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Gynogenesis in the African catfish, *Clarias gariepinus* (Burchell, 1822). Optimizing the induction of polar-body gynogenesis with combined pressure and temperature shocks

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Abstract

The effect of a simultaneous temperature and hydrostatic pressure shock applied a few minutes after activation of the egg was evaluated by monitoring the short-term survival rate of polar-body gynogenetic embryos of African catfish, *Clarias gariepinus* (Burchell, 1822). The aim was to evaluate the interdependence of temperature and pressure in retaining the second polar body. The temperature shock varied between 4°C and 44°C in increments of 4°C, while the pressure shock varied between 0.1 and 68.8 MPa in increments of 13.8 MPa. Highest survival rates were attained at a pressure between 40 and 55 MPa, regardless of the temperature at which the shocks were applied. Optimal survival rates of shocks applied at lower temperatures required longer durations. If reliability to induce retention of the polar body is of prime concern, hydrostatic pressure shocks of 55 MPa at room temperature are recommended.

Introduction

Ploidy manipulation of teleost embryos is a well-accepted method in experimental research and commercial production (Chourrout 1988; Ihssen, McKay, McMillan & Phillips 1990). Although conditions are species-specific, the strategy to induce gynogenesis, androgenesis, triploidy, tetraploidy or any form of polyploidization combines the irradiation of sperm or egg, and the application of a physical or chemical shock some time after activation of the egg. Physical shocks to induce ploidy manipulation include temperature shocks (cold and warm), hydrostatic pressure shocks, chemical shocks (ether, colchicine, deuterium oxide and specific enzyme inhibitors), or combinations of the above mentioned. Combined shocks have been documented in just a few cases. Streisinger, Walker, Dower, Knauber & Singer (1981) successfully combined temperature and chemical shocks to induce homozygous gynogenesis in zebra fish, *Daniodanio rerio* (Hamilton). They drew attention to the easy screening of haploid and homozygous progeny of recessive mutants. Combined cold and chemical shocks have been tested with success in rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Lou & Purdom 1984; Shelton, Macdonald & Johnstone 1986) and Atlantic salmon, *Salmo salar* L. (Johnstone, Knott, MacDonald & Walsingham 1989). The only record of a combined hydrostatic pressure and cold shock to manipulate the genome, involves tilapia, *Oreochromis mossambicus* (Peters) and *O. niloticus* (L.) (Myers 1986). Because neither pressure nor temperature shocks were effective in inducing tetraploidy, two combined conditions were tested: a pressure of 52 MPa at a temperature of 7.5°C and a suboptimal regime of 45 MPa and 6.5°C. The former condition was the most effective although the tetraploid fish proved to be less vital than their diploid counterparts.

In a previous paper, we evaluated the efficiency of either hydrostatic pressure shocks or temperature shocks to induce meiogynogenesis (Volckaert,

Galbusera, Hellemans, Van den Haute, Vanstaen & Ollevier 1994). Cold and pressure shocks were the most efficient in terms of survival rate. A further optimization by combining pressure and temperature shocks to retain the second polar body in fish, unique to the literature, is the next step. The present study systematically screens the survival rate of the embryo of the African catfish, *Clarias gariepinus* (Burchell, 1822), submitted to retention of the polar body within a temperature range of 4–44°C at a hydrostatic pressure less than 69 MPa. We observe that diploidization is nearly independent of temperature at a pressure of 40–55 MPa, but is dependent on the duration of the shock.

Materials and methods

The stock of African catfish, the artificial fertilization, the UV irradiation of the sperm and the hatching procedure have been documented in Volckaert *et al.* (1994). In brief, eggs were stripped from GnRH-treated mature females and sperm was collected from testes operatively isolated from mature fish. The sperm was diluted in extender and UV irradiated. Normally fertilized and pressure-treated embryos were raised at 28°C; survival rates were scored in duplicate at 12, 24 and 72 h post treatment. All experiments included an indirect test of manipulation efficiency: the survival rate of normally fertilized embryos (control). Only experiments with a 100% haploid induction rate in the control experiment were analysed. Direct tests of manipulation efficiency by means of paternity testing with multiple-locus DNA fingerprinting were carried out in selected cases according to Volckaert *et al.* (1994). More accurate tests of paternity with microsatellite DNA fingerprinting, although not explicitly reported in this paper, confirmed the efficiency of the UV treatment (Galbusera, Volckaert, Hellemans & Ollevier 1996; Galbusera *et al.* unpublished data).

Procedures different from those in the above-mentioned paper include the temperature-controlled pressure shocks and the statistical analysis.

