Introduction to the Second Edition

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Aquatic species cryopreservation has grown substantially in the past decade since publication of the first edition of this book. Cryopreservation has progressed from its original status as something of a research diversion or oddity, to a quirky unexploited technology with unrealized promise, to its current status as a viable strategy available for incorporation into broader programmatic frameworks as a supporting technology, with the potential to launch commercial development into new areas such as improvement, maintenance, and distribution of genetic resources. Despite this vigorous growth and progress, problems still remain before repositories based on cryopreservation can begin to provide more than basic utility, typically in self-contained programs. Successful entities and activities need to develop interconnections; projects must combine into programs which can coalesce into systems capable of crossing from research to large-scale application and cooperation across commodities and countries. As such, a roadmap is needed to identify routes available for future application of this technology.

In addition, scientific study in this area, although burgeoning, requires a blueprint to lay out plans for unification and codification as a true research field. "Those attempting to utilize the available literature on cryopreservation in aquatic species will encounter a number of problems. The literature is distributed across numerous journals and disciplines, and there is a lack of standardization in terms, protocols and reporting of results. Those new to the field are often confronted with successful protocols that cannot be repeated, unsuccessful experiments that cannot be interpreted, and contradictory findings even within a single species." Such was the assessment of the state of the scientific literature for aquatic species cryopreservation in the first edition of this volume in 2000. With the rapid expansion of this literature in the past decade (see below), this situation could be considered to be even worse today.

Moreover, cryobiology remains a developing science with a rudimentary theoretical framework, and cryopreservation research is often empirical with advances made by trial and error. It should also be noted that the term "fish" is an artificial collective of more than 25,000 species characterized more by differences than by similarities. To discuss cryopreservation within fish or aquatic species is thus a balancing act of attempting to generalize observations into basic principles while recognizing the considerable diversity that exists across these organisms. As such, a basic philosophical approach should be developed by those working in the field. Until a consensus can be reached, an initial working approach could include the following concepts:

1) Be aware of the differences among entities such as species and user groups;
2) Focus on the commonalities across groups and technologies;
3) Generalize technology development to the extent possible;
4) Target broad application of findings, and
5) Work to reduce barriers to communication and integration across communities (e.g., species, commodity groups, or private and public sectors).

Further compounding these difficulties in utilizing the aquatic species cryopreservation literature is the lack of standardization in protocols, terminology, and reporting. Because of this hodgepodge, it is problematic or impossible to make valid direct comparisons among the results of most published studies (Table 1).

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Table 1. Examples of major factors under-appreciated for aquatic species that need to be defined, controlled, and reported to enable direct comparisons of sperm cryopreservation results for standardization and application (based on Yang et al. 2010).

Step or process	Factors to be defined	Relevance to cryopreservation
Source of animals, housing, and conditioning	Strain and source	Variation among populations or mutant lines can influence results
	Size and age	Can affect gamete quality and quantity (report body weight, length, age)
	Maturity	Reproductive condition (report sperm volume, or testis mass, and GSI*)
	Culture conditions	Water quality parameters, temperature, salinity, and light:dark cycles
	Male selection	Using best males may not represent normal variation (report selection criteria)
Sample handling, preparation, and freezing process	Initial quality	Major influence on post-thaw quality (requires assessment and definition)
	Sperm density	Can affect cryopreservation and fertilization (a major uncontrolled variable unless set)
	Cryoprotectant	Type and final concentration is a critical factor (should be defined and reported)
	Motility	Sperm motility prior to addition of cryoprotectant (should be defined and reported)
	Equilibration time	Requires tight control of methods and temperature (should be defined and reported)
	Packaging	Affects heat transfer (type, size, and materials should be defined and reported)
	Biosecurity	Such as sealing of containers and disinfection (should be defined and reported)
	Cooling rate	Critical cryobiological factor (should be defined with start and finish temperatures)
	Storage time	Duration can differentiate freezing and super-cooling (should be defined and reported)
Egg collection and use of thawed sperm samples for fertilization	Pooling of eggs	Sometimes used to provide sufficient numbers for experiments (should be reported)
	Thawing process	Warming temperature, duration, and rate (should be defined and reported)
	Post-thaw motility	Necessary to estimate effects of cryopreservation (should be defined and reported)
	Fertilization method	Can influence gamete activation especially for thawed sperm (should be reported)
	Sperm-to-egg ratio	Concentration and volume of sperm for fertilization (should be defined and reported)
	Egg quality	Fresh sperm can be used to evaluate fertility of eggs (should be reported)
	Fertilization rate	Reporting of exact definition of fertility criteria should be compulsory
	Hatching rate	Reporting of absolute or relative values should be identified (report both)

