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# Bio-banking: An Emerging Approach for Conservation of Fish Germplasm

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**Abstract**

Global biodiversity is declining with substantial losses of populations, species and habitats causing a significant impact on human life. Holistic and integrated approach for biodiversity conservation requires concrete and urgent action. Although there is increasing concern over the loss of species and degradation of habitat, still more innovative efforts are required. Biobanking holds immense promise to provide biomaterial for conservation of fish germplasm. Establishment of repositories along with gene banks could provide opportunities for collaboration among the leading experts in the fields of fish ecology, physiology, and cryobiology to synthesize effective ways for conservation of fish genetic resources, and discuss what needs to be done to increase the impact in the next century. The article reviews different causes for loss of fish biodiversity, the ways to overcome the situation and advocates cell line repository as an alternative aid to conserve fish germplasm.

**Keywords:** Biodiversity; Conservation; Cell line repository; Cryopreservation

## Introduction

Conservation of fish genetic resources requires a combination of sound research, careful coordination of efforts and intensive management of the resources. The collaborative research effort has improved the identification and taxonomic categorization of the world's fish species [1]. The WorldFish Center, comprised of eight international agencies, has engaged in an ambitious effort to catalog global fish populations. This institution has launched fish base, a web-based resource that provides detailed information on 28,000 fish species representing 96% of those known to science. Improving the effectiveness of conservation action requires a better understanding of the needs for such action across species, the extent to which it is being applied and the effects it has had in preventing species extinctions. Based on an analysis of commercial catch data from 64 large marine ecosystems (LMEs) spanning from 1950 to 2003, [2] Worm et al. [2] reported that an increasing proportion of fisheries were in a "collapsed" state. Of all the aquatic vertebrate taxa, fishes are experiencing the greatest decline in species numbers sending a clear, unequivocal signal that something is wrong, as their extinction rates rise to unprecedented levels. Concerns about biodiversity arise because present extinction rates are exceptionally high.

Aquatic ecosystems throughout the world are experiencing serious threats to both biodiversity and ecosystem stability and many conservation strategies have been developed to overcome the crisis. Fish cell lines are recognized as one of the essential live materials for the conservation of fish germplasm. Fish cell lines have increased tremendously in number covering a wide variety of species and tissues of origin such as the fin, kidney, muscle, liver, embryos etc, since the development of the first permanent fish cell line in 1962 from gonad tissue of rainbow trout [3]. So far, more than 283 fish cell lines from freshwater and marine fishes have been reported across the world [4]. During the last decade, there has been a consistent effort to develop new cell lines across the world and particularly in India more than 50 fish cell lines have been developed. These cell lines have been maintained and cryopreserved in a National Repository of Fish Cell Line. The cell line repository would serve as a biobank for the conservation of fish germplasm. The article reviews status and scope of biobanking in conserving fish germplasm.

### Factors affecting fish biodiversity

Threats to global fish biodiversity could be categorized under five headings: overexploitation; aquatic pollution; flow modification; destruction or degradation of habitat; and invasion by exotic species. Their combined and interacting influences have resulted in the declination of fish population and reduction of fish biodiversity worldwide. Irrational fishing practices, environmental aberrations in the form of a reduction in water volume, increased sedimentation water abstraction and pollution over the years make freshwater fish biodiversity a declining trend. The growing tendency of human population coupled with other biotic and abiotic factors is the root cause of the loss of biodiversity. Moyle and [5] listed five broad categories as responsible for reduced biodiversity of aquatic organisms, these being competition for water, habitat alteration, pollution, species introduction and commercial exploitation. [6] considered the biological changes that environmental degradation brings about and enumerated pollution, increased sedimentation, flow alteration and water diversion as the main causes. The number of threatened species is likely to increase rapidly in regions where human population growth rates are high. Protection of freshwater biodiversity is perhaps the ultimate conservation challenge because it is influenced by the

upstream drainage network, the surrounding land, the riparian zone, and in the case of migrating aquatic fauna downstream reaches.

Overfishing, pollution, climate changes are the major factors that affect marine fish biodiversity. In all oceans, a number of fish stocks targeted in fisheries have collapsed because they have been overfished or fished above their maximum sustainable levels. Fishing pressure is so intense in some marine systems that over much of the world the biomass of fish targeted in fisheries (including that of both the target species and those caught incidentally) has been reduced by 90% relative to levels prior to the onset of industrial fishing Millennium Ecosystem Assessment.