Temperature control of pressure shocks

The temperature of a hydrostatic pressure vessel of 0.67 l filled with mineral oil was regulated with a water jacket surrounding the pressure vessel, which was connected to a water bath. Temperature could be controlled between 0°C and 50°C with an accuracy of 1°C. Experiments were carried out at a temperature between 4°C and 44°C in increments of 4°C, and a pressure between 0.1 and 68.8 MPa in increments of 13.8 MPa (megapascals; 1 atm = 0.101 MPa). Before and after the experiment, the zygotes were incubated in a 28°C incubation tank. During the experiment, the embryos were enclosed in a 2-ml syringe, which was sealed off with a needle and a rubber stopper, and transferred to the pressure vessel. The impact of short-term enclosure on the eggs was negligible (judged by survival rate of normal controls treated in a standard water bath and controls immersed in an oil bath in a 2-ml syringe). All experiments were carried out either at 3 or 4 min after activation of the egg (time 0 corresponds to the addition of water to a mixture of eggs and sperm) and during 2, 10 or 20 min depending on the water temperature. Previous experiments revealed that treatment time was inversely related to temperature (Volckaert *et al.* 1994).

Statistical analysis

Data were standardized to relative survival rates at 12, 24 or 72 h post activation (i.e. per cent survival of the treated embryos expressed relative to the diploid control) to reduce the maternal effect among experiments. The relative survival rates were normalized by arcsin transformation (Θ = asin $\sqrt{p}$) (Sokal & Rohlf 1994). Relative survival rates higher than 100% were arbitrarily set at 100%. Analysis of variance, contrast analysis, correlation analysis and contour plotting of the data was carried out with the statistical package Statistica (release 4.5, StatSoft Inc., USA).

Results

The relative survival rates of meiogynogenetic African catfish at 72 h differed considerably among treatments. No survival was observed at atmospheric pressure; increasing hydrostatic pressures induced better survivals. Pressures of 55 MPa induced the highest survivals while pressures of 69 MPa resulted in decreasing survival rates (Fig. 1). The factors temperature, pressure or the combination of both influenced survival at 12, 24 and 72 h in most conditions. However, no interaction between

