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Short communication

Induction of triploidy in *Rhamdia quelen* (Siluriformes, Heptapteridae) by double-temperature shock

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ABSTRACT. This study evaluated sequential double-temperature shock (heat and cold) to induce triploidy in *Rhamdia quelen*. Fertilized eggs were heat shocked at 37, 39 and 41°C at 2 min post-fertilization and then cold shocked at 1°C for 20 min. Intact eggs were used as controls. Egg viability in control groups was higher (65.5 ± 37.0%) than that at 37°C (58.2 ± 37.7%), 39°C (1.8 ± 0.3%) and 41ºC (0%). The survival of control hatchery (64.6 ± 36.8%) was higher compared to treatments at 37ºC (24.4 ± 15.5%) and 39ºC (0.6 ± 0.07%). Triploid percentages were 98.5% at 37°C and 100% at 39°C. Therefore, while double-temperature shock induced 100% triploidy in *R. quelen*, it also significantly decreased embryo survival and increased the deformity larvae.

Keywords: *Rhamdia quelen*, silver catfish, polyploidy, temperature shock, flow cytometry.

The silver catfish *Rhamdia quelen* (Quoy & Gaimard, 1824) from the Neotropical region is distributed from central Argentina to southern Mexico (Silvergrip, 1996). In southern Brazil, the culture of this species is increasing based on its growth rate, cold tolerance, and marketability as a boneless species (Baldisserotto *et al.*, 2004; Fracalossi *et al.*, 2004). Production in Santa Catarina State, the main region for culturing this species, has increased significantly in recent years, from 25 ton in 2005 to 833 ton in 2012 (Silveira, 2014). Despite all these favorable characteristics, early sex maturation in males results in decreased performance when compared with females. This problem is related to gonadal development which delays somatic growth (Fracalossi *et al.*, 2004; Huergo & Zaniboni-Filho, 2006), in turn affecting the overall profitability of the fish industry. One possible solution involves the large-scale culture of triploid fish because, in general, triploids are sterile and do not present problems related to early reproductive maturation. Previous studies in the literature have reported on triploidization in *Rhamdia quelen*. For example, Vozzi *et al.* (2003) obtained triploids by heat shock, while Silva *et al.* (2007) obtained triploids by cold shock. Huergo & Zaniboni-Filho (2006) experimentally obtained silver catfish triploids using hydrostatic pressure, but it was never evaluated on a large scale, likely a result of the low egg quantity typical of this method (Tiwary *et al.*, 2004). Since these methods have shown poor performance and since large-scale triploidy is difficult to carry out in *R. quelen*, heat followed by cold shock, *i.e.*, double-temperature shock, is an alternative.

Nam *et al.* (2004) used double shock in *Misgurnus mizolepis* (Günther, 1888) for tetraploid induction. To the best of our knowledge, this is the first attempt to use this method to produce triploids. Temperature shocking is generally easy and can be applied on a large scale, as observed in salmonids (Benfey & Sutterlin, 1984; Sanches *et al.*, 2011). Therefore, this study aimed to evaluate sequential double-temperature shock to induce triploidy in *R. quelen* and then measure the effects on reproduction.

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Twelve females (740 ± 53 g) and eight males (634 ± 39 g) were selected. The selected broodstock was distributed in 0.5 m^3 hapas (nylon mesh net cages) (3 hapas with 4 females each and 8 males in a single hapa). Each hapa was placed in a 2.5 m^3 concrete tank with constant aeration (dissolved oxygen >6 mg L^{-1}), and the temperature was set at 25.0 ± 0.3ºC. Only the females were induced to spawn using a single dose of carp pituitary gland at 5 mg kg^{-1} (Amaral-Junior, 2007). In order to avoid synchronic ovulation of all females, groups of females were induced at 1-h intervals. Oocytes were collected by stripping 10 h afterwards, and only oocytes with good quality, *i.e.*, those showing translucence and homogeneity of color, shape and size, were used in the fertilization experiments. The sperm was stripped on the egg mass and homogenized. Gametes were then activated using hatchery water (Ribolli & Zaniboni-Filho, 2009). In order to estimate the amount of eggs for subsequent incubation procedures, an aliquot (0.5 g) from each batch was fixed in 4% formalin. The number of eggs was checked (Wirtz & Steinmann, 2006) and estimated as 1,356 ± 73 eggs g^{-1}. Twenty milliliters of fertilized eggs (total amount of 1,401.33 ± 21.06 eggs) were distributed in twelve circular floating incubators with a nylon mesh in the bottom (0.4 mm and total volume of 900 mL). These incubators were placed in a 2,500-L tank with constant water flow, temperature of 25.0 ± 0.3ºC, dissolved oxygen of 6.8 ± 0.7 mg L^{-1} and pH of 7.8 ± 0.5.

