Development of a Sperm Cryopreservation Approach to the Fish Biodiversity Crisis in Bangladesh

Mostafa A. R. Hossain, Md. Nahiduzzaman and Terrence R. Tiersch

Introduction

Currently there are valid scientific descriptions and proper identifications for about 24,600 living species of fishes within 482 families and 57 orders (Nelson 1994). Among the six normally recognized freshwater zoogeographic regions (Helfman et al. 1997), the Oriental Region includes Bangladesh, India, Pakistan, Nepal, Bhutan, Sri Lanka, the other Southeast Asian countries, the Philippines and most of the Indonesia. Without doubt, freshwater biodiversity is facing crisis worldwide, but the severity of this threat in the Oriental Region is more intense than any in other geographical area (Dudgeon 2005).

Bangladesh has a unique position in the sub-tropical region, situated within the deltas of three great rivers - the Ganges, the Brahmaputra and the Meghna - covering a total area of 14.3 million ha. It has 290 rivers of which 54 are international, and numerous ponds, *beels* (relatively large waterbodies with static water in the Ganga-Brahmaputra flood plains of Bangladesh), *haors* (wetlands in the northeastern part of Bangladesh which are a bowl or saucer shaped shallow depressions), *baors* (an oxbow lake, found mostly in moribund deltas as in northeastern Bangladesh), lakes, flood plains, brackish, and marine water bodies. The country is rich in the diversity of fish species and is ranked third in aquatic biodiversity in Asia behind China and India, with approximately 300 freshwater and brackish fish species (Hussain and Mazid 2001). Asia represents about 40% of the world total of species of plants and animals and its aquatic environment is the most diverse in the world (Asian Development Bank 2000).

The vast wetlands, wide river-fed systems, abundant rainfall and warm temperatures play a significant role in the ecosystem diversity of Bangladesh. Enormous freshwater fisheries resources feed millions of people living in the Delta. Bangladesh produces about 2.7 million metric tons of fish per year and about 81% of the total production comes from inland resources of which 42% are from culture and 39% are from capture fisheries (DoF 2010). Increasing human population pressure requires accelerated fish production in the country, yet fish seedstock quality is deteriorating. Negative selection, inbreeding and interspecific hybridization in hatcheries have resulted in poor growth, performance and has become a serious constraint to increasing fish production in Bangladesh (Sarder et al. 2005).

The major threats to freshwater biodiversity in Bangladesh are overexploitation, water removal, pollution, massive destruction or degradation of fish habitat, and introduction and invasion of exotic species. Rapid extraction of fish seedstock (for aquaculture) as well as broodfish (for seed production and consumption) from natural waterbodies combined with destructive and unregulated fishing practices (e.g., use of destructive traps, pesticides, gillnets, and complete dewatering of waterbodies) has threatened a number of valuable native species. Loss of aquatic habitat due to siltation, anthropogenic activities such as dam construction (mainly for flood control, irrigation and drainage), and unregulated construction of polders (natural depressions enclosed by embankments), hydroelectric generation, and construction of road networks have been major causes of freshwater species loss. In addition, freshwater

Hossain, M. A. R., M. Nahiduzzaman, and T. R. Tiersch. 2011. Development of a Sperm Cryopreservation Approach to the Fish Biodiversity Crisis in Bangladesh. In: *Cryopreservation in Aquatic Species,* 2nd Edition. T. R. Tiersch and C. C. Green, editors. World Aquaculture Society, Baton Rouge, Louisiana. Pp. 852-861.

resources are subject to severe competition among multiple human stakeholders such as crop farming, aquaculture, and industrial usage. This article aims to describe the present status of freshwater fish biodiversity in Bangladesh and current and emerging conservation strategies including sperm cryopreservation for cultured and wild species.

Status of the Freshwater Fishery

Capture fisheries, which are second only to agriculture in the overall economy of Bangladesh, comprise nearly 5% of the gross domestic product (GDP), 23% of gross agricultural production and 6% of total export earnings. It accounts for about 6% of the total protein intake and about 60% of animal protein intake in the diet of the people of Bangladesh (DoF 2010). The fisheries sector provides full-time employment to an estimated 1.2 million fishermen, and an estimated 10 million households are partly dependent on fishing full or part-time for family subsistence in the floodplain. The total fish production according to the 2008-09 survey was 2.7 million metric tons (DoF 2010) (Table 1).