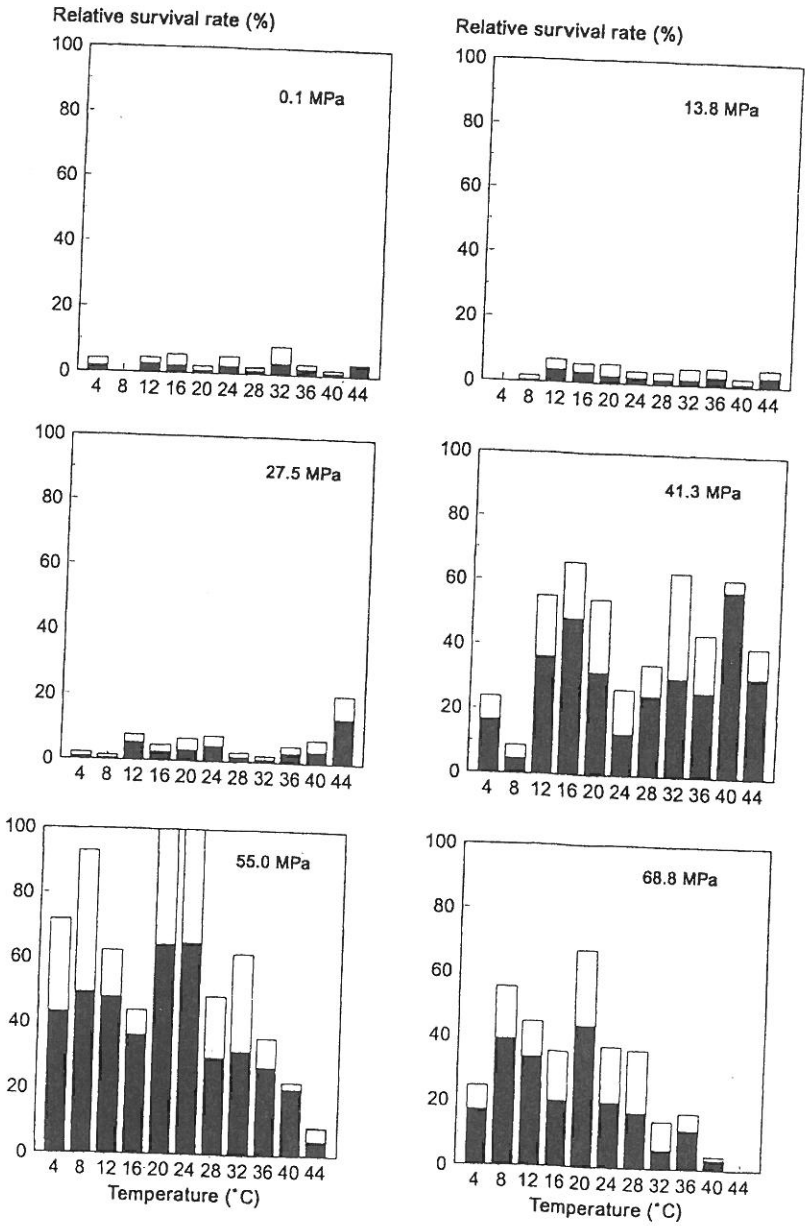

Figure 1 Relative survival rate (mean (dark bars) and standard deviation (clear bars)) of *Clarias gariepinus* embryos which were pressure and temperature shocked at 4–44°C between 0.1 and 68.8 MPa during 2 min measured at 72 h post activation.

pressure and temperature in affecting the survival rates was observed at 24 h (Table 1).

The extent of the effect of temperature and pressure on survival rate was studied in contour plots and with contrast analysis. Visual inspection of a three-dimensional plot of the survival rate at 72 h shows that pressure applied at 50–60 MPa at lower temperatures gave the best survival rates of meiogynogens (Fig. 2). At higher temperatures a pressure of 40–50 MPa gave the best survivals. Regardless of temperature, a pressure of 55 MPa induced the best survival rates. Exponential regression analysis of the arcsin-transformed survival rate at 12, 24 and 72 h at a shock duration

Survival rate at	Duration (min)	Factors		
		Temperature	Pressure	Temp. and pressure
12 h	2	**		
	10	**	**	*
	20	**	**	*
24 h	2			
	10	*	**	
	20	*	**	
72 h	2	*	**	**
	10		**	**
	20	**	**	**

Table 1 Analysis of variance of the factors temperature and pressure applied at various durations and affecting relative survival rates of meiogynogenetic embryos of *Clarias gariepinus*. *, $P < 0.05$; **, $P < 0.005$

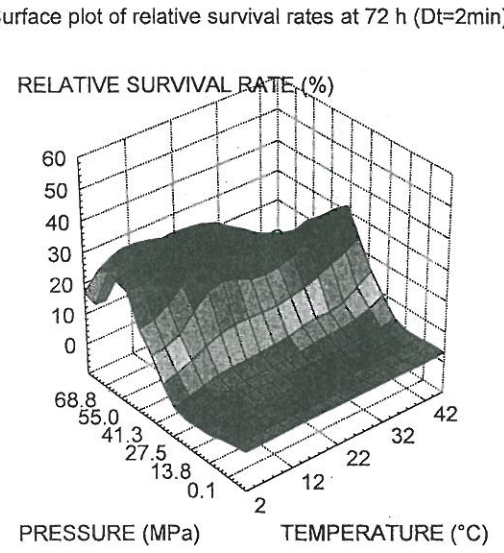

Figure 2 Contour plot (fifth-order polynomial interpolation) of relative survival rates of *Clarias gariepinus* embryos shocked between 4°C and 44°C and between 0.1 and 68.8 MPa during 2 min measured at 72 h post activation.

of 2 min models respectively. 0%, 0% and 29% of the total variance. At 72 h, survival rate integrates temperature and pressure in the following exponential model:

$$SUR = -3570.87\, e^{8.182344 - 0.00023TEMP + 0.000166PRES}$$

where *SUR* denotes relative survival rate of arcsin-transformed data (%), *TEMP* is temperature (°C) and *PRES* is pressure (MPa).

A posteriori contrast analysis (ANOVA) between high/low temperature and high/low pressure indicates that 22°C and 38 MPa differentiate survival at 72 h post activation ($P < 0.002$).

At lower temperatures (up to 16°C), shocks of longer duration positively influenced the induction of meiogynogenesis (Fig. 3). A cold shock of 10 min induced the highest survivals at 27.5 MPa, while a shock of 20 min induced highest survivals at a lower pressure of 13.8 MPa. The highest survivals (44%) were observed at a shock of 20 min and a pressure of 13.8 MPa.

No analysis of variance was performed because of the asymmetry of the data matrix.

Discussion

When applying temperature and pressure shocks during a short period to produce meiogynogenetic fish, pressure seems to influence survival rate more so than temperature. On average, the gradients crossed with pressure are much sharper than those crossed with temperature.