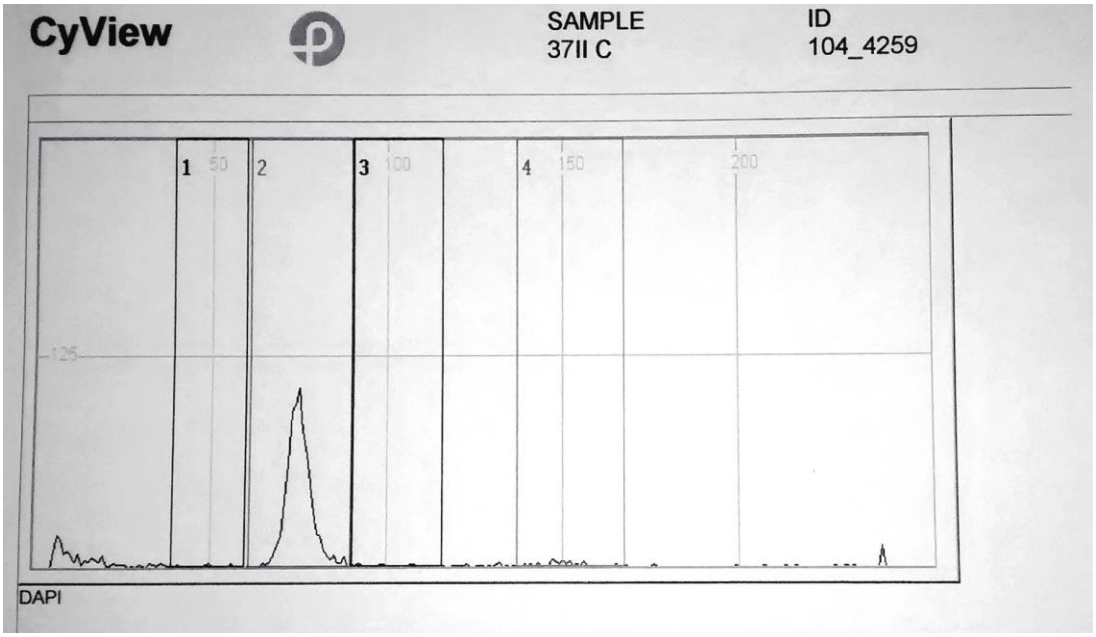
The incubators were distributed in triplicate between treatments: control (no temperature shock) and three treatments with double-temperature shock. In these teatments, the eggs with double-temperature shock were first submitted to heat shock in water bath at 37, 39 and 41ºC for 2 min, starting from 2 min post-fertilization (mpf), followed by cold shock at 1ºC (ice) for 20 min. Immediately afterwards, eggs were incubated at 25°C. Intact eggs not undergoing temperature shock were used as control groups.

Egg viability was measured at late gastrula stage (~12 h post-fertilization (hpf)) in a sample of ~260 embryos (Zaniboni-Filho, 1992; Rizzo *et al.*, 2003). Survival of hatchery and the percentages of normal and abnormal larvae were measured at 30 hpf. At 60 hpf, 25 larvae were fixed from each egg batch in acetic methanol (methanol:acetic acid 3:1) for confirmation of ploidy status by flow cytometry (Lamatsch *et al.*, 2000). For flow cytometric analysis, we used a CyStain® DNA 2 step kit (PartecGmbh, Germany). Briefly, one larva was lysed with 120 µL of solution A in order to isolate the nuclei. Nuclei were then stained with 1.5 mL of DAPI (4,6-diamidino-2-phenylindole) solution. The resultant suspension was then filtered in a 30 µm mesh (CellTrics ® Partec Gmbh, Germany), followed by measurement of DNA content by flow cytometry (PartecCyFlow Ploidy Analyzer, PartecGmbh, Germany). The relative DNA content was obtained by the histograms (Fig. 1). In order to detect small variations in DNA content, we used somatic cells of *Astyanax altiparanae* (Garutti & Britski, 2000) as a control group during flow cytometry analysis. To evaluate the temperature-dependent viability of eggs, survival of the hatchery and number of deformed larvae, data were grouped by treatment and structured in contingency tables using chi-square test (χ^2; $\alpha = 0.05$) (Zar, 2010). Partitioning of contingency tables was used to show the treatments that produced significant differences between the proportions. Data were shown as mean ± standard deviation. Voucher specimens were deposited in the Zoological Museum of the Federal University of Rio Grande do Sul (UFRGS 18258 to 18266).

