Cryopreservation: A Viable Tool for Sustainable Catfish Aquaculture Industry in Nigeria

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Abstract
Catfish aquaculture industry, a private-driven sub-sector with huge investment has today become a formidable economic venture in Nigeria. One major factor for this spectacular growth was the development of modern techniques for production of seed. However, the process of induced propagation of African catfish was still based on long standing technique of sacrificing male for fertilization with its attendant problems. Cryopreservation is now being explored globally to solve these challenges and promotes viable aquaculture production as obtained in livestock industry. But the level of adoption of this technology is relatively low in Nigeria hence the need for this awareness. A successful and sustainable cryopreservation method will require integrated practices for sample collection, refrigerated storage, freezing, thawing, rule for use and disposal, transfer agreements and database development. Some of these procedures have been standardized for catfish with room for improvement before commercial production commenced.

Keywords: Catfish industry; Sustainable; Sperm storage; Fish seed

Introduction
Fish is a nutritious food, containing proteins of high biochemical value to man. It undoubtedly occupies one of the highest places on the food list of Nigerians. Unfortunately, increase in Nigeria population coupled with ever rising awareness of the nutritional value of fish has scandalously depleted both the marine and freshwater fishery [1]. Hence, aquaculture remains a valuable mean of supplementing natural marine and freshwater productivity where maximum sustainable yield of many lakes and rivers have been attained. In Nigeria, the prominence of aquaculture as fish food source is growing with greater contributions from catfish farming, though the pace of development has been slower than expected. The unprecedented growth witnessed was made possible through the expansion of semi-intensive, small-scale pond aquaculture and development of techniques for hatchery production of seed.

Owing to this, Nigeria freshwater aquaculture has moved from experimental stage to a formidable industry dominated by catfish culture which constituted more than 80% of her annual aquaculture production [2]. Clarias gariepinus is a benthopelagic, dioecious, omnivorous fish widely tolerant to extreme environmental conditions. It is an indigenous fish to Nigeria with good nutritional value and adequate market demand. A 205 g African catfish contain 16.8 g protein, 5.7 g fat, 5.42 mg/kg-1 iron, 46.65 mg/kg-1 calcium and 360 mg phosphorus. African catfish may attain a weight of 20 kg in nature and 2 kg/year under culture [1]. It is a fast growing species introduced to culture in Nigeria since 1970 and has gained considerable acceptance in a number of African countries [3]. Among the cultivable fishes, African catfish has attracted more attention of ecologists and fishery managers because of its impressive growth rate and possibility of high stocking density [4]. Fish producers ranked African catfish as the most preferred species followed by Tilapia on the basis of market demand and growth rate [3].

The seed of African catfish is artificially produced in hatcheries along with others indigenous fish. More than 500 hatcheries (Government and Private) have been established to meet the demand of fry and at present 89 percent of total seed demand are produced in the hatcheries [2]. However, the quantity and quality of fish seeds produced remained a major concern. Fish seed demand far outstrips the supply [5], and this has been linked to scarcity of viable broodstock especially depletion of male broodstocks due to sacrificial method mostly used. Also, the seed quality has been deteriorating over the years due to inbreeding, hybridization and improper broodstock management. Therefore, it is becoming increasingly difficult to come across pure breed of African catfish. Cryopreservation is now being encouraged as a mean of conserving the male broodstock, curbing the collapse of pure breed of indigenous species and enhancing sustainable seed production.

Cryopreservation is a process in which a living cell is frozen, stored and thawed for fertilizing egg without losing its viability. Omitogun [6], had earlier noted that one way of expanding aquaculture in Nigeria is by devising a means of preserving genetic resources of our broodstock for all year round supply of fish seed through cryopreservation. Successful fry production using cryopreserved sperm has been reported for many economically important fish species [7,8]. It has been estimated that sperm from more than 200 fish species has been successfully cryopreserved. Unfortunately, the technology of cryopreservation has not been reasonably adopted in Nigeria aquaculture industry probably due to lack of awareness/technological challenges. This paper therefore, draws the attention of stakeholder in Nigeria aquaculture industry to the need for the adoption of cryopreservation as a tool for boosting catfish production.

What is Cryopreservation?
The term cryopreservation is used to describe the freezing and long-term storage of living cells, tissues and organs. Tiersch [9], define cryopreservation as a process where biological materials such as cells and tissues are preserved by cooling to very low temperatures, typically, -196°C (in case of liquid nitrogen), yet remain viable after exposure to temperatures above 0°C. Successful cryopreservation is well established for sperm from more than 200 fish species has been successfully cryopreserved. Unfortunately, the technology of cryopreservation has not been reasonably adopted in Nigeria aquaculture industry probably due to lack of awareness/technological challenges. This paper therefore, draws the attention of stakeholder in Nigeria aquaculture industry to the need for the adoption of cryopreservation as a tool for boosting catfish production.

