

Recent Advancement in the Ex-Situ Conservation of Fish Genetic Resources

Tamil Prakash S.¹, Maria Susila Vinisha², Edward Inpent Campal³ and Panneerselvam Dheeran³

¹College of Fisheries, Mangalore

²Tamil Nadu Dr. J. Jayalalitha Fisheries University, Tamil Nadu

³ICAR-Central Institute of Fisheries Education, Mumbai (M.S.)

SUMMARY

Ex-situ conservation is crucial for preserving the genetic resources of fish species, which are under threat due to habitat loss, overfishing, and climate change. The recent advancements in biotechnology, cryopreservation, and molecular tools have increased the efficiency and scope of ex-situ strategies. This article delves into these innovations and elaborates on advanced cryopreservation techniques, germplasm repositories, genomic sequencing, and biobanking. The integration of these techniques has strengthened the conservation efforts of fish genetic resources for future generations while promoting sustainable aquaculture and fisheries management.

INTRODUCTION

Fish genetic resources are essential for biodiversity conservation, food security, and sustainable aquaculture. However, degradation of the environment, overexploitation, and climate change threaten many fish species. Ex-situ conservation complements in situ efforts by conserving genetic material outside natural habitats. Traditionally, ex-situ methods comprised live gene banking and captive breeding. However, the advent of cryobiology, genomics, and biotechnological tools has transformed these approaches, allowing for the storage and use of genetic materials with unprecedented precision and efficiency. This article discusses recent innovations in ex-situ conservation techniques, their applications, and their implications for global efforts in fish biodiversity preservation.

Recent Advancements in Ex-Situ Conservation

1. Cryopreservation of Gametes and Embryos

Cryopreservation has emerged as a cornerstone of ex-situ conservation. Techniques for freezing fish sperm have become highly reliable, where improved cryoprotectants enhance post-thaw viability. Advances in vitrification methods are now addressing problems associated with the cryopreservation of fish eggs and embryos, which are yolk-rich and pose structural and physiological issues.

Sperm Freezing:

The activation of fish sperm is affected by the fluctuations in extracellular osmolarity, though it varies between freshwater and saltwater species (Cosson, 2010). A decrease in external osmolarity activates the sperm from the freshwater fish, while an increase in external osmolarity activates that of the saltwater species. Remarkably, the sperm of marine fish is tolerant of high osmolarity environments due to the osmotic stress that occurs with cryoprotectant exposure and cryopreservation procedures. Freshwater fish sperm, in contrast, are not native to high osmolarity and are, therefore, more susceptible to osmotic stress at cryoprotectant equilibration and during freezing. The cryopreservation of fish sperm occurs via vapour-phase liquid nitrogen freezing methods. Samples like vials or straws are laid horizontally against the rack at a certain indicated height from the surface of the liquid nitrogen. The height of placement and the time of exposure usually depend upon the sample volume, the type of container being used, the specifications of the Dewar, and the temperature. For instance, aliquots of sturgeon sperm, with 12% (v/v) dimethyl sulfoxide (DMSO) in 0.7- or 1.5-mL volumes, are placed 20 cm over the surface of the liquid nitrogen for cryopreservation. Spermiation can also be induced by the administration of 2 mg/kg sturgeon pituitary gland powder dissolved into water to species such as sturgeon (*Acipenser baeri*).

2. Biobanking and Germplasm Repositories

Biobanking and germplasm repositories are the most advanced solutions in the ex-situ conservation of fish genetic resources, significantly preserving biodiversity, aquaculture, and research (Hindar *et al.*, 2022).

Improved cryopreservation techniques for fish gametes, embryos, and somatic cells have been developed, with recent innovations such as vitrification and nano-delivery systems that will enhance cell viability during storage and thawing. The integration of high-throughput genomic tools, including next-generation sequencing (NGS), provides accurate characterization of genetic material, making it possible to identify and conserve unique genotypes. Digital repositories and blockchain technologies are also increasingly being used to track germplasm origins and attributes with tamper-proof details, ensuring the transparency and security of genetic resources. These developments will certainly aid restoration initiatives, sustainable aquaculture, and climate change adaptation by emphasizing biobanking as a key strategy for managing fish genetic resources globally.