At ambient pressure, cold shocks tend to be more efficient to induce retention of the second polar body in warm-water fish (such as tilapia and African catfish), while heat shocks are more efficient in cold-water fish (such as salmonids) (Ihssen *et al.* 1990). Cold and warm are defined relative to the normal breeding temperature of the species concerned. Also, warm-water shocks seem more suitable to prevent the first mitotic division (Hussain, Penman, McAndrew & Johnstone 1993). According to the literature, pressure shocks are in general efficient in inducing retention of the second polar body between 40 and 80 MPa (Streisinger *et al.* 1981; Lou &

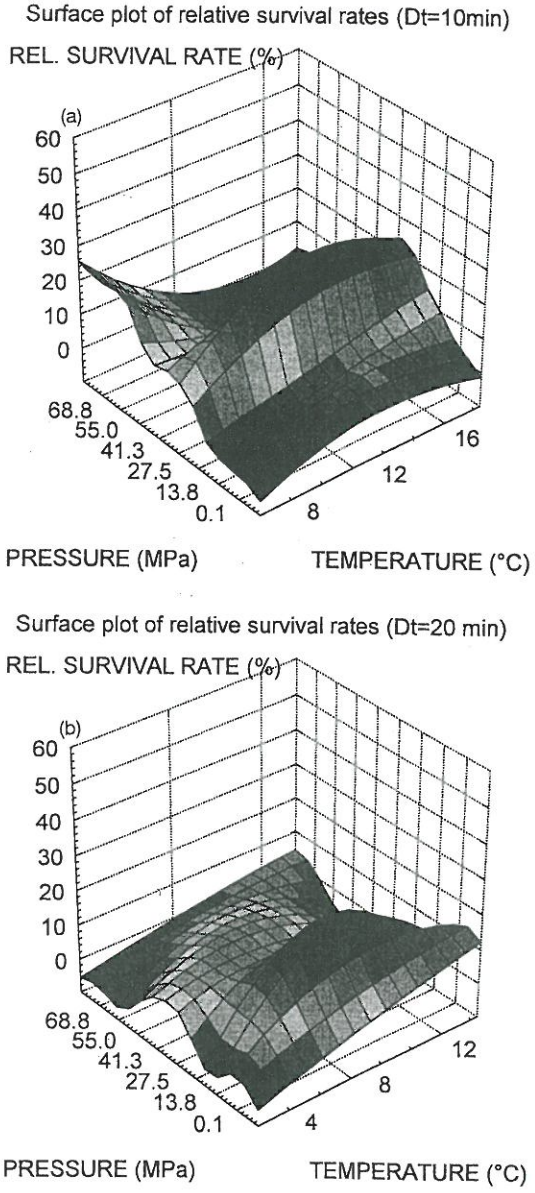

Figure 3 Contour plot (fifth-order polynomial interpolation) of relative survival rates of *Clarias gariepinus* embryos shocked between 4°C and 16°C and between 0.1 and 68.8 MPa during 10 min (Fig. 3a) and 20 min (Fig. 3b) measured at 72 h post activation.

Purdom 1984; Hussain *et al.* 1993). A previous study by Volckaert *et al.* (1994) focused on the efficiency of either temperature or pressure shocks to induce meiogynogenesis. Few of their results are comparable with those of this study. Similar low survival rates at 40°C 3 and 4 min post activation were observed in the previous study. The cold shocks cannot be compared because they were applied at different durations. The pressure shocks at room temperature (20°C) are comparable: 27%, 43% and 33% (this study) in comparison with 35%, 58% and 34% at 41, 55 and 69 MPa. A high variability among replicates was observed in both studies. Maternal effects including overmaturation, general fitness and epigenetic effects might interfere (Hörstgen-Schwark 1993).

The physical shock conditions resulting in viable fry combine factors typical for normal diploid fish and factors specific to the retention of the polar body. Embryos of African catfish normally hatch between 20°C and 35°C at atmospheric pressure and have a lower threshold temperature of 14.5°C (Haylor & Mollah 1995). Water of less than 1°C induces developmental arrest while water warmer than 44°C at atmospheric pressure irreversibly damages the zygote. Pressures above 70 MPa induce irreversible cellular damage.

If reliability is of prime concern in applications, meiogynogenesis should be induced in African catfish with a short pressure shock of 55 MPa at room temperature. Working at room temperature avoids the need for temperature control. If a press is not available, then a cold shock at 5°C during 30 to 40 min is recommended.

Future research on the impact of pressure and temperature on the embryonic development of fish should concentrate on the optimization of mitotic or homozygous gynogenesis. Survival rates are much lower in this case, mostly due to the expression of recessive lethal mutants.

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