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On-site evaluation of commercial-scale hybrid catfish production using cryopreserved blue catfish sperm

E Hu [a,1], Brian Bosworth [b], Jeff Baxter [c], Terrence R. Tiersch [a,*]

[a] Aquaculture Research Station, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA
[b] USDA Catfish Genetics Research Unit, Stoneville, MS 38756, USA
[c] Baxter Land Company, 114 E. Speedway, Dermott, AR 71638, USA

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ABSTRACT

Cryopreservation is an effective tool for conservation of genetic resources and is becoming increasingly used worldwide with aquatic species. Broadening the application of this technology to a commercial scale through high-throughput approaches has become essential for use with aquatic species. This study addressed high-throughput sperm cryopreservation of blue catfish at an industrial level. Our objectives were to: 1) optimize the sperm volume used for thawed sperm; 2) evaluate commercial application of high-throughput cryopreserved sperm with standard hatchery techniques, and 3) initiate evaluation of the fertility relationship between individuals and pooled samples. The results showed that a doubling of the previously established volume did not produce significant improvement in fertilization. The working volume of thawed sperm (2 ml at a concentration of 1×10^9/ml for batches of 100–150 ml channel catfish eggs) was practical. There was no significant difference in fry production after artificial fertilization of 2 million eggs with cryopreserved or fresh sperm. Pooled sperm samples and the individual samples used to form the pools produced similar fertilization rates. Blue catfish sperm is valued as a genetic material for hybrid catfish production, and cryopreservation makes genetic material management possible. This study initiates industrialization of this technology for use with aquatic organisms, and because the technology can be generalized, expands the opportunities for application to other species. High-throughput cryopreservation of blue catfish sperm provides new capabilities and can maintain sperm quality sufficiently to support commercial hybrid production.

1. Introduction

The dairy industry has used high-throughput sperm cryopreservation since the 1940s (Pickett and Berndtson, 1974). There is a billion-dollar global market specifically for cryopreserved cattle germplasm according to the National Association of Animal Breeders-Certified Semen Services (www.naab-css.org). Sperm cryopreservation technology currently serves the purposes of animal breeding, preservation of genetic diversity, and medical research. In human reproduction applications, US sperm banks export frozen semen of human donors to more than 60 countries to help infertile parents, and this industry has grown from less than $1 billion in 1988 to more than $4 billion in 2012 (Newton-Small, 2012). Thus genetic improvement in the dairy industry and assisted reproduction in human medicine each provide an example of genetic resource utilization. The networking of cryopreserved sperm has proven invaluable to human society. Although cryopreservation of fish sperm started at the same time as dairy (Blaxter, 1953), large-scale application of cryopreserved germplasm of aquatic species is not currently utilized. In the past decades, substantial effort and resources have been applied to protocol development in different laboratories with a variety of species. Despite this, cryopreservation remains at a research level in aquatic species. However, there is recognition of the value of this technology. A survey among fish culturists indicated common interest in genetic improvement obtainable by adopting cryopreservation into existing procedures (Boever, 2006), such as among hybrid catfish hatcheries. The expanding demand for genetic improvement in fish culture can be addressed by cryopreservation, and recent development of high-throughput processing (Hu et al., 2011) provides the necessary technical prerequisites.

As the largest foodfish aquaculture industry in the United States (Harvey, 2006), the catfish industry has been challenged recently with global competition and increased costs of feed and fuel (USDA-NASS, 2011). A number of efforts, including hybrid production, are being implemented by catfish farmers to deal with those challenges. By fertilizing eggs of channel catfish (*Ictalurus punctatus*) with sperm from blue catfish (*Ictalurus furcatus*), hybrid catfish can be produced with improvements in growth rate, disease resistance and feed conversion (Dunham and Masser, 2012). Although hybrid catfish are currently in high demand by the industry, the production capacity for hybrids is

* Corresponding author at: 2410 Ben Hur Road, Baton Rouge, LA 70820, USA. Tel.: +1 225 765 2848; fax: +1 225 765 2877.
E-mail address: ttiersch@agcenter.lsu.edu (T.R. Tiersch).
[1] Current affiliation: Genetic Resources Management Consulting L.L.C., Baton Rouge, LA 70820, USA.

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constrained primarily due to the lack of natural hybridization between these species and the consequent need for artificial spawning that involves killing of the males for testis collection (Dunham and Masser, 2012). In addition, limited availability of blue catfish males can constrain hybrid production (Avery et al., 2005). The use of artificial spawning and incorporation of cryopreserved sperm collected from blue catfish in peak spawning condition enables fertilization of channel catfish eggs while they are also at peak spawning condition, overcoming biological limitations and maximizing hybrid production. Initial studies tested the feasibility of using a commercial dairy bull facility for cryopreservation of blue catfish sperm (Lang et al., 2003) and a specific high-throughput cryopreservation process has been established for this species and is ready for testing at a commercial level (Hu et al., 2011).

During the cryopreservation process, from before freezing to after thawing, sperm undergo biological, chemical, and physical stresses (Leibo, 2011). The frequency of damage to sperm is often estimated by the difference between initial motility and post-thaw motility (Rurangwa et al., 2004). The differences in motility between fresh and thawed sperm can affect the final ratio of motile sperm to eggs (Tiersch et al., 1994) which can in turn affect fertilization rates (Hu et al., 2011; Makeeva and Emel'yanova, 1993; Saksena et al., 1961; Small and Bates, 2001). The use of additional thawed sperm at fertilization to compensate for losses in motility has been used as a potential solution to reduced fertility (Tiersch et al., 1994). However, due to the cost and availability of blue catfish males, the efficient use of limited sperm must be carefully managed (Avery et al., 2005) to avoid unnecessary increased costs in fry production. In addition, commercial hatchery operators have expressed interest in the utility of increasing the volume of sperm to ensure maximal egg fertilization.

