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Triploidy induction by cold shock in the South American catfish, *Rhamdia quelen* (Siluriformes) (Quoy & Gaimard, 1824)

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Abstract

The catfish *Rhamdia quelen* (Siluriformes: Pimelodidae) has been widely produced for human consumption in the Southern and South-eastern Brazil, Argentina and Uruguay. Therefore, studies have been conducted to obtain a greater knowledge of its biology and production improvement, including its growth and food conversion which are compromised by the precocious sexual maturation, interfering with somatic growth. Thus, the possible use of sterile triploids is an interesting option for its culture. The best parameter for cold shock triploidy induction was achieved at 4 °C, 3 min after fertilization for 20 min resulting in 97.9±1.16% of triploid fish. This cold shock conditions resulted in a survival rate of 65.4±5.34%. The nucleoli staining with silver nitrate ($AgNO_3$) proved to be a practical and efficient tool to investigate the ploidy of the animals from the treatments. The Nucleolar Organizer Region (NOR) is located on one chromosome per haploid set at a secondary constriction.

Keywords: *Rhamdia quelen*; Catfish; Chromosomal manipulation; Triploidy; Cold shock

1. Introduction

The average annual rate of growth of overall aquaculture production has been 10.5% in the last 10 years (FAO, 2004). Latin America was responsible for 2.46% of the world aquaculture production in 2004, reaching 1118.053 t (FAO, 2006).

Brazil has a substantial potential for tropical aquaculture: availability of land, freshwater sites, favourable climate, and a growing local demand for fish. The majority of aquaculture production stems from freshwater finfish. The major native species produced are the round fish 'tambaqui' (*Colossoma macropomum*) and the 'pacu' (*Piaractus mesopotamicus*). From the catfish group, the 'surubim' (*Pseudoplatystoma coruscans*) and the 'jundiá' (*Rhamdia quelen*) are the most representative species (Borghetti et al., 2003).

The South American catfish *R. quelen* is a Siluriformes belonging to the Pimelodidae family (Silfvergrip, 1996). This species inhabits rivers from Argentina to the South of Mexico (Perdices et al., 2002), reaches 50 cm in length and 3 kg in body weight, and produces an average of 50,000 eggs/kg of body weight of female (Narahara et al., 1989; Mérigoux et al., 2001). The 'jundiá' or silver catfish is omnivorous (Salhi et al., 2003; Meyer and Fracalossi, 2004), shows an early

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gonadal maturation (around 6 months old) and is able to reproduce more than once per reproductive cycle. The cultivation of *R. quelen* is well consolidated in the South of Brazil, where the estimated production was 547 t in 2004 (IBAMA, 2005).

According to Narahara et al. (1985), *Rhamdia hilarii* (synonym of *R. quelen*) is commonly sexually mature at 1 year of age, may reach maturity as early as the age of 6 months. This sexual precocity hinders its growth and, as a result, its performance under culture conditions. Aquaculture has been boosted by biotechnology improvements, such as, hormone induced spawning, QTL markers, and sterility induction through sexual and chromosomal manipulation (Hulata, 2001).

Production of sterile fish is useful to the fish industry because during the gonadal development and maturation the fish mobilizes part of the absorbed nutrients and/or part of the body reserves for gonadal development and gametes production (Henken et al., 1987). Thus, growth and feed conversion rates may be negatively affected and the percentage of fillet or marketable product may decrease (Purdom, 1983; Thorgaard, 1986). It is also important to mention the potential genetic introgression threat to wild populations posed by the escape of diploids (non-sterile) from fish farms (Na-Nakorn et al., 2004).

Triploidy induction for fish farming has been applied to several species of salmon (Johnston et al., 1999), trout (Bonnet et al., 1999), carp (Basavaraju et al., 2002) and catfish (Manickam, 1991; Linhart and Flajshans, 1995) in combination or not with other genetic tools. These chromosomal manipulations might result in gynogenetic, androgenetic and polyploid individuals, as well as in the establishment of sterile or endogamic genetically improved broodstocks for commercial production.

Since previous investigations have already evaluated the pressure (Huergo and Zaniboni Filho, 2006) and heat shock (Vozzi et al., 2003) conditions for *R. quelen* triploid production, the present study aims at investigating the parameters for a cold shock method result in low mortality and deformity by which mass production of sterile individuals can be produced for aquaculture purposes.

2. Materials and methods

2.1. Origin of fish stock and induced reproduction

The broodstock used was obtained from a commercial fish farm located in the Southeast of Brazil. Seven males and five females were induced for gamete release with carp pituitary extract (4 mg/kg for females and 1 mg/kg for males). At the moment of gametes release, one female and one male were manually stripped for release of eggs and sperm that were gently mixed. Water was then added to achieve fertilization.

2.2. Induction of triploidy

Given that no previous protocols of cold shock have been established to induce triploids in *R. quelen*, other Siluriformes triploidy induction protocols were used as a baseline to set the parameters for this species (Wolters et al., 1981; Richter et al., 1987; Vejaratpimol and Pewnim, 1990; Manickam, 1991).

A preliminary trial was conducted so that the variables would be adjusted. The parameters for the cold shock (CS) trials were set thereafter as follows: temperature of shock $\theta = 4$ °C and 7 °C (±0.5 °C); time of beginning cold shock after fertilization (t) = 3 min; duration of cold shock (d) = 20, 40 or 60 min (Table 1).

2.3. Experimental design and egg incubation

A pool of fertilized eggs from different spawners was distributed in triplicate for each trial and one control group. The temperature and dissolved oxygen were monitored with an oxygen and temperature meter (YSI-55, YSI, Yellow Springs, Ohio).

The fertilization was carried out in water at a temperature of 25.5±2.4 °C. Approximately 1500 eggs for each treatment were cold shocked and then incubated in 2-l round-bottomed plastic jars with constant water flow to ensure gentle movement of eggs. The control group was treated in the same way, except for the water temperature that was maintained at 25.5±2.4 °C.

Table 1
Treatments for cold shock triploidy induction and their corresponding survival and triploidy rates

Treatment	Temperature (°C)	Time after fertilization (min)	Shock duration (min)	Survival rate (%)	Triploidy rate (%)
1			20	65.4±3.1[a]	97.9±1.16[a]
2	+4.0±0.07	3	30	7.8±3.1[c]	42.0±6.68[b]
3			40	0.1±0.04	*
4			20	52.6±1.6[b]	92.0±8.47[a]
5	+7.0±0.07	3	30	1.2±0.9[d]	*
6			40	0.5±0.3	*
Control	+25.5±2.40	–	–	100%	0

* = Treatments with low survival rates, triploidy rates unattainable.
[a,b,c,d]Statistical difference ($P<0.01$).