Table 1. Status of water areas and fish production in Bangladesh in 2008-09 (after DoF 2010).

Resource type	Water area (ha × 1,000)	Production (MT × 1,000)	Percent of total production
Inland Fisheries			
Capture			
1. Rivers and estuaries	853.9	138.2	
2. *Beel*	114.2	79.2	
3. Floodplain	2,832.8	879.5	
4. Kaptai lake	68.8	8.6	
5. Sundarbans	177.7	18.5	
Capture Total	4,047.3	1,124.0	41.6
Culture			
1. Ponds and ditches	305.0	912.2	
2. *Baors*	5.5	5.4	
3. Coastal shrimp farms	218.9	145.6	
Culture total	528.4	1,063.2	39.4
Inland total	4,575.7	2,186.7	81
Marine fisheries	-	514.6	19
Country total	-	2,701.3	100

However in recent years, due to several man-made and natural causes, aquatic biodiversity, especially diversity of fish and other non-fish aquatic organisms in open waters, has been declining sharply. During the 1940s and 1950s fish supplied about 95% of the animal protein requirement of the people in Bangladesh, which dropped to 80% in the 1980s (Karim and Ahsan 1989), and to 60% in recent years (DoF 2010). The number of listed freshwater fish species in Bangladesh is 260 comprising 158 Genera, 52 Families and 13 Orders (Table 2). Many of the listed species, however, are under severe threat and are rarely available in natural waters.

Recent studies have shown that the number of freshwater fishes has been declining at an alarming rate. There are 54 species of freshwater fishes are threatened in Bangladesh, and require immediate measures to protect and conserve them (IUCN-Bangladesh 2000) (Table 3). Among the threatened species, 14 are categorized as vulnerable, 28 as endangered, and 12 as critically endangered; another 66 species are classified as data deficient, and only146 as not threatened. Based on the Red Book of the World Conservation Union (IUCN) and reports from different countries, 56 cyprinid species are listed as threatened in 14 Asian countries. This list is not comprehensive and hence there is the likelihood that far more fish species than those reported are either extinct or threatened (Froese and Torres 1999).

Table 2. Freshwater fish species of Bangladesh grouped in 13 Orders.

Order	Family	Genus	Species
Anguiliformes	5	6	7
Osteoglossiformes	1	2	2
Elopiformes	1	1	1
Clupeiformes	3	12	16
Cypriniformes	4	36	86
Siluriformes	13	36	62
Cyprinodontiformes	1	1	1
Syngnathiformes	1	3	3
Synbranchiformes	2	4	6
Perciformes	14	46	59
Beloniformes	3	5	7
Pleuronectiformes	3	4	7
Tetraodontiformes	1	2	3
Total	52	158	260

Conservation Strategy

As more fish species become threatened or endangered, there is tremendous need to preserve disappearing genetic material as well as to conserve the existing gene pools. The ideal strategy for conservation of threatened and endangered fish species is through *in-situ* protection (i.e., habitat restoration) of the native habitat. Unfortunately, most habitat damages are irrevocable and where remediation is possible it is costly and requires a great deal of time, as the restoration process is slow. One alternative is to maintain *ex-situ* conservation (outside the natural environment) as live populations or in a cryopreserved sperm bank (Pullin et al. 1991). The different initiatives taken at government and non-government levels towards *in-situ* and *ex-situ* conservation of freshwaters fishes are highlighted below.

In-situ Conservation

The inland open water fishery resources of Bangladesh are among the richest in the world. They have contributed more than 90% of the country's fish production in the past. However over the last four decades these resources have experienced, as stated above, significant declines due to man-made and natural causes such as overfishing, destructive fishing, loss and destruction of habitats, poor policy, and a lack of planning and management. Fish stocks, particularly, broodstocks have been depleted below replaceable levels. As a result, both fish biodiversity and overall production have been severely affected. The government has taken different measures for protection, conservation, and management of fisheries resources for sustainable production. The National Water Policy has recently emphasized reserving wetlands for fish in a reversal of past trends. Of the other measures taken, fish sanctuaries have been considered as an important tool for protection and conservation with a policy shift towards community participation. The restoration of 'fish sanctuaries' (the deeper parts of the floodplains and river channels where fish survive during lean periods, and where they grow and attain maturity for spawning in the next monsoon) is particularly important. Fish sanctuaries provide shelter and dry-season refuge, provide protection from predators and fishermen, act as feeding grounds, and serve as breeding and nursery grounds.