A protocol for high-throughput cryopreservation of blue catfish sperm was developed with the assistance of automated equipment (Hu et al., 2011). When the number of straws that could be produced reached a commercial level, quality assurance became the most critical aspect for blue catfish sperm cryopreservation (Hu, 2012). Laboratory-scale neurulation testing and hatchery-scale neurulation (fertilization) testing have shown that the cryopreserved blue catfish sperm had consistent quality that was minimally influenced by individual variation in males (Hu et al., 2013). However, evaluation is needed at the commercial production level to realistically demonstrate feasibility and efficiency for the use of cryopreserved blue catfish sperm in hybrid fry production.

With large-scale application of cryopreserved sperm, routine hatchery use and genetic improvement applications are feasible and the value of sperm is greatly increased. Proper handling and utilization of sperm also become essential to practice. Because thawed sperm had a larger variation in motility among individuals than did fresh sperm (Hu et al., 2011), and testing of the individual fertility of males is typically not feasible in commercial hatcheries due to time and space limitations, sperm samples from several blue catfish males are routinely pooled in commercial hatcheries (Avery et al., 2005). The effects of pooling sperm on subsequent fertilization are not known and could have important implications for commercial use of cryopreserved sperm. Although the influence of factors such as motility and concentration on fertility of sperm from individual males is becoming more studied (Rurangwa et al., 2004), it will be important to understand how the pooling of sperm samples can influence commercial applications. For example, will aggregate fertilization of the pool be characterized by a few outstanding individuals, and should sperm of similar quality be pooled together selectively? The overall goal of this study was to address high-throughput sperm cryopreservation of blue catfish at a commercial level. The objectives were to: 1) evaluate double-dosage of thawed sperm in hybrid fry production; 2) evaluate commercial application of high-throughput cryopreserved sperm with standard hatchery techniques, and 3) evaluate the fertility relationship between individuals and pooled samples.

2. Material and methods

2.1. Fish

Blue catfish males (D&B strain, originally from Crockett, TX) were obtained from Baxter Land Company Fish Farm (Arkansas City, Arkansas; 33°34′58.64″N, 91°15′18.45″W). The males were 4-to-6 yr old, and ranged from 61 to 97 cm, and 2.8 to 9.8 kg. Prior to transportation, males were selected based on observable secondary sexual characteristics indicative of maturity (e.g. well-muscled head and dark coloration) (Avery et al., 2005). A hauler with a high-pressure oxygen supply was used to transport fish from the farm to the Aquaculture Research Station of the Louisiana State University Agricultural Center (Baton Rouge; 30°22′07.32″N, 91°10′27.90″W) in February of 2010 and 2011. The males were placed in aerated outdoor 405-m^2 ponds and fed commercial broodstock diets (Aquaxcel, Cargill™, 45% protein) for 3 to 4 weeks until early May. Fish were collected by seining and moved into indoor tanks within a recirculating system 2 d before processing. The system used bubble-washed bead filters that were back-flushed every 2 d. The water quality parameters were: pH 7.0–8.0, total ammonia-nitrogen 0.1–0.8 mg/l, nitrite 0.04–0.30 mg/l, alkalinity 39–125 mg/l, hardness 44–126 mg/l, temperature 28 ± 1 °C, and dissolved oxygen 4.3–6.5 mg/l. Guidelines from the Institutional Animal Care and Use Committees (IACUC) of Louisiana State University were followed for animal care in this study.

2.2. Sperm collection

At the beginning of the channel catfish spawning season (April), blue catfish males were killed by a sharp blow to the head, and were rinsed with Hanks' balanced salt solution at an osmolality of 300 mOsmol/kg (HBSS300) to remove low osmolality fluids that could cause activation of sperm during dissection (Bates et al., 1996). The body weight and standard length were measured, after which the testes were removed by dissection. Tared weigh boats (catalog number: 02-203-501, Fisher Scientific) were used as containers for testes. HBSS300 was added to the weigh boat to prevent desiccation and sperm activation. The testes were blotted on a paper towel to remove blood and adherent tissues. The entire testis was weighed and the anterior portion of each testis was collected and weighed separately. Only the anterior portion (Sneed and Clemens, 1963) was used for sperm collection by crushing in HBSS300. The volume of HBSS300 (ml) used was two times the mass (g) of the crushed testis. The suspension was filtered through a mesh series consisting of a 7.62-cm round mesh strainer (1-mm mesh), a 15.24-cm round mesh strainer (0.5-mm mesh), and a 200-μm mesh filter to screen out tissues. Sperm suspensions were processed and labeled for each male.

2.3. Determination of sperm concentration

Fish sperm concentration has been found to be highly correlated with absorbance readings (Cuevas-Uribe and Tiersch, 2011; Dong et al., 2007; Tan et al., 2010) and a microspectrophotometer (NanoDrop 1000, Thermo Scientific, Wilmington, DE) was used to measure the absorbance of serially diluted sperm suspensions with 2-μl aliquots using a wavelength of 600 nm to estimate sperm concentration. The conversion equation between absorbance and concentration was:

$$\text{sperm concentration(cells/ml)} = \text{absorbance} \times 5.12 \times 10^8 - 4.07 \times 10^7 \left(R^2 = 0.960\right)$$

(Hu et al., 2011).