## Habitat loss and degradation

Habitat destruction and associated degradation and fragmentation are the greatest threats not only to terrestrial species but also to aquatic species. Habitat degradation caused by soil erosion, siltation and turbidity due to deforestation in the catchment areas results in the alteration of ecological conditions. The changes in the ecological condition become uncongenial for the survival of the endemic fish. Levels of habitat destruction are probably their greatest where human population density is among the highest and industrial activity widespread and long established. Habitat loss remains the primary cause of fish population declination. The fast-growing industries have brought a whole new range of impacts and a new scale of human activity. Most industrial fisheries are either fully or overexploited, and the impacts of overharvesting are coupled to destructive fishing techniques that destroy habitat, as well as associated ecosystems such as estuaries and wetlands.

## Pollution

Pollution of rivers and lakes by domestic wastes, sewage and industries effluents results in the deterioration of water quality which in turn declines fish population. It also affects reproductive capability of fish and sometimes even pollutants cause sex conversion. The visible outcome of pollution is stress and mass mortality of individuals. In cases of genotoxic pollutants, the effect can be more damaging but subtle. A number of fishes have been displaced or eliminated from their original habitats [7]. Toxic products, such as heavy metals and synthetic organic compounds, are most conveniently and cheaply dump into nearby rivers causing severe pollution. The industrialization of farming was made possible on the back of the easy availability of synthetic chemical fertilizers and pesticides, both of which have caused pollution of natural ecosystems through eutrophication and toxicity to non-target organisms respectively.

## Climate change

Changes in climate have significant impacts on biodiversity and ecosystems, including causing changes in species distributions, population sizes, the timing of reproduction or migration events, and an increase in the frequency of pest and disease outbreaks. By the end of the twenty-first century, climate change and its impacts may be the dominant direct driver of biodiversity loss and changes in ecosystem services globally (Millennium Ecosystem Assessment). Foremost among these is the effect of some atmospheric pollutants on climate. Most species are adapted to a relatively narrow range of environmental conditions. Their evolution is a result of the interaction between genes and environment. The species present today are here because they have been able to adapt to changing conditions in the past, but conditions in the future could change faster than ever before and many species will not be able to evolve sufficiently rapidly to persist.

## Over-exploitation and unsustainable use

Overexploitation is one of the most serious threats to many aquatic species and populations. Over-exploitation of the world's fish resources has caused serious decline in fish populations, and there is widespread concern that the world oceans will be unable to supply fish products for future generations. About 80 percent of the world marine fish stocks for which assessment information is available are fully exploited or overexploited. About three quarters (75%) of the world's commercial marine fisheries are either fully exploited (50%) or overexploited (25%). Big fish in the ocean have experienced a 55 percent decline in last 40 years. Half the world's fish are small, in the open seas and not exploitable and the fish that remain are fish humans aren't so interested in catching or eating Millennium Ecosystem Assessment, 2005.

## Introduction of invasive alien species

Ecologists, conservation biologists and managers widely believe that invasions by non-native species are a leading cause of recent species extinctions [8,9]. The introduction and spread of non-native species have become a global ecological and conservation crisis as invasive organisms are increasingly altering terrestrial and aquatic communities worldwide. The spread of invasive alien species and disease organisms has increased because of increased trade and travel, including tourism. Increased risk of biotic exchange is an inevitable effect of globalization (Millennium Ecosystem Assessment).

## Strategic approaches for conservation

Globally threatened species frequently require a combination of conservation responses to ensure their continued survival. These responses encompass research, species-specific actions, site and habitat based actions, policy responses and communication and education [10]. A majority of the threatened species require substantially greater effort to improve their status. While many species already receive some conservation attention, many others do not. Species can be saved and many already have been saved from extinction (IUCN/WCU). Cryobanking offers a tool to manage the genetics of small populations by storing genetic diversity while it is still high and extending the effective generation time through reintroduction of stored genes at a later date [11]. Additionally, cryobank could be a practical tool for exchanging genetic material between captive and wild populations, improving the efficiency of captive breeding, translocation, reintroduction, and population supplementation programs [12]. Gamete cryopreservation is a secure method for ex situ preservation of genetic diversity and genetically improved materials, thus providing opportunities to reconstruct the original genetic make-up, reestablish the improved nucleus population and establish genetic linkage among different generation and /or runs. Cryopreservation is a long-term storage technique to preserve the biological material without deterioration for an extended period of time at least several thousands of years. The ability to preserve and store both maternal and paternal gametes provides a reliable source of fish genetic material for scientific and aquaculture purposes as well as for conservation of biodiversity [13]. Genetic and taxonomic information serve as a backbone for the implementation of plans to protect and preserve threatened and endangered species. This information should be supported by comprehensive knowledge of the aquatic landscapes needed to protect