Table 3. Threatened fish species of Bangladesh (after IUCN-Bangladesh 2000).

English name	Scientific name	Status	English name	Scientific name	Status
Grey featherback	*Notopterus notopterus* *	VU	Reticulate loach	*Botia lohachata*	EN
Bengal eel	*Anguilla bengalensis*	VU	Giant river catfish	*Aorichthys seenghala*	EN
Reba	*Labeo ariza*	VU	Stripped catfish	*Batasio tengara*	EN
Ticto barb	*Puntius ticto*	VU	Butter catfish	*Ompok bimaculatus* *	EN
River catfish	*Aorichthys aor*	VU	Pabda catfish	*Ompok pabda* *	EN
Day's mystus	*Mystus cavasius*	VU	Indian butter catfish	*Ompok pabo* *	EN
Gangetic ailia	*Ailia punctata*	VU	Silond catfish	*Silonia silondia*	EN
Grey eel catfish	*Plotosus canius*	VU	Squarhead catfish	*Chaca chaca*	EN
Gangetic mud eel	*Monopterus cuchia*	VU	Wrestling halfbeak	*Dermogenys pusillus*	EN
Glassy prchlet	*Chanda nama*	VU	Deocata pipe fish	*Microphis deokata*	EN
Indian glassy fish	*Pseudambassis ranga*	VU	Spotted scat	*Scatophagus argus*	EN
Gangetic leaffish	*Nandus nandus*	VU	Mud pearch	*Badis badis*	EN
Snakehead	*Channa orientalis*	VU	Frail gourami	*Ctenops nobilis*	EN
Spiny eel	*Macrognathus aculeatus*	VU	Giant snakehead	*Channa marulius*	EN
Featherback	*Chitala chitala**	EN	Zig-zag eel	*Mastacembalus armatus*	EN
Carplet	*Barilius bendelisis*	EN	Boga labeo	*Labeo boga* *	CR
Carplet	*Barilius vagra*	EN	Nandil	*Labeo nandina* *	CR
Bengala barb	*Bengala elanga*	EN	Baitka	*Labeo pangusia* *	CR
Glass barb	*Chela laubuca*	EN	Olive barb	*Puntius sarana* *	CR
Minor carp	*Crossocheilus latius*	EN	Tor masheer	*Tor tor* *	CR
Minor carp	*Labeo bata* *	EN	Rita catfish	*Rita rita*	CR
Black carp	*Labeo calbasu* *	EN	Schilbeid catfish	*Clupisoma garua*	CR
Kuria labio	*Labeo gonius* *	EN	Schilbeid catfish	*Eutropiichthys vacha*	CR
Cotio	*Osteobrama cotio*	EN	Yellow tail catfish	*Pangasius pangasius* *	CR
Trout barb	*Raimas bola*	EN	Gangetic goonch	*Bagarius yarrellii*	CR
Rasbora	*Rasbora rasbora*	EN	Sisor	*Sisor rhabdophorus*	CR
Nectic loach	*Botia dario*	EN	Barca snakehead	*Channa barca*	CR

*Commercial or aquaculture interest; VU, Vulnerable (high risk of extinction in the medium-term); EN, Endangered (very high risk of extinction in the near future); CR, Critically endangered (extremely high risk of extinction in the immediate future).

The Bangladesh government has established a number of fish sanctuaries under different development projects since 1980, with the most intensive efforts in the last decade, and is planning for more fish sanctuaries in the country (Table 4). However, in many cases sanctuaries are not sustainable and there have been little or no monitoring efforts to properly assess management performance, problems, and constraints to ensure sustainability. In addition, Bangladesh has established several training and research institutes working at fish biodiversity and conservation in addition to the scientists and teachers working in more than ten universities and non-governmental organizations. Among other major activities, establishment of fish passages and fish-friendly structures (FFS), stock enhancement in floodplains, and creation of social awareness are ongoing through public and private initiatives.

