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Short communication

MEIOTIC GYNOGENESIS IN IDE (*LEUCISCUS IDUS* L.) INDUCED BY HIGH-TEMPERATURE SHOCK

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ABSTRACT. Ide spermatozoa were genetically inactivated using ultraviolet (UV) irradiation. The highest survival of haploid embryos was noted in the group treated with UV for 5 min (dose 1920 J m^{-2}). High-temperature shock influenced the suppression of the second polar body in ide oocytes. The highest survival rate of diploid gynogenotes (below 10%) was observed in groups shocked at 12 min after egg insemination for 3 min. Eggs shocked later in time exhibited lower survival rates.

Key worlds: IDE (*LEUCISCUS IDUS*), GYNOGENOTES, HIGH-TEMPERATURE SHOCK

Artificial gynogenesis is a method of producing specimens from female gametes without the genetic contribution of male gametes (Kucharczyk 1996). This genome manipulation is comprised of two steps: first – the genetic inactivation of spermatozoa and the second – environmental shock. To produce viable gynogenetic diploids, the eggs are activated with genetically inactivated spermatozoa and treated to inhibit the second polar body or suppress the first mitotic cleavage with environmental shock applied shortly after egg activation (Chourrout et al. 1980). Ide, *Leuciscus idus* L., is a species of interest to aquaculture (pond culture, ornamental fish) and angling. Additionally, in the wake of some wild populations becoming extinct, different methods of restoring their genetic material should be tested. This paper presents results of inducing gynogenesis in ide using UV treated spermatozoa and heat-shock.

Wild ide spawners were caught in Mazurian lakes (Olsztyn District, Poland), and Orfe, the golden variety of ide, spawners were sampled at Oleśnica Hatchery. The fish

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were transported to the hatchery and kept in 1000 l tanks in which temperature (12-15°C) and photoperiod (L:D – 14:10) were controlled. Spawners received injections of human chorionic gonadotropin (hCG) and acetone-dried common carp pituitary extract (CPE) to induce gamete maturation (Kucharczyk et al. 1998). In each experiment milt was collected from a few males. All manipulations of spermatozoa were carried out according to Kucharczyk (1999). The UV lamp (30 W, 6.4 W m^{-2}) was switched on for at least 30 min before the onset of irradiation. Before the sperm was irradiated, control samples of eggs (100-150 eggs in each sample) were fertilized with a small volume (0.05 ml) of undiluted sperm (egg quality control: groups C1 and C2), and with 0.5 ml of diluted but non-irradiated sperm (sperm irradiation control: groups DB1 and DB2). The quantity of sperm used to fertilize control eggs depended on the spermatozoa concentration, so that in controls (undiluted sperm) and in treated groups (diluted sperm) the same number of spermatozoa was used for egg insemination. Experimental groups of eggs were fertilized with 0.5 ml of diluted and irradiated sperm (irradiation time ranged from 1 to 9 min, UV irradiation dose ranged from 384 to 3456 J m^{-2}). After sperm irradiation, additional control groups of eggs were fertilized with 0.5 ml of diluted, non-irradiated sperm (diluted sperm viability control: groups DE1 and DE2). In the second experiment the eggs were fertilized with UV treated spermatozoa (for 5 min) and then exposed to heat-temperature shock (32°C) applied 12, 14 or 16 min after egg-fertilization (shock duration 3 and 5 min). The new control group I (irradiated milt, eggs not heat shocked) was added. The eggs were incubated in a laboratory recirculating system at 14°C, which was established to be the optimal temperature for ide embryonic development (Kucharczyk 1999). All groups were in duplicate.

The survival at the eleutheroembryo stage (free embryos) in all experimental groups inseminated with genetically inactivated spermatozoa was much lower than in the control groups (Fig. 1). These results were in contrast with embryo survival to the eyed-egg stage, where no significant differences between treated and control groups were observed. The highest percentages of hatched haploid embryos were observed in the group in which the eggs were inseminated with sperm exposed to UV irradiation for 5 min (dose 1920 J m^{-2}). Similar data for ide (Kucharczyk 1999) and other cyprinids were reported by Komen et al. (1988) for carp, *Cyprinus carpio* L., and by Kucharczyk et al. (1996) for common bream, *Abramis brama* (L.). For other fish species the optimal UV doses used to inactivate spermatozoa were different (i.e., Chourrout et al. 1980), but these irradiation doses varied depending on the fish species, the type of sperm diluents, ratio of

