarzyna Ługowska

Siedlce University of Natural Sciences and Humanities, Faculty of Exact and Natural Sciences,
Institute of Biological Sciences,
Prusa 14, 08-110 Siedlce, Poland

The study was conducted on the eggs (roe) and sperm of grass carp (*Ctenopharyngodon idella* Val.) obtained during artificially induced spawning. The effect of Roundup on eggs was assessed using the following concentrations of the herbicide: 0.0 (control), 0.1, 0.2, 0.5, 2.0, 5.0 and 10.0 mg/l. Sperm motility was measured at concentrations of 0.0 (control), 0.1, 0.5, 2.0, 5.0, 10.0, 20.0 and 50.0 mg/l. The study showed that the herbicide increased swelling of grass carp eggs and significantly reduced the duration of sperm motility.

KEY WORDS: grass carp, glyphosate, fish eggs, sperm

Grass carp is a phytophagous cyprinid fish able to consume large quantities of aquatic macrophytes (van der Zweerde, 1990), and therefore it is widely cultured as a 'natural weed control' (Pipalova, 2006; Verma et al., 2009). Due to its restrictive spawning requirements, natural reproduction of grass carp can take place only in its native habitat, the Amur River in Asia (Shireman and Smith, 1983). In all countries where the species has been introduced, it must be artificially reproduced in hatcheries. The biology of the species is well known (Cross, 1969; Michewicz et al., 1972; Krupauer, 1989; Bozkurt and Ogretmen, 2012), and there has been extensive research on the effects of many environmental (Galloway and Kilambi, 1984; Glasser et al., 2004; El-Fiky, 2002; Vajargah and Hedayati, 2017) and anthropogenic (Jezierska et al., 2002; Lugowska et al., 2002; Jezierska et al., 2009; Matasin et al., 2011; Ahmed et al., 2012; Vajargah and Hedayati, 2017) factors on grass carp. However, data on the effects of herbicides on this species are scarce. Therefore the aim of the present preliminary study was to determine the effects of Roundup on grass carp gametes.

[#]Corresponding author e-mail: katarzyna.lugowska@uph.edu.pl
Received: 11.03.2020
Accepted: 15.05.2020

Material and methods

The study was conducted on eggs and sperm of grass carp, obtained from the hatchery of the Samoklęski fish farm in Kamionka, Poland. The material was transported in a refrigerator (at 5ºC) to the laboratory of the Department of Animal Physiology (Siedlce University of Natural Sciences and Humanities). About 2 hours after collection, a mixture of eggs from three females was fertilized with a mixture of sperm from three males by the method described by Lugowska (2009). To assess the effect of Roundup (active substance glyphosate, 170 g/l, Monsanto Europe S.A./N.V.) on egg swelling, 25 fertilized eggs were placed in Petri dishes with the following concentrations of Roundup: 0.0 – control, 0.1, 0.2, 0.5, 2.0, 5.0 or 10.0 mg/l. To establish the time and magnitude of complete swelling, the diameters of whole eggs and yolks were measured 20, 40, 60 and 120 min after fertilization using a stereoscopic microscope (1.6×12 magnification) with measuring eyepieces.

The percentage of swelling was calculated by the following formula:

$$x = (c - d) \times 100 / d$$

where:

x – swelling (as increase in egg diameter)

c – egg diameter

d – yolk diameter

Sperm motility was measured using a light microscope (40×12.5 magnification). Spermatozoa from each of three males (10 µl) were activated by mixing with 10 µl of clean tap water or solutions of Roundup (0.0 – control, 0.1, 0.5, 2.0, 5.0, 10.0, 20.0 or 50.0 mg/l) on a glass slide. The time of sperm activity was measured from the moment of activation until cessation of movement (5 replicates for each concentration). The water temperature during the experiments was 24°C.

Statistical analysis of the results was performed using STATISTICA 10 software. Normality of distribution was tested using the Shapiro-Wilk test, and homogeneity of variance using Levene's test. Egg swelling showed normal distribution, and the results were analysed by ANOVA, followed by Tukey's post-hoc test. For sperm motility, for which the data did not meet the criteria for ANOVA, a non-parametric Mann–Whitney U test was performed. The level of significance was set at $p < 0.05$. The data were presented as means ± SD.