Figure 1. A typical fish sanctuary constructed with bamboo poles and water hyacinth in a river. Additional habitat is created at the bottom with bushy tree branches.

Table 4. Fish sanctuaries established in Bangladesh under different projects through 2007.

	Fish Sanctuaries			
Project name	**Number**	**Water area (ha)**	**Area (ha)**	**Beneficiary***
Fourth Fisheries Projects (FFP)	75	39,040	1,361	59,712
Community Based Fisheries Management-2 (CBFM-2)	164	9,359	85	14,618
Patuakhali Barguna Aquaculture Extension Project (PBAEP)	18	545	26	2,118
International Fund for Agricultural Development (IFAD)	18	525	10	1,477
Fisheries Resource Development Project (FRDP)	15	1,434	-	-
Management of Aquatic ecosystem through Community Husbandry (MACH) project	56	-	277	-
Hilsa Sanctuary	4	-	-	-
Joboi beel Project	4	-	-	-
Fish Habitat Restoration Project (FHRP)	3	642	4.50	
Fisheries Development and Management Programme (FDMP)	20	738	-	2,335
Total	377	52,283+	1,764+	80,260+

*The professional and part-time fishers who receive benefits from the sanctuary. This includes catches for subsistence and family consumption from the site of the sanctuary.

Ex-situ Conservation by Sperm Cryopreservation

Recently there has been expanded development of cryogenic sperm banks for fish in Europe and North America. These sperm banks are more cost effective than maintaining live gene banks which require dedicated facilities, labor and high costs. Cryogenic gene banking avoids the risk of genetic contamination and requires little space and minimal facilities. The sperm cryopreservation protocols for different fish species seem variable and species-specific. Although fish are the main protein source in Bangladesh and other countries in the sub-continent, and the fish biodiversity and production from open water are declining, little attention has been paid to cryopreservation of fish sperm. In India, protocols have been developed with varying success only for a few aquacultured and endangered species (Chao et al. 1992, Rana 1995, Ponniah 1998). The trials have mainly concentrated on development of extenders, activation media, dilution rates, activation periods, and sperm-to-egg ratios among species.

In Bangladesh, research on fish sperm cryopreservation was started in early 2004. Like Indian scientists, research in Bangladesh concentrated on basic conditions such as suitable combinations of extenders and cryoprotectants, optimal dilution ratios (milt:diluent), optimal cryoprotectant concentrations, and assessing the fertilization and hatching success for cryopreserved sperm. The studies have focused on aquacultured or commercial species and so far none of the threatened species have been considered (Table 5). The results of the trials are variable. The extenders used for different species are egg-yolk citrate, urea-egg yolk, 0.8% NaCl, 0.9% NaCl, Mounib A and B solutions, modified Kurokura solution, Kurokura-1, Kurokura-2, Alsever’s solution, modified fish Ringer, 0.6% NaCl, 0.3 M glucose solution, 0.3 M fructose solution, and 0.3 M and 0.6 M sucrose solutions among others. Cryoprotectants such as dimethyl sulfoxide (DMSO), glycerol, ethanol, methanol, ethylene glycol, and dimethylamine (DMA) have been combined with the extenders. Egg-yolk citrate and DMSO were the most used extenders and cryoprotectants. Different combinations and compositions of extenders have been used to dilute sperm in different ratios to increase motility or viability after thawing. Unfortunately, osmolality and pH of the seminal plasma and medium were not reported in any of these experiments.

A number of cooling protocols have been used for fish sperm cryopreservation (Leung and Jamieson 1991). In Bangladesh, however, in almost all trials, a two-step freezing protocol has been used from ambient temperature (25 C) to -4 °C at a rate of 4 °C per min, and then from -4 °C to -80 °C at a rate of 10 °C per min. One-step freezing was applied only in tilapia, Indian major carp (*L. calbasu*) and olive barb from 20 °C to -80 °C at a rate of 10 °C per min.