species within the natural habitat (in situ) and outside the natural habitat (ex situ).

## Tissue preservation

Many other organizations in the world like Integrated Digitized Bio collections (iDigBio) in the United States, maintain DNA banking facilities and genetic resources repositories that accomplish collections of DNA or RNA or preserved tissues suitable for genetic and genomic studies of biodiversity. Marine Environmental Specimen Bank (ESB) is designed to cryogenically bank environmental specimens as part of ongoing research and monitoring programs conducted in the marine and coastal environments including banked specimens like mussels, oysters, fish tissues, marine sediments, marine mammal tissues, bird eggs and feathers, and sea turtle tissues [14]. Many of the DNA barcoding project going on throughout the world insists on tissue collection along with voucher specimen for DNA extraction. Under a megaproject on DNA Barcoding Indian fishes, huge amount of tissue material from more than 250 marine fish and 117 freshwater fish species have been preserved in the tissue bank [15]. In general, tissues from fish species have been stored in alcohol or freezers for taxonomic, DNA Barcoding, genetic or phylogenetic analysis. However, it is widely accepted that the quality of DNA and other macromolecules (e.g. RNAs, proteins and lipids) are stored in -20$^0$C freezers declines over time and there is no guarantee they will be of sufficient quality for the aforementioned investigations or any of the research opportunities generated by the emergence of next generation sequencing and other high-throughput technologies [16]. Tissues and cells should be stored in a way that allows the broadest range of downstream uses for research and conservation purposes. In combination with artificial reproductive technology (ART), cryogenically stored tissues and cells can be used to rescue endangered animals. In the case of endangered species, tissue cryopreservation, freezing of gonadal tissue would allow for the rescue of reproductive material from deceased animals [17].

## Sperm preservation

Successful cryopreservation of fish sperm have been achieved for more than 200 fish species and many fish species have been adequated for the purpose of cryobanking [18-20]. Cryopreservation of sperm have been established for freshwater and marine fish species, including carp, salmonids, rainbow trout, catfish, cichlids, medakas, whitefish, pike, milkfish, grouper, cod, and zebrafish [21-27]. Species-specific sperm cryopreservation protocols have been developed for 14 species at NBFGR, Lucknow, India. The technique has been tested for 12 species through the production of progeny using cryopreserved sperm [28]. Cryopreservation of fish sperm is relatively common in the breeding and management of fish species, including salmonid, cyprinids, silurids, sturgeons [29-32].The creation of a cryopreserved sperm bank is an effective strategy for protecting the biodiversity of fish population and provides the opportunity to preserve the sperm samples of the most valuable males, which can be used in reproductive technologies in hatchery conditions. Sperm cryobanking could be a real alternative to breeding in captivity in order to preserve genetic diversity [33]. Sperm cryopreservation is a valuable conservation tool with applications in genome security as well as wider practical uses in minimizing the potential risk of disease transmission and removing animal welfare concerns associated with transporting live animals between breeding facilities [34]. A fertilization and hatching rate of 95% using the frozen-thawed sperm has been reported for the common carp and these results are not significantly different from fresh sperm [30]. Generally, high survival and fertilization capacity has been obtained in marine frozen-thawed spermatozoa when compared to freshwater species [35,36]. As the majority of fish display In Vitro Fertilization (IVF) is relatively simple to perform, involving the combination of gametes in an environment without the need for subsequent embryo transfer or implantation as required in mammals. Coupled with sperm cryopreservation, IVF techniques provide the means to increase genetic diversity among captive populations, exchange genes between captive and wild cohorts, as well as increase the gene pool within fragmented wild populations.