Results and discussion

Grass carp eggs swelled completely within 120 minutes of fertilization (Fig. 1a, b). In the control, the swelling percentage was 181.8%. Incubation in water containing Roundup (except for 0.1 mg/l – 180.1% swelling) caused a significant increase in swelling, with the highest results obtained at concentrations of 0.5 and 5 mg/l (229.3% and 230.1%, respectively). The results are opposite to those obtained in a previous study on common carp eggs (Lugowska, 2018), where the same concentrations of Roundup caused a significant

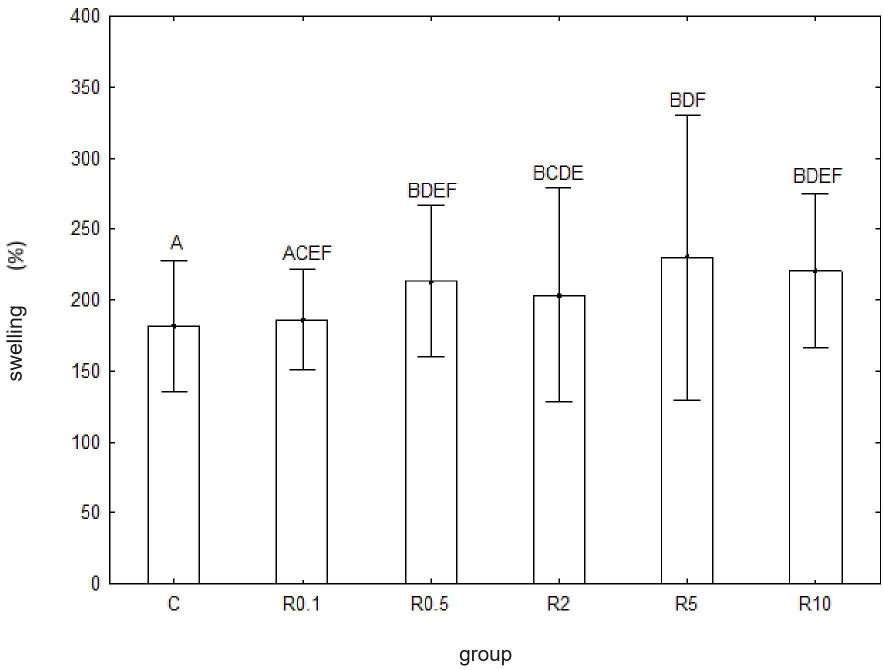

Fig 1a. The effect of Roundup on swelling of grass carp eggs 120 min after fertilization (Tukey's post-hoc test – different superscript letters indicate significant differences between groups)

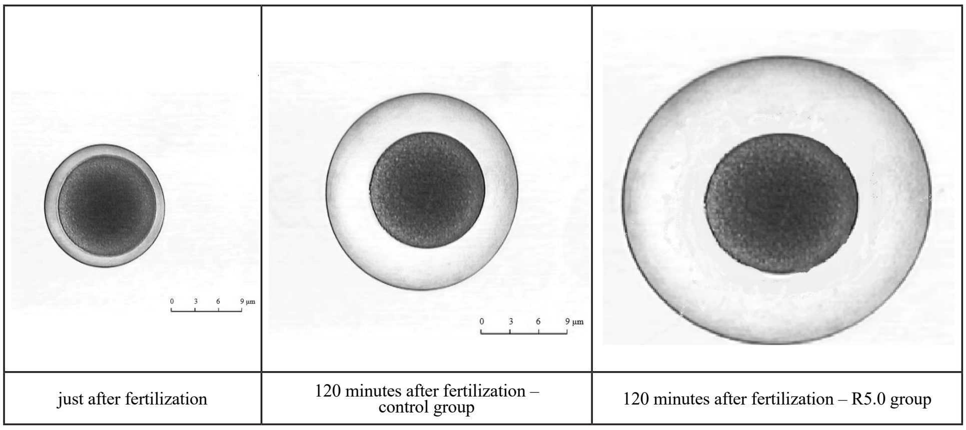

Fig. 1b. Swelling of grass carp eggs

decrease in swelling. Other available data also show that adverse environmental conditions reduce swelling of fish eggs (Witeska et al., 1995; Jezierska and Slominska, 1997; Calta, 2001; Sikorska and Lugowska, 2005). The mechanism of the effect of herbicides on fish egg swelling remains unknown. The changes in egg swelling caused by Roundup are probably due to disturbances in water uptake and ion exchange between the perivitelline fluid and the external environment and/or to changes in the physical properties of the egg surface.