Although breeding success could not be adequately addressed in all of these studies, cryogenic protocols have been developed for nine aquacultured species in Bangladesh. The fertilization of eggs with the cryopreserved sperm was successful and the hatching rate varied between 0% and 90%. The fish produced from the eggs fertilized by cryopreserved sperm performed well and there was no significant deviation in growth when compared with fish produced from eggs fertilized by fresh sperm (Sarder et al. 2005).

Freshwater biodiversity is under threat all over the world, however, the intensity of threat in Bangladesh, is exceptional. In addition, commercial and subsistence fish farmers and hatchery owners have long been complaining about inbreeding problems such as retarded growth rate, poor reproductive performance, morphological deformities, increased incidence of disease, and mortality of hatchery-produced seed (Hussain and Mazid 1999). The extensive stocking in natural water bodies of fish seed of genetically poor quality is also a major concern.

Table 5. Cryopreservation research summary of some fish species in Bangladesh.

Species	Best combination*	Maximum (percent) Post-thaw motility	Fertilization	Hatching	Reference
Indian Major carp *Catla catla*	2	83	--	--	Sarder 2004
	3	83			Islam 2004
	1	82			
Indian major carp *Cirrhinus cirrhosus*	1	83	--	--	Sarder 2004
	2	79			Sultana 2004
Indian major carp *Labeo rohita*	1	85	90	90	Rahman 2006
	4	83			Rafiquzzaman 2004
	2	81			
Silver carp	1	75	--	--	Ahmed 2004
Hypophthalmichthys molitrix	6	70			Khan, 2004
	7	60			Hossain 2005
Bighead carp	1	75	40	10	Khan 2004
Aristicthys nobilis	6	70			
	7	60			
Common carp	8	86	--	15	Salam 2005
Cyprinus carpio	9	82			
Silver barb *Barbonymus gonionotus*	1	70	--	27	Hasan-ud-doulha 2004
	2	78			Shafin 2006
	10	74			
Nile tilapia	1	80	20	20	Rafiquzzaman 2004
Oreochromis niloticus	5	70			
Indian major carp *Labeo calbasu*	4	74	60	15	Ongoing
	11	68			
Olive barb	4	69	70	30	Ongoing
Puntius sarana	9	66			
	12	63			
Butter catfish *Ompok bimaculatus*					Ongoing
Zig-zag eel *Mastacembelus armatus*					Ongoing
Striped snakehead *Channa striatus*					Ongoing
River catfish *Rita rita*					Ongoing

*Best combinations in terms of post-thaw motility reported for different species. 1: Egg-yolk citrate with DMSO; 2: Urea-egg yolk with DMSO; 3: Egg-yolk citrate with methanol; 4: Alsever's solution with DMSO; 5: Egg-yolk citrate with methanol; 6: Egg-yolk citrate with DMSO and glycerol; 7: Urea-egg yolk with Glycerol; 8: Alsever's solution with ethanol; 9: Alsever's solution with methanol, and 10: Modified Alsever's solution with methanol, 11. 0.9% NaCl with DMSO, and 12. 0.9% NaCl with glycerol.

Fish sperm cryopreservation assists conservation of fish biodiversity through gene banking of endangered species, and assists aquaculture by providing flexibility in spawning of females and selective breeding through synchronizing artificial reproduction, efficient utilization of semen, and maintaining genetic variability of broodstocks (Tiersch 2000, Lahnsteiner 2004). The technique also ensures preservation of genetic materials of superior wild fish populations and enables gene transfer from wild and hatchery stocks (Cloud et al. 1990, Tiersch et al. 1998).

Conclusions

Genetic stock conservation for wild and domesticated fishes is very important, as the genetic diversity of species develops through non-recurring evolutionary events over millions of years. It is vital in improving the standard of living through sustainable utilization of fish genetic resources in a populous country like Bangladesh. Quality seed production largely depends on the availability of superior genetic material. Therefore cryopreservation techniques can potentially help to preserve and supply quality germplasm from genetically superior broodstocks. Thus, cryogenic techniques can assist in the conservation of biodiversity, to bring back threatened species to natural environment with restocking programs, as well as in improving aquaculture production. Cryogenic sperm banks for fish need to be established as means of germplasm conservation in Bangladesh. The country has a well-established network for the delivery of cattle and goat semen at governmental and non-governmental levels. Through the Department of Livestock (DoL), the government provides frozen cattle semen throughout the country. The largest non-government organization (NGO) of Bangladesh - Building Resources Across Communities (BRAC) presently produces 500,000 doses (straws) per annum and operates through 89 semen stations and 1,279 technicians nationwide to supply cattle and goat semen. If the protocols were properly developed and public and private entrepreneurs can be made aware about the long-term prospects, cryopreserved fish sperm could be integrated with the existing network for country-wide delivery and distribution (Lang et al. 2003).