## Oocyte and embryo preservation

Early studies on ovarian tissue cryopreservation were performed in mouse [37], and has been proven to be effective on other species such as sheep [37,38], goat [39] and pig. Ovarian tissue cryopreservation can be a viable alternative to cryopreservation of oocytes or embryos [40-42]. Ovarian tissue cryopreservation has attracted much scientific and public attention due to its potential use in human infertility treatment, in safeguarding the reproductive potential of the endangered species and in genome banking of genetically important lab animal strains [43]. Cryopreservation of ovarian tissues is advantageous over the oocytes as they can be cultured and cryopreserved in small pieces which are rich in primary follicles. Cryopreservation of structurally intact tissues is more beneficial to cells, since it can retain all the tissues potential. The development of cryopreservation methods for oocyte and embryos in aquatic species is challenging. After many decades of research the successful cryopreservation of eggs, embryos and larvae has been documented in four bivalve species (pearl oysters, Sydney rock, Pacific) and one finfish species [44-50]. Cryopreservation of fish oocyte has been studied [51-55]. Immature oocytes can be an alternative for the mature eggs because of their smaller size [56]. However, there is no practical technique available to induce the small oocyte to mature in vitro. A technique to obtain the mature eggs from the late stage oocytes is available. Thus, the combination of this technique and their cryopreservation could be a breakthrough [13].

The studies also indicated that in cryopreserved ovarian tissues, the ovarian follicles remain in their natural three-dimensional structure where they may be protected from physical stress and damage. In these studies the percentage of viable follicles, stromal cells and vasculature was similar to the fresh tissues before freezing [57]. Although several studies have been undertaken on zebrafish oocyte cryopreservation at different stages [54,55,58] they resulted in compromised viability. Hence, cryopreservation of zebrafish ovarian tissue provides a promising alternative for zebrafish oocytes cryopreservation. Studies on cryopreservation of fish ovarian tissues would need to be accompanied by the development of in vitro culture method of these tissues as zebrafish ovarian tissue cryopreservation and in vitro culture method has not been studied systematically although some studies have been carried out by culturing of isolated zebrafish oocytes [59]. Cryopreservation of early stage zebra fish oocytes using the controlled slow freezing has been reported by Tsai et al. [13] Studies on the cryopreservation of invertebrate oocytes and eggs over the past several decades have been extraordinarily difficult to achieve [27,60,61]. However, it was found that intracellular crystallization occurred in the starfish oocytes at a relatively high temperature that was very close to the temperature of extracellular ice formation. In order to avoid this problem, [62] successfully cryopreserved starfish oocytes using a ultra-rapid freezing technique, called vitrification.

Cryopreservation of embryos has become an integral part of assisted reproduction. Successful cryopreservation of embryos is important because the biodiversity of both the paternal and maternal genomes will be preserved [13]. Factors limiting fish embryo cryopreservation include their multi compartmental biological systems, high chilling sensitivity, low membrane permeability and their large size, which gives a low surface area to volume ratio [63]. Although cryopreservation of the embryos has not been fully achieved, considerable progress has been made in understanding the conditions required for fish embryo cryopreservation and this would undoubtedly assist the successful protocol design in the future [13]. Cryopreservation of fish embryo is not viable, mainly because of the same limitations as in fish oocytes, i.e., high chilling sensitivity and low membrane permeability. Attempts to cryopreserve invertebrate larvae have been relatively more successful than the finfish. In penaeid prawns, successful survival of thawed larvae has been reported from a freezing temperature of $-40^0$C. Cryopreservation of isolated embryonic cells is another option for preserving both maternal and paternal genome.

## Cell culture preservation

Cryopreservation of biomaterials as cell cultures has emerged as an important resource for the conservation of aquatic biodiversity. With the development of cell culture techniques, many cell lines have been established from important commercial and aquaculture fish species and have been successfully cryopreserved for their long term conservation. Few embryonic cell lines (ES cells) of endangered species has become of immense value in aquatic biotechnologies which provide an important tool for protecting the endangered species.