The motility of grass carp spermatozoa decreased significantly as the Roundup concentration increased (except at 5.0 mg/l). The duration of movement in the control was 102.2 s, as compared to just 42.6 s at the highest herbicide concentration (Fig. 2). In a study on common carp, the same concentrations of Roundup caused only a slight decrease in the motility of spermatozoa, with a significant effect observed only at 20 mg/l (Lugowska, 2018). That study also discussed possible causes of the reduction in the sperm motility of common carp (Lugowska, 2018). Although the mechanisms of Roundup toxicity for fish sperm are still unknown, a review article by Hatef et al. (2011) indicates critical components determining sperm motility (the plasma membrane, axoneme, and ATP) which are targets for environmental contaminants (including herbicides). Damage to the plasma membrane disturbs the membrane potential required for the initiation of sperm motility, resulting in a decrease in the number of motile spermatozoa. In the axoneme, the target of

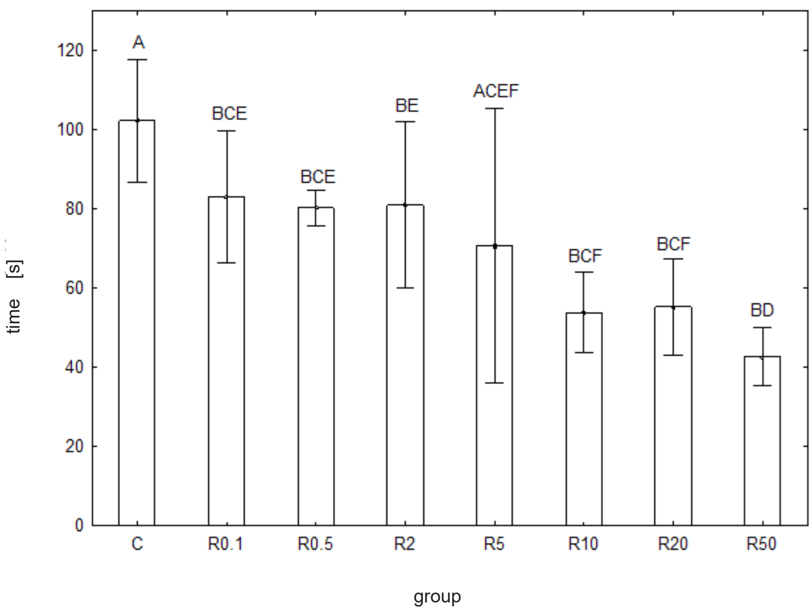

Fig 2. The effect of Roundup on motility of grass carp sperm (Mann–Whitney U test, $p < 0.05$ – different superscript letters indicate significant differences between groups)

herbicides is the cAMP second messenger, involved in the initiation of sperm motility via transfer of membrane potentials to the axoneme. At the molecular level, only one study to date has shown the effect of a herbicide on the adenylate energy charge (AEC), which was shown to decrease as ATP decreased and AMP increased.

Conclusions

This paper presents the results of a preliminary study on the effect of Roundup on grass carp. The results indicate that even at low concentrations this herbicide can significantly affect egg swelling and sperm motility, which is of crucial importance for successful fertilization and the further development of fish.

REFERENCES

Ahmed M.S., Ahmed K.S., Mehmood R., Ali H., Khan W.A. (2012). Low dose effects of cadmium and lead on growth in fingerlings of vegetarian fish, grass carp (*Ctenopharyngodon idella*). The Journal of Animal & Plant Sciences, 22 (4): 902–907.

Bozkurt Y., Ogretmen F. (2012). Sperm quality, egg size, fecundity and their relationships with fertilization rate of grass carp (*Ctenopharyngodon idella*). Iranian Journal of Fisheries Sciences, 11 (4): 755–764.

Calta M. (2001). Effects of aqueous cadmium on embryos and larvae of mirror carp. Indian Journal of Animal Sciences 71: 885–888.

Cross D.G. (1969). Aquatic weed control using grass carp. Journal of Fish Biology 1: 27–30.

El-Fiky N.K. (2002). The influence of water pH oh the embryonic development of grass carp, *Ctenopharyngodon idella*. Egyptian Journal of Aquatic Biology and Fisheries, 6 (3): 233–261.