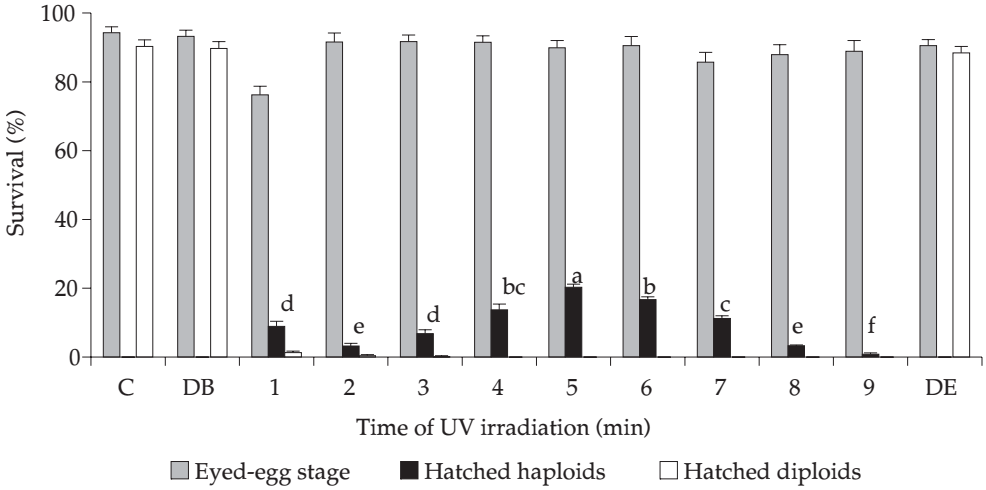

Fig. 1. Effect of UV treatment of ide sperm. Groups are described in the Material and Methods section. Groups (hatching rate of haploids) marked with the same letter did not differ statistically.

dilution, sperm irradiation method, etc. The heat-shock applied to induce gynogenesis in ide was not very efficient (Fig. 2). The highest percentage of gynogenotes was observed in groups exposed to a shock 3-min in duration applied 12 min after egg insemination. Generally, in the groups shocked later in time, lower survival was noted.

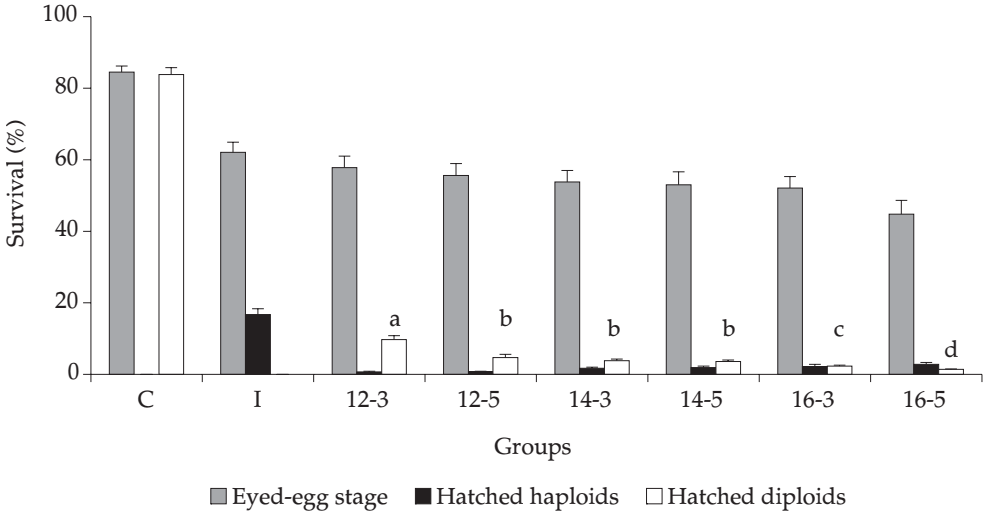

Fig. 2. Effect of heat-temperature shock on the induction of gynogenesis in ide. Groups are described in the Material and Methods section. Groups (hatching rate of diploid gynogenotes) marked with the same letter did not differ statistically.

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STRESZCZENIE

MEJOTYCZNA GYNOGENEZA U JAZIA (*LEUCISCUS IDUS* L.) WYWOŁANA SZOKIEM TERMICZNYM

Plemniki jazia zostały genetycznie inaktywowane w wyniku zastosowania promieniowania ultrafioletowego (UV). Najwyższą przeżywalność embrionów haploidalnych zanotowano w grupie naświetlanej 5 min (dawka 1920 Jm^{-2}) (rys. 1). Zastosowanie szoku termicznego spowodowało zatrzymanie drugiego ciałka kierunkowego w oocytach. Najwyższą przeżywalność diploidalnych gynogenotów (około 10%) zaobserwowano w grupie poddanej szokowi w 12 min od aktywacji oocytów i czasie ekspozycji wynoszącym 3 min (rys. 2). Przy późniejszym zastosowaniu szoku przeżywalność embrionów była znacznie niższa.