Cryopreservation of blastomeres can maintain the genetic diversity of both, nuclear genome and mitochondrial DNA [64]. Blastomeres from the early embryos of fish still retain pluripotency [65] and their cryopreservation may be a promising approach to preserve the genotypes of zygotes and reconstitution of the organism. The studies demonstrated that germ-line cells from the cryopreserved blastomeres could develop into mature gametes of chimeric fish because the blastomeres were not damaged by cryopreservation [66-71]. An embryonic stem cell like cell culture system was developed from the inner cell mass of Labeo rohita. Cryopreservation of blastomeres has been successful in several fish species [72-75]. In recent years, more than 50 fish cell lines belong to 24 species were established in India and these well characterized cell lines have been maintained and cryopresreved in the National Repository of Fish Cell Line at National Bureau of Fish Genetic Resources, Lucknow [76].

Hofmann et al. [77] reported that although the loss of species is dire, 20% more fishes might have been lost if not for global conservation efforts. Cellbanking would provide additional resource material in the form of cell line to make the conservation programme more effective. Cell cryobanking has a hopeful future for conservation of fish germplasm. Short term and continuous cell cultures from a variety of fish species have been reported in the past and applied in virological, toxicological, and cytogenetic studies [78,79]. There has been a tremendous increase in the development of fish cell lines in the recent years from India [80-85]. This approach has enormous potential for conservation of germplasm of aquaculture and endangered fish species. This could be used to pinpoint the best opportunities for recovering cells, sperm and eggs to the biobank, and indeed the suitability of various hormone regimens to induce or manipulate reproductive processes. The researchers active in fish cell culture are sharing information reality but more importantly are genuinely embracing the conservation-effective approach of interacting with other biologists in complementary scientific disciplines, stakeholders, wildlife managers and key end-users to achieve conservation outcomes. Efforts must continue to include these in regional policy documents along with opportunities to train not just fellow researchers but other academic, governmental and NGO practitioners in the future [12]. The recent progress in cryopreservation of spermatogonia cells that can be obtained without sacrificing donor fish and the rapid advances made in IVF technology [12], the prospective collection of cryopreserved spermatozoa and using it to propagate a threatened fish species has now become a reality in fishes.

## Cell line repository

The establishment of national and international fish cell banks is one of the most active resources for resurrecting an almost lost species. Cell line repository provides essential biomaterial to the scientific community by establishing, verifying, maintaining, and distributing cells cultures and DNA derived from cell cultures. Much of the biomedical research on medicine, genetics, drug discovery, vaccine development, reconstructive medicine, basic science, HIV testing/treatment, and cell biology have been conducted using cultured cells obtained from major repositories such as American Type Culture Collection (ATCC) or from fellow researchers. An estimated 15–20% of the time, cells used in experiments have been misidentified or cross-contaminated with another cell line. ATCC, along with the Coriell Institute for Medical Research, European Collection of Cell Cultures (ECACC), Deutsche Sammlung von Mikrorganismen and Zellkulturen (DSMZ), and the Japanese Collection of Research Resources, all have received cell line submissions that, upon authentication, were determined to have been misidentified by the depositor. This poses a huge threat to the quality of publications and legitimacy of research findings produced from any of these cell cultures. For this reason, these repositories now authenticate cell line submissions and monitor cross-contamination.

## Importance of fish cell culture

In vitro culture systems offer several advantages as experimental tools. These systems allow cellular phenomenon to be studied in a controlled and completely defined environment, independent of the complexities and variability of systemic or larger physiological controls. Cellular behaviour, such as movement and experimental approaches like cellular imaging can only be studied conveniently in cultures.

Development of cell lines from various tissues of fish is desirable for developing cell models for in vitro study of their cellular physiology, molecular biology, genetics, immunology, endocrinology, nutrition, comparative biology and biotechnology [86]. The availability of fish cell lines, since the 1960s, has begun to make impacts in scientific research, but at a much slower rate than with mammalian cell lines. Early work with fish cell lines was initiated with RTG-2, a gonadal cell line derived from rainbow trout [3], mainly for virological studies. In the almost 50 yr since then, fish cell lines have grown in number covering a wide variety of species and tissues of origin and an array of applications. Fish immunology [87-90], toxicology [89,90], ecotoxicology [91-93] endocrinology [94], virology [3], biomedical research [79], disease control [95], biotechnology and aquaculture [88] and radiation biology [96] are some of the areas in which fish cell lines have made significant contributions. Many fish cell lines have been

derived from dissociated adult or embryonic tissues [97] but few have been characterized for tissue of origin with appropriate markers [94]. The model systems have been developed to demonstrate the utility of fish cells as sources of special adaptations and exaggerated physiological systems in epithelial ion transport, endocrinological studies, the cellular stress (heat shock) response, thermotolerance, cancer biology, and environmental toxicology (Hightower and Renfro 1988). Recently, fish have emerged as a suitable model and a promising alternative to the classical mammalian systems to study vertebrate development, in particular, skeletogenesis to complement in vivo developmental studies and identify signaling pathways involved in development processes, fish cell lines have been developed, in particular, bone-derived cells [98].