Egg viability ($\chi^2 = 752.78$; $P < 0.05$), deformed larvae ($\chi^2 = 350.57$; $P < 0.05$) and survival of hatchery ($\chi^2 = 380.54$; $P < 0.05$) were significantly dependent on heating temperatures (Table 1). The percentage of triploid fish produced directly increases as heat shock temperature increases, *e.g.*, from 98.6% (37ºC) to 100% (39ºC) ($\chi^2 = 165.89$; $P < 0.05$). At the same time, however, survival dramatically reduces from 24.4% (37ºC) to 0.6% (39ºC). The production of abnormal larvae also increases by 30% when the temperature changes from 37 to 39ºC (Table 1). No surviving embryos were observed at 41ºC.

Induction of triploidy was measured until lethal temperature had been reached. Therefore, the temperature evaluated in this study fell within a good range. Considering the survival and the percentage of abnormal larvae, it can be concluded that the number of viable larvae was severely decreased with increasing temperature. As observed in this study, induction of triploidy was achieved at temperatures close to lethal; this similarity makes it necessary to evaluate the duration of temperature treatments in future studies.

According to Nam *et al.* (2004), the sequential combination of heat and cold shocks decreases survival rates, but such severe treatment also improves the percentage of polyploids. Previous studies using the double-shock temperature was not found to induce triploidy in Silver catfish. Others studies with silver catfish obtained good results after either heat or cold shock. Vozzi *et al.* (2003), for example, observed egg viability at 65.4 ± 3.1%, survival at 33.4% and triploidy at 74.8% after heat shock at 36°C for 5 min, similar to our study at 37°C for 2 min, followed by 1ºC for 20 min. Silver catfish triploidy was also obtained after cold shock at 4°C applied 3 min after fertilization for 20 min,



Regions				
Name	Particle	Center	CV%	Median
DAP_1	17	50.00	12.20	50
DAP_2	752	77.29	4.84	75
DAP_3	22	101.00	6.72	101
DAP_4	47	151.00	4.58	151

Figure 1. Cytometric histogram of silver catfish (*Rhamdia quelen*) larvae treated with double-temperature shock.

Table 1. Development and ploidy status of silver catfish (*Rhamdia quelen*) after temperature treatments. Different letters in rows denote differences after partitioning chi-squared test ($P < 0.05$). *The number of analyzed larvae was grouped by treatment, n: individuals number.

Parameters	Heat shock temperature (ºC)		Control
	37	39	
Egg viability (%)	58.2 ± 37.7^{b}	1.8 ± 0.33^{c}	65.5 ± 37.0^{a}
Survival of hatchery (%)	24.4 ± 15.5^{b}	0.6 ± 0.07^{c}	64.6 ± 36.8^{a}
Abnormal larvae (%)	47.4 ± 9.6^{b}	65.2 ± 8.38^{a}	0.0^{c}
Analyzed larvae* (n)	72	29	66
Triploids (n, %)	71^{a} (98.6)	29^{a} (100.0)	0^{b} (0.0)

resulting in 97.9 ± 1.2% of triploid fish and a survival rate of 65.4 ± 5.3% (Silva *et al.*, 2007). Huergo & Zaniboni-Filho (2006) exposed silver catfish to pressure shock for 5 min at 3.4473950×10^{7} Pa applied either 2 or 5 mpf to induce 100% triploidy. However, this treatment resulted in low survival (20.5 to 29.1%). The percentage of abnormal larvae was increased in our study when compared with Vozzi *et al.* (2003) and Silva *et al.* (2007), but higher percentages of abnormal larvae were reported by Huergo & Zaniboni-Filho (2006) by pressure shocking, reaching 40-80% of embryonic deformity in treatments, albeit with better triploidy percentages.

In conclusion, this study, for the first time, evaluated the application of double-temperature shock for the production of silver catfish. Compared to other methods, a high percentage of triploids were produced - up to 100%. At the same time, however, this strategy significantly decreased embryo survival and increased the number of deformed larvae. Therefore, future studies are required in order to determine the best temperatures and durations of double-shock treatments

to improve the efficiency of this method for large-scale aquaculture.

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