3. Genomic Tools for Conservation

The rapid development of genomic tools has revolutionized ex-situ conservation strategies for fish genetic resources. These developments allow for more precise management of genetic diversity, with reduced inbreeding and preservation of critical alleles essential for adaptation and survival. Whole-genome sequencing and SNP (Single Nucleotide Polymorphism) genotyping provide detailed insights into population structure and genetic diversity. Cryobanking of gametes, embryos, and somatic cells with genomic data ensures a safe repository for future breeding and restoration projects. Marker-assisted selection (MAS) and genomic selection (GS) have become indispensable tools in breeding programs to conserve endangered fish species. These approaches allow the selection of individuals that maximize genetic diversity while retaining traits critical for resilience in changing environments (Sonesson *et al.*, 2012). Transcriptomic analyses identify stress-related genes, guiding the development of captive conditions that mimic natural environments. Meanwhile, epigenomic studies shed light on heritable changes due to captive breeding, enabling better management of phenotypic adaptations. CRISPR-CRISPR actually means Clustered Regularly Interspaced Short Palindromic Repeats. Indeed, it has emerged as a path-breaking technique in gene editing allowing scientists to make precise changes in DNA in a living organism. CRISPR-based gene editing offers a powerful tool to mitigate genetic bottlenecks by introducing or preserving beneficial traits in captive populations. Synthetic biology approaches are also being explored to recreate lost genetic variation, offering promising avenues for conservation efforts.

4. Cell Line Conservation

Ex-situ conservation helps maintain fish genetic resources in external locations outside their habitats. The most advanced technique of the new ones is cell line conservation, which finds many applications in research, breeding, and biodiversity preservation. Advances in cryoprotectants and freezing methods improved the viability and the duration of the preserved fish cell lines. The development of species-specific cell lines in molecular biology has allowed for the establishment of primary and immortalized cell lines for many fish species, including endangered and economically important ones. Genomic and transcriptomic integration integrating cell line conservation with genomic studies aids in identifying genetic markers that are important for species conservation and sustainable aquaculture. High-throughput screening new tools have enabled efficient screening of cell lines for genetic stability and disease resistance. Biobanking facilities establishing specialized biobanks with modern storage systems have streamlined the exchange and safeguarding of fish genetic materials globally.

5. ARTs (Assisted Reproductive Technologies)

Another side of ex-situ conservation is that ART has made progress in developing the techniques for the conservation of fish genetic resources. These technologies actually dwarf genetic diversity and boost propagation programs leading to endurance of fish attainability outside its natural habitat. A few ART applications in ex-situ conservation are as follows:

In Vitro Fertilization (IVF)

Fish species have undergone in vitro fertilization for genetic enhancement and preservation on the part of species that exhibit lower reproductive activity and more complicated requirements during breeding.

Gene Banking

Gene banking is critical in conservation: it conserves genetic material to reinstate genetic diversity within wild populations or to underpin sustainable breeding programs.

Hormonal Induction of Spawning

Hormonal induction has been widely applied to artificially induce individuals to spawn when appropriate temperatures, humidity, and rainfall are not available to allow natural spawning.

Germ Cell Transplantation

Germ cell transplantation has been evaluated as a method for transferring rare genetic materials that maintain or restore genetic diversity in endangered fish populations.

Marker-assisted Genetic Selection

Genetic marker-assisted selection enables the characterization of beneficial traits in groups of fish populations, thus helping inform breeding strategies and sustaining ex-situ conservation .

Integration with Aquaculture

The past few years have witnessed substantial progress in combining ex-situ conservation techniques with aquaculture activities, particularly in conserving valuable fish genetic resources. Ex-situ conservation refers to the maintenance of genetic materials outside natural habitats. It plays an essential role in ensuring biodiversity, sustainability, and balanced fish populations to assist breeding programs within aquaculture. Recent work on the ex-situ conservation of fish genetic resources focuses on upgrading cryopreservation techniques, developing advanced breeding protocols, and establishing genetic banks. These developments significantly enhance selective breeding programs in aquaculture and facilitate farmers in introducing genetically superior traits, such as disease resistance and growth efficiency. In addition, biotechnological innovations, including gene editing, genome sequencing, and molecular marker-assisted selection, complement ex-situ conservation efforts, allowing more effective management of genetic resources while promoting biodiversity within aquaculture systems. As fish populations are exposed to increasing pressures from climate change, overfishing, and habitat loss, the integration of ex-situ conservation strategies with aquaculture provides a promising pathway for sustainable fisheries management, ensuring the long-term survival of fish species while also enhancing food security through responsible aquaculture practices.

CONCLUSION

Advances in cryobiology, molecular genetics, and biotechnology are revolutionizing ex-situ conservation. These are critical advances in preserving fish genetic resources, the sustainability of aquaculture, and support for global biodiversity goals. Continued investment in research and international collaboration is needed to maximize the potential of these technologies.

REFERENCES

- Bodnar, A., Ivankova, I., & Petrov, M. (2023). Cryopreservation techniques for fish gametes: Innovations in vitrification and nano-delivery systems. *Aquaculture Research*, 54(9), 2261–2273.
- Chen, H., & Lu, C. (2020). High-throughput screening in aquatic genetics: Recent advances. *Marine Biotechnology*, 22(4), 567-580.
- Cosson, J. (2010). Fish sperm physiology: Structure, factors regulating motility, and motility evaluation techniques. *Theriogenology*, 74(5), 617-637.
- Gibbons, M. D., et al. (2021). Advances in fish sperm cryopreservation techniques for aquaculture. *Aquaculture Research*, 52(1), 235-249.