## Importance of cell line repository

The main objectives of the cell line repository are to receive, identify, maintain, store and supply cell lines to the researchers for R and D work. Repository facilitates collection and cataloguing of fish cell lines at one place. Repository provides characterized and quality-controlled cell lines without sparing time to develop as on required at a nominal cost. Cell lines can be made available to those who are unable to develop such lines themselves through cell banking. The cell line repository is instrumental for in vitro research in fish biotechnology and conservation of fish germplasm. The repositories serve as an "insurance" against the loss of cell lines within an individual laboratory.

Successful conservation of species requires integrated management efforts to sustain available genetic diversity (IUCN-World Conservation Union). These efforts include programs to protect and manage animal populations within their natural, native habitat (in situ conservation) as well as supporting programs that manage populations, individuals, gametes, and/or embryos outside of natural environments (ex situ conservation). IUCN-World Conservation Union recognizes that the efficiency and efficacy of intensive conservation efforts can be increased many folds by applying recent advances in reproductive technology. Germplasm banks offer a high degree of security against the loss of diversity. Ancillary conservation benefits include banks for basic and applied research including repositories of serum, DNA and cultured cell lines from germplasm donors that permit studies on disease status, geographical differentiation of populations and cellular physiology [99]. One of the most important criteria for conservation purposes is the establishment of primary culture from a variety of tissue sources that result in healthy, chromosomally stable and long lived cell lines. Cell culture facility at NBFGR has been instrumental in the development of many fish cell lines from different tissues of important fish species and all the developed cell lines are being maintained in NRFC.

## Status of cell line repository

Major cell line repositories including American Type Culture Collection (ATCC), European Collections of Cell Cultures (ECACC), German Collection of Microorganisms and Cell Cultures (DSMZ) have received cell line submissions from researchers across the world and authenticate all cell line submissions. To date, out of over 3,400 cell lines deposited at the American Type Culture Collection (ATCC), only 43 cell lines could be found that are of aquatic animals, and only 17 fish cell lines are usable and available for dissemination to the researchers globally.

The European Collection of Cell Cultures (ECACC) was established in 1984 as a cell culture collection to provide service to the research community and provide an International Depository Authority recognised patent depository for Europe. Over the last 25 years, ECACC has expanded and diversified to become one of the premier collections of authenticated cell cultures in the world and this remains the core of ECACC's business. The collections currently hold over 40,000 cell lines representing 45 different species, 50 tissue types, 300 HLA types, 450 monoclonal antibodies and at least 800 genetic disorders. ECACC has developed a comprehensive range of cell culture services and diversified into new product areas such as high quality genomic DNA extracted from cell lines.

The American Type Culture Collection (ATCC) is a private, nonprofit biological resource center whose mission focuses on the acquisition, authentication, production, preservation, development and distribution of standard reference microorganisms, cell lines and other materials for research in the life sciences. Established in 1914, the ATCC has developed into the global leader in research and development expertise for identifying, characterizing, preserving and distributing a wide range of cell lines and microbes. ATCC serves researchers internationally by characterizing cell lines, bacteria, viruses, fungi and protozoa, as well as developing and evaluating assays and techniques for validating research resources and preserving and distributing biological materials to the public and private sector research communities. ATCC's collections include a wide range of biological materials for research, including cell lines, molecular genomics tools, microorganisms and byproducts. The organization holds a collection of more than 4,000 human, animal and plant cell lines and an additional 1,200 hybridomas. The molecular genomics collection at ATCC contains 8 million cloned genes from a host of species, including human, mouse, soybean, rat, monkey, zebrafish and several disease vectors. ATCC's microorganism collection includes a collection of more than 18,000 strains of bacteria from 900 genera, as well as 2,000 different types of animal viruses and 1,000 plant viruses. In addition, ATCC maintains collections of protozoans, yeasts and fungi with over 49,000 yeast and fungi strains from 1,500 genera and 2,000 strains of protists.