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of sperm is a useful technique that can assist reproductive efforts by allowing spawning to take place whenever females are ready. It reduces the need for males, and can increase flexibility and genetic diversity in spawning protocols. Cryopreservation has proved its worth in the animal husbandry and milk production, and can also be a useful tool for quality seed production in hatchery.

The Need for Cryopreservation of Sperm

Fish farming has remained an important industry in several countries including Nigeria, where mass seed production through modern breeding techniques is imperative to meet the demand of ever growing investors. It is noteworthy that obtaining fish seed for pond stocking at the right time depend on correct and careful planning of broodstock management. Therefore, optimal broodstock husbandry management remains a key issue for reproduction control. One of the critical limiting factors to catfish seed production has been the seasonality in availability of viable broodstocks which make fingerlings scarce and thus expensive during dry season. Studies of hatchery operation have also suggested that loss of male broodstock is common during breeding operation. Omitogun [11], emphasized that a major catfish aquaculture constrain is the shortage of reliable males for breeding.

Cryopreservation, an economic tool for genetic resources allows for increase number of offspring from genetically superior males, more efficient transport of semen for later use, and all year round supply of male gametes [8]. The possibility of improve productivity in aquaculture by cryopreservation is very promising as it helps farmers to worry less about keeping male broodstocks. Cryopreservation of fish sperm provides a tool by which reproduction is optimized during larva production, thereby improving breeding and fish conservation programs. This technology is new to aquaculture industry in Nigeria but has long been adopted in livestock production.

The use of cryopreserved gamete in fish breeding is intrinsically desirable in African catfish aquaculture production. According to Tiersch [12], the benefits of cryopreservation in aquaculture include the following:

- Cryopreservation can be use to improve existing hatchery operations by providing sperm on demand and simplifying the timing of induced spawning.
- Frozen sperm can enhance efficient use of facilities and create new opportunities in the hatchery by eliminating the need to maintain live males, potentially freeing resources for use with females and larvae.
- Valuable genetic lineage such as endangered species, research models, or improved farm strains can be protected by storage of frozen sperm.
- Cryopreservation opens the door for rapid genetic improvement because it can be used in breeding programs to create improved lines and shape the genetic resources available for aquaculture.
- Cryopreserved sperm of aquatic species will at some point become an entirely new industry itself. Large, highly valuable global markets for cryopreserved sperm of aquatic species are on the horizon.

General Procedures of Cryopreservation in Freshwater Fishes

The potential applications of biotechnology in aquaculture include the establishment of cryopreservation technology in cultured fin fish. Practically, both short-term (in deep freezer at -35°C) and long-term cryopreservation (in liquid nitrogen, LN, at -296°C) was developed for catfish sperm [11]. A successful and sustainable technology will require integrated practices for sample collection, refrigerated storage, freezing, thawing, rule for use and disposal, transfer agreements and database development (Figure 1). This technique must start from selection of healthy and matured male broodstock, usually aged 7 months to 2 years [11] and body weight range of 600 g to 2 kg [13]. According to Chew and Zulkafli, the cryopreservation of the semen of freshwater fishes falls under the following general procedures.

- Sperm is collected by hand stripping, or by testis collection and homogenization. Immediately after collection, sperm is stored at 4°C in immobilizing medium. Most sperm can be stored for several days in this medium.
- For cryopreservation, one to 3 volumes of freezing extender such as Ringer’s solution, sugarcane water, coconut water, soybean milk, skim milk, taps, caps, Milk in ringer, Honey in ringer, etc. is added to sperm and the mix is loaded into 500 µL straws.
- Freezing is performed in liquid nitrogen vapors, using either a programmable freezer or a Styrofoam frame floating 3 cm above liquid nitrogen. Straws are then stored in liquid nitrogen tanks (-196°C).
- Thawing is performs in a water bath at 37°C for no more than

![Figure 1: Flow chart of Semen cryopreservation.](image-url)
10 sec. Thawed sperm should be immediately used for fertilization, without any washing or recovery step.

Conclusion

African catfish is an excellent food fish with adequate market demand. However, the quality and quantity of fish seeds produced for pond stocking remain a major concern for sustainable aquaculture. Therefore, cryopreservation is encouraged as a means of enhancing viable seed production. Technology for cryopreservation of semen could be developed among fish hatchery operators in Nigeria catfish aquaculture industry. The procedure includes selection of broodstock, sperm collection, refrigerated storage, freezing, thawing, rule for use and disposal, transfer agreements and database development.

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