Galloway M.L., Kilambi R.V. (1984). Temperature preference and tolerance of grass carp (*Ctenopharyngodon idella*). Journal of the Arkansas Academy of Science, 38: 36–37.

Glasser F., Mikolajczyk T., Jalabert B., Baroiller J.F., Breton B. (2004). Temperature effects along the reproductive axis during spawning induction of grass carp (*Ctenopharyngodon idella*). General and Comparative Endocrinology, 136: 171–179.

Hatef A., Alavi S.M.H., Butts I.A.E., Policar T., Linhart O. (2011). The mechanisms of action of mercury on sperm morphology adenosine-5-triphosphate content and motility in *Perca fluviatilis* (Percidae; Teleostei). Environmental Toxicology and Chemistry, 30: 905–914.

Jezierska B., Ługowska, K., Witeska M. (2002). The effect of temperature and heavy metals on heart rate changes in common carp *Cyprinus carpio* L. and grass carp *Ctenopharyngodon idella* (Val.) during embryonic development. Archives of Polish Fisheries, 10: 153–165.

Jezierska B., Ługowska K., Witeska M. (2009). The effects of heavy metals on embryonic development of fish (a review). Fish Physiology and Biochemistry, 35: 625–640.

Jezierska B., Słomińska I. (1997). The effect of copper on common carp (*Cyprinus carpio* L.) during embryonic and postembryonic development. Polish Archives of Hydrobiology, 44: 261–272.

Krupauer V. (1989). Bylozrave ryby [Herbivorous Fish]. Mze CR a Cesky rybársky svaz, SZN, Praha, pp. 115 (in Czech).

Ługowska K. (2009). Embryonic development of barbel (*Barbus barbus*). The Israeli Journal of Aquaculture – Bamidgeh, 61: 68–72.

Ługowska K. (2018). The effects of Roundup on gametes and early development of carp. Fish Physiolology and Biochemistry, 44 (4); 1109-1117 (DOI/:10.1007/s10695-018-0498-9).

Ługowska K., Jezierska B., Witeska M., Sarnowski, P. (2002). Deformations of newly hatched grass carp larvae. Acta scientiarum Polonorum, series: Piscaria, 1: 15–21.

Matasin Z., Orescanin V., Jukic V.V., Nejedli S., Matasin M., Tlak Gajger I. (2011). Heavy metals in mud, water and cultivated grass carp (*Ctenopharyngodon idella*) and bighead carp (*Hypophthalmichthys molitrix*) from Croatia. Journal of Animal and Veterinary Advances, 10 (8): 1069–1072. DOI: 10.3923/javaa.2011.1069.1072.

Michewicz J.E., Sutton D.L., Blackburn R.D. (1972). Water quality of small enclosures stocked with white amur. Hyacinth Control Journal, 10: 22–25.

Pipalova I. (2006). A review of grass carp use for aquatic weed control and its impact on water bodies. Journal of Aquatic Plant Management, 44: 1–12.

Shireman J.V., Smith Ch.R. (1983). Synopsis of biological date on the grass carp *Ctenopharyngodon idella* (Cuv. and Val., 1844). FAO Fish Synopses 135, Rome: FAO.

Sikorska J., Ługowska K. (2005). Wpływ kadmu na rozwój embrionalny karpia (Cyprinus carpio L.). Komunikaty Rybackie, 3: 6–8 (in Polish).

Vajargah M.F., Hedayati A. (2017). Toxicity Effects of Cadmium in Grass Carp (*Ctenopharyngodon idella*) and Big Head Carp (*Hypophthalmichthys nobilis*). Transylvanian Review of Systematical and Ecological Research 19.1 (2017), “The Wetlands Diversity” (DOI: 10.1515/trser-2017-0004).

Verma D.K., Routray P., Dash C., Dasgupta S., Jena, J.K. (2009). Physical and biochemical characteristics of semen and ultrastructure of spermatozoa in six carp species. Turkish Journal of Fisheries and Aquatic Sciences, 9: 67–76.

Witeska M., Jezierska B., Chaber J. (1995). The influence of Cd on common carp embryos and larvae. Aquaculture, 129: 129–132.

Zweerde W. van der (1990). Biological control of aquatic weeds by means of phytophagous fish. In: A.H. Pieterse and K.J. Murphy (eds.) Aquatic Weeds The Ecology and Management of Nuisance Aquatic Vegetation. Oxford Univ. Press, Oxford, pp. 201–221.