The National Animal Cell Repository at National Centre for Cell Science, Pune, India provide services to receive, identify, maintain, store, cultivate and supply animal and human cell lines, hybridomas and intracellular obligate parasites, to establish new cell lines, to conduct postgraduate training programs, seminars, and to serve as a National Reference Centre for tissue culture and tissue banking. This Facility is a source for hundreds of cell cultures and different cell lines for several research institutions in the country. At present, the total number of culture strains is 1127, of which about 300 are available for distribution to users on registration. Approximately 510 researchers from 275 institutes have registered with NCCS for the same. The repository has initiated programmes to develop, immortalise and characterize cell lines from different tissue/tumor types.

The National Bureau of Fish Genetic Resources, Lucknow established a National Repository of Fish Cell Lines with the financial assistance from Department of Biotechnology, New Delhi during 2010 to receive, characterize for identification and authentication, maintain and distribute cell lines to interested stakeholders for research and development purpose. The facility also aims to provide support for training and education to stakeholders and to serve as a National Referral Centre of Indian and exotic fish cell lines.

The success story of cell line development in the country has raised the need to conserve the cell lines in one secured place. Considering the importance of conservation of fish germplasm for endangered Indian exotic fishes to maintain the fish biodiversity at National level recently, National Repository of Fish Cell lines (NRFC) has been established at National Bureau of Fish Genetic Resources, NBFGR, Lucknow, India with the financial assistance from Department of Biotechnology (DBT), Govt. of India, New Delhi. The cell lines available at NRFC are subjected to rigorous characterization and quality assurance procedures in order to guarantee their authenticity, purity and performance. At present, 50 fish cell lines from 24 different fish species are being maintained and cryopreserved in the NRFC. These cell lines were deposited by various research groups working on fish cell line including the researchers at NBFGR. The NRFC provides technical services viz. authentication (DNA barcoding, protein profiling, karyotyping), sterility testing mycoplasma, bacterial, fungal, yeast etc., cryo-storage and distribution of characterized cell line, dissemination of the fish cell culture technology through training and workshop, Web based information service for deposition of cell lines and request for cell lines.

## Challenges and Key Issues

There should be a collective effort to acquire fish genetic material for biobank which can contribute to conservation programs. These biobanks should actively receive 'deposits' in the form of tissue, cell and disperse material for captive management to support conservation and breeding programme. A significant challenge is to collect and maintain biomaterial and dispense them for conservation programme. The contributors to the biobank should have enough confidence in sharing the material without having any conflict of interest. Species prioritization is required so that targeted endangered species can be protected in the biobank. The preplanning aspect of the biobank should also include generating a wealth of knowledge on more basic integrative science focused on the species physiology and reproductive biology, which will be crucial to successful assisted breeding technologies using biobanked gametes [12]. The accomplishment of the Barcode of Life initiative iBOL and FishBOL provides excellent opportunities for the conservation community to utilize the resources for the biobank. The Specimen Central initiative for human cells, tissues DNA/RNA and other reagents should be used as model for computerizing herpetological museum specimen holdings, tracking and management transactions, and for mobilizing specimen occurrence data to the Internet [12] Kouba et al. 2013 outlined several overarching topic areas that will be the focus of future efforts designed to promote a wider acceptance of cell banking and associated biotechnologies as a conservation strategy for amphibians. Similar approach may be adopted for the conservation of fish germ plasm. Organization of training and capacity building through communication portals, websites, social media, symposia, and workshops would be of ideal effort to spread the concept and importance of biobanking in conserving fish germplasm. In this context, recently organized international conference and exhibition on tissue preservation and biobanking, July 20-22, 2015, Barcelona, Spain generated would be beneficial for popularizing and sensitizing researchers and industrialist about the biobanking approach for conservation of fish germplasm. Similar type of workshop was also organized on National workshop on Fish Cell Line: Development and Storage at National Bureau of Fish Genetic Resources, Lucknow for spreading the concept of biobanking fish cell lines. A well-coordinated efforts anticipated by participatory organizations supported by a meticulous plan would help to accelerate progress in utilizing biobanking approach for conserving fish germplasm. This would also facilitate in vitro research.

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