It is urgent to care for the biodiversity of these beautiful and valuable fishes – the pride, heritage, and livelihood of Bangladesh before they are lost forever. Researchers (in Bangladesh and overseas), policy makers, governmental and NGOs and national and international donors should come forward to conserve the fish species using *in situ* and *ex situ* techniques.

Acknowledgments

Our research on sperm cryopreservation of Bangladeshi fish is supported by an ongoing project funded by United States Department of Agriculture (USDA). This manuscript has been approved for publication by the Director of the Louisiana Agricultural Experiment Station as number 2010-244-5261.

References

Asian Development Bank. 2000. Sustainable development in Asia. Retrieved July 14, 2008, http://www.adb.org/documents/books/sustainable_dev/chap_1.pdf.

Ahmed, A. U. 2004. Effects of diluents and dilution rates on cryopreservation of silver carp (*Hypophthalmichthys molitrix*) spermatozoa. M.S Thesis. Department of Fish. Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Chao, N. H., H. P. Tsai and I.C. Liao. 1992. Short-term and long-term cryopreservation of sperm and sperm suspension of the grouper, *Epinephelus malabaricus* (Bloch and Schneider). Asian Fisheries Science 5(1):103-116.

Cloud, J. G., W. H. Miller and M. J. Levanduski. 1990. Cryopreservation of sperm as a means to store salmonid germ plasm and to transfer genes from wild fish to hatchery populations. The Progressive Fish-Culturist 52:51-53.

DoF (Department of Fisheries). 2010. National Fisheries Week Compendium 2010 Department of Fisheries, Ministry of Fisheries and Livestock, The Peoples Republic of Bangladesh, 120 pp.

Dudgeon, D. 2005. River management for conservation of freshwater biodiversity in monsoonal Asia. Ecology and Society 10(2):15 Retrieved July 14, 2008, www.ecologyandsociety.org/vol10/iss2/art15/.

Froese, R. and A. Torres. 1999. Fishes under threat: an analysis of the fishes in the 1996 IUCN Red List. *In:* Towards Policies for Conservation and Sustainable Use of Aquatic Genetic Resources. ICLARM Conference Proceedings 59. R. S. V. Pullin, D. M. Bartley and J. Kooiman, editors. Pp. 131-144.

Hasan-ud-doula, M. 2004. Effects of diluents and dilution ratios on the cryopreservation of thai sharpunti (*Barbodes gonionotus*) spermatozoa. M.S. Thesis. Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh.

Helfman, G. S., B. B. Collette and D. E. Facey. 1997. The Diversity of Fishes. Blackwell Science, Inc., Malden, Massachusetts, USA.

Hossain, M. A. 2005. Effects of selected cryodiluents on the motility and fertility of silver carp (*Hypophthalmichthys molitrix*) spermatozoa. M.S. Thesis. Department of Fisheries Biology and Genetics. Bangladesh Agricultural University, Mymensingh, Bangladesh.

Hussain M. G. and Mazid M. A. 1999. Broodstock management status and some suggestions to control negative selection and inbreeding in hatchery stocks in Bangladesh. Naga - The ICLARM Quarterly 22 (4): 24-27.

Hussain, M. G. and M. A. Mazid. 2001. Genetic improvement and conservation of carp species in Bangladesh. Bangladesh Fisheries Research Institute and International center for Living Aquatic Resources Management, 73 pp.

Islam, M. S. 2004. Cryopreservation of catla (*Catla catla*) spermatozoa for conserve gene pool and facilitating artificial insemination technique. M.S. Thesis. Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh.

IUCN-Bangladesh, 2000. Red Book of Threatened Fishes of Bangladesh. IUCN-The World Conservation Union xii,116 pp.

