Theriogenology 88 (2017) 254–263

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Natural and artificial spawning strategies with fresh and cryopreserved semen in *Rhamdia quelen*: Reproductive parameters and genetic variability of offspring

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ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form 11 September 2016
Accepted 12 September 2016

Keywords:
Controlled fertilization
Cryopreservation
Microsatellite
Offspring
Reproduction

ABSTRACT

This study evaluated the reproductive parameters and genetic variability of offspring of *Rhamdia quelen* from mating by natural spawning and by controlled fertilization with fresh and cryopreserved semen. After hormonal manipulation, three *R quelen* pairs were used for natural spawning in high-flow tanks, three pairs were used for mating with fresh semen, and another three pairs were used with cryopreserved semen. Matings were performed in triplicate. For matings with fresh and cryopreserved semen, semen from each male was used to individually fertilize oocytes from each female. Pools of semen and oocytes were made, and aliquots of these gametes were taken for fertilization. No differences ($P > 0.05$) were detected for absolute or relative fecundity, fertilization rate, or egg hatching rate. The use of fresh semen led to a higher ($P < 0.01$) percentage of normal larvae. The use of fresh semen in pooled mating resulted in a greater effective number of alleles and a higher expected heterozygosity. Offspring from natural spawning presented higher observed heterozygosity and a lower inbreeding coefficient. The highest inbreeding coefficient was found in offspring from individual matings using fresh semen. Regarding paternal contributions, a single male dominated in matings with fresh semen, whereas two males dominated in natural spawning and matings using cryopreserved semen. The use of gamete pools for mating and natural spawning resulted in higher genetic variability of offspring, and mating using cryopreserved semen found no effects on genetic variability of offspring but did reduce the percentage of normal larvae. Other reproductive parameters were not influenced by spawning strategies.

1. Introduction

Rhamdia quelen is a neotropical fish that occurs from southern Mexico to central Argentina [1]. It is an important species for fish farming in South America and is also considered a biological model for breeding studies [2].

Captive breeding of tropical fish generally involves hormonal induction to promote final maturation and spawning. Most processes use the extrusion technique to remove gametes followed by controlled fertilization of oocytes [3] using fresh or cryopreserved semen [4]. Natural spawning, in which there is no human interference during the release

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0093-691X/$ – see front matter © 2016 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.theriogenology.2016.09.029

of gametes, has also been studied in neotropical fish, as it can reduce mortality and increase fertilization rates [3,5].

The use of inappropriate protocols for spawning and/or controlled fertilization can result in loss of genetic variability of offspring [5–7]. This decline in genetic variability can occur through selection and breeding of specimens or by using a small number of parents for mating [8]. These actions can result in inbreeding and problems for adaptability and survival of broodstock [9].

In addition, some authors suggest that cryopreservation of semen may also affect hatching rates and genetic variability of offspring because despite the benefits associated with cryopreservation, it can damage sperm and impair its functionality [10], with associated reductions of fertilization rates [4]. Moreover, recent studies have indicated that cryopreservation of semen can damage the DNA of cells through fragmentation [10–13].

Therefore, studies designed to determine protocols for spawning and/or controlled fertilization that promote the maintenance of genetic variability of offspring are needed, as their results can support breeding programs or wildlife biodiversity conservation programs. As such, the objective of this study was to evaluate the reproductive parameters and genetic variability of offspring of *R quelen* generated using natural spawning and controlled fertilization with fresh or cryopreserved semen.

2. Material and methods

2.1. Animals and experimental design

This experiment was carried out in accordance with the guidelines of the Brazilian College for Animal Experimentation (http://www.cobea.org.br).

A total of 27 females (270.18 ± 21.80 g) and 27 males (209.20 ± 13.07 g) of *R quelen* were used from a broodstock housed in 200-m^2 excavated ponds with masonry walls and dirt floors, in which water was added only to compensate for losses by evaporation and seepage. Fish were previously fed with commercial feed containing 32% crude protein and 3200 kcal gross energy/kg.

Three reproduction assays were performed. In the first assay (assay 1), aliquots of fresh sperm from three males were used to fertilize the oocytes of three females apiece; matings were performed so that semen from each male was used to fertilize the oocytes from three separate females (individual mating). Then, identical volumes of fresh semen from each male were mixed to form a sperm pool, and the same was done with the oocytes from three females. From these pools, samples of sperm and oocytes were taken for controlled fertilization (pooled mating). Each mating was performed in triplicate. In another assay (assay 2), the same procedures for controlled fertilization were employed with another three males and three females, but they used cryopreserved semen instead of fresh semen. Finally, in the third assay (assay 3), another three males and three females were housed in circular tanks with high water flow after receiving the last hormonal application to promote natural spawning and subsequent uncontrolled fertilization of oocytes (natural mating). Natural matings were also performed in triplicate.

The first experiment was conducted with a completely randomized design in a 2 × 2 bifactorial structure, with four treatments and three replicates (Fig. 1). The experimental factors (treatments) were the use of fresh or cryopreserved sperm, and individual or gamete pool mating.

The second experiment was conducted in a completely randomized design with five treatments and three replicates (Fig. 1). The treatments consisted of (1) individual and

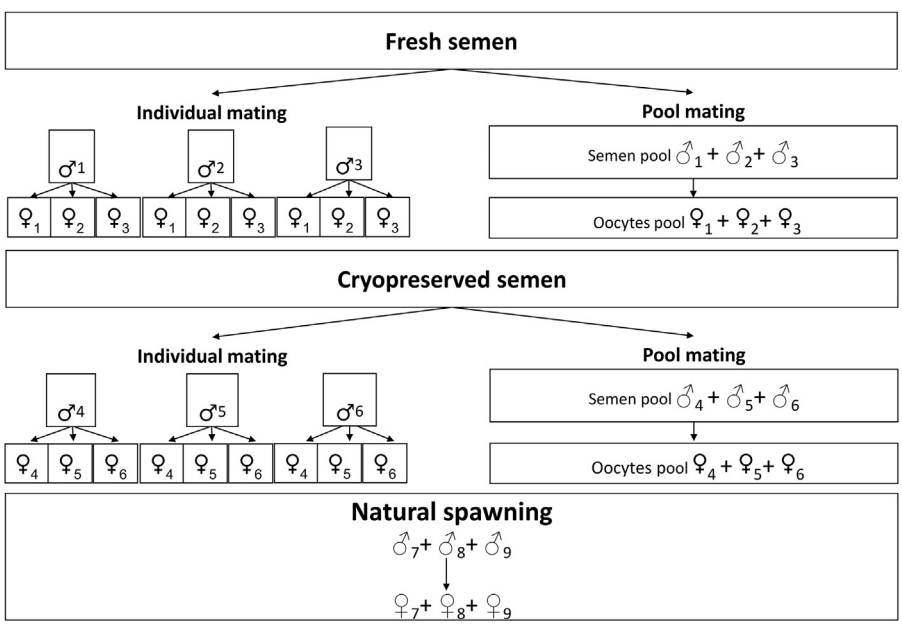

Fig. 1. Experimental design adopted for *Rhamdia quelen* mating.