Karim, M. and A. K. M. Ahsan. 1989. Policy Recommendation on Fisheries Development in Bangladesh. Ministry of Fisheries and Livestock, Government of Bangladesh, Dhaka.

Khan, M. G. Q. 2004. Cryogenic freezing of silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristicthys nobilis*) spermatozoa for gene resource conservation. M.S. Thesis. Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Lahnsteiner F. 2004. Cryopreservation of semen of the Salmonidae with special reference to large-scale fertilization. Methods in Molecular Biology 253:1-12.

Lang, P. R., K. L. Riley, J. E. Chandler and T. R. Tiersch. 2003. The use of dairy protocols for sperm cryopreservation of blue catfish (*Ictalurus furcatus*). Journal of the World Aquaculture Society 34(1): 66-75.

Leung, L. K. P. and B. G. H. Jamieson. 1991. Live preservation of fish gametes. *In:* Fish Evolution and Systematics: Evidence From Spermatozoa. B. G. M. Jamieson, editor, Cambridge University Press, Cambridge, England. Pp 245-269.

Nelson, J.S. 1994. Fishes of the World. 3rd Edition. John Wiley and Sons, Inc. New York, 620 pp.

Ponniah, A. G. 1998. An integrated approach to gene banking of India's fish germplasm resources. *In:* Action Before Extinction, Proceedings of an International Conference on Conservation of Fish Genetic Diversity. B. Harvey, editor. World FisheriesTrust, Victoria, British Columbia, Canada. Pp. 129-140.

Pullin, R. S. V., A. E. Eknath, T. Gjedrem, M. Tayamen, M. Julie and A. Tereso. 1991. The genetic improvement of farmed tilapias (GIFT) Project. Naga, the ICLARM Quarterly 14(2):210-315.

Rafiquzzaman, S. M. 2004. Cryopreservation of *Labeo rohita* and *Oreochromis niloticus* spermatozoa for quality seed production. M.S. Thesis. Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Rahman, A. K. M. A. 2007. Cryopreservation of spermatozoa of *Labeo rohita* (Hamilton) and its effects on fertilization and hatching. M.S. Thesis. Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Rana, K. 1995. Preservation of gametes. *In:* Broodstock Management and Egg and Larval Quality. N. R. Bromage and R. J. Roberts, editors. Blackwell Science Ltd., Oxford. Pp 53-75.

Salam, M. A. 2005. Cryopreservation of common carp (*Cyprinus carpio* L.) spermatozoa. M.S. Thesis. Department of Fisheries Biology and Genetics. Bangladesh Agricultural University, Mymensingh, Bangladesh.

Sarder, M. R. I., M. A. Salam, S. M. Shafin, M. G. Hussain and M. S. Islam. 2005. Cryopreservation of sperm of common carp, *Cyprinus carpio* and silver barb, *Barbonymus gonionotus* for genetically improved seed production. Bangladesh Journal of Fisheries Research, 9 (1):1-3.

Sarder, M., R., I. 2004. Application of cryopreservation in the Indian major carps (*Catla catla*, *Labeo rohita*, *Cirrhinus cirrhosus*) and other culture species for their quality seed production. SUFER, DFID final Report, May 2004.

Shafin, M. S. 2006. Cryopreservation of spermatozoa of silver barb, *Barbonymus gonionotus* (Bleeker). M.S. Thesis. Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Sultana, R. 2004. Cryopreservation of mrigal (*Cirrhinus cirrhosus*) spermatozoa with special emphasis on cryodiluent composition, cryoprotectant concentration and sperm: cryodiluent ratio. M.S. Thesis. Department of Fisheries Biology and Genetics. Bangladesh Agricultural University, Mymensingh, Bangladesh.

Tiersch T. R., C. R. Figiel, W. R. Wayman, J. H. Williamson, G. J. Carmichael and O. T. Gorman. 1998. Cryopreservation of sperm of the endangered razorback sucker. Transactions of the American Fisheries Society 127:95-104.

Tiersch, T. R. 2000. Introduction. *In:* Cryopreservation in Aquatic species. T. R. Tiersch and P. M. Mazik, editors. World Aquaculture Society, Baton Rouge, Louisiana, USA. Pp xix-xxvi.