*GSI, gonadosomatic index, the percentage of testis weight in relation to the body weight.

More insidious is the problem caused by usage of particular terms such as “percent motility” or “percent fertilization” to represent a spectrum of widely varied activities and endpoints that are partially reported or not defined; such terms are often directly compared with the assumption that the conclusions are meaningful. This is a basic impediment to the pursuit of scientific research and has been addressed in a number of sections and chapters throughout this volume.

In brief, cryopreservation addresses the freezing, cryogenic storage and thawing of living material. Gametes or early life stages (e.g., embryos and larvae) are collected and suspended in an extender solution. The material to be frozen is usually evaluated for quality (e.g., motility of sperm) and can be maintained by refrigeration prior to the actual freezing and thawing processes. Fertilization success of gametes and subsequent development of early life stages are the first demonstration of cryopreservation success. Numerous factors such as cooling and thawing rates can influence formation of ice crystals, cell dehydration and maintenance of cell integrity. The addition of permeating cryoprotectant agents (e.g., dimethyl sulfoxide, methanol or glycerol) can minimize cell damage associated with ice formation. Most cryoprotectants, however, are toxic to cells and must be diluted with an extender solution prior to the addition of sperm. Cryoprotectant concentration and equilibration time (for the cryoprotectant to permeate the cell) can influence cryopreservation success. Moreover, this process can be species-specific or male-specific (although the exact magnitude and sources of variation are yet to be identified), and it can even depend on the timing when sperm are collected during the spawning season. These factors can lead to fertilization success rates that are variable among and within species. Previous reviews on fish sperm cryopreservation notwithstanding (e.g., Horton and Ott 1976, Scott and Baynes 1980, Stoss 1983, Billard et al. 1995, Rana 1995, Tiersch and Mazik 2000, Cabrita et al. 2009) the literature on this subject remains fragmented and disjointed, and protocols for cryopreservation vary considerably from study to study.

With respect to the human dimension, cryopreservation researchers working with aquatic species comprise a heterogeneous lot. Consider the variation presented by the authors of this volume, who likely provide a representative sample (perhaps even a healthy percentage) of the total population of active workers around the world. The more than 140 authors represent more than 20 countries (including some 25 states in the United States) and at least 75 different organizations and departments ranging from veterinary and medical schools to land-grant schools and community colleges to tribal governments to state, provincial and federal agencies to private organizations. Numerous disciplines are represented including conservation and molecular genetics, animal breeding, histology, endocrinology, physiology, basic cryobiology, veterinary medicine, animal science, oceanography, fisheries, hatcheries and aquaculture. These authors report on more than 70 species of amphibians, fishes, bivalves, gastropods, crustaceans and polychaetes. In contrast, much larger, more homogeneous groups often pursue study of cryopreservation within a single species (e.g., cattle, swine, or humans).

These and other issues have driven the genesis of this second edition. This volume recognizes protocol development as the foundation for the current status of this field, but places it in the context of pathway development (rather than the typical, narrow, single-entity research approach), and proceeds from there into commercial application and programmatic development. These activities will require a broad, comprehensive picture to emerge as this field moves forward, and it is hoped that this volume can at least serve to open some of the necessary discussions. This edition has added two new sections (gamete quality analysis, and international perspectives) and is not directly organized along the steps in the cryopreservation process. As such, cryopreservation process steps are presented across multiple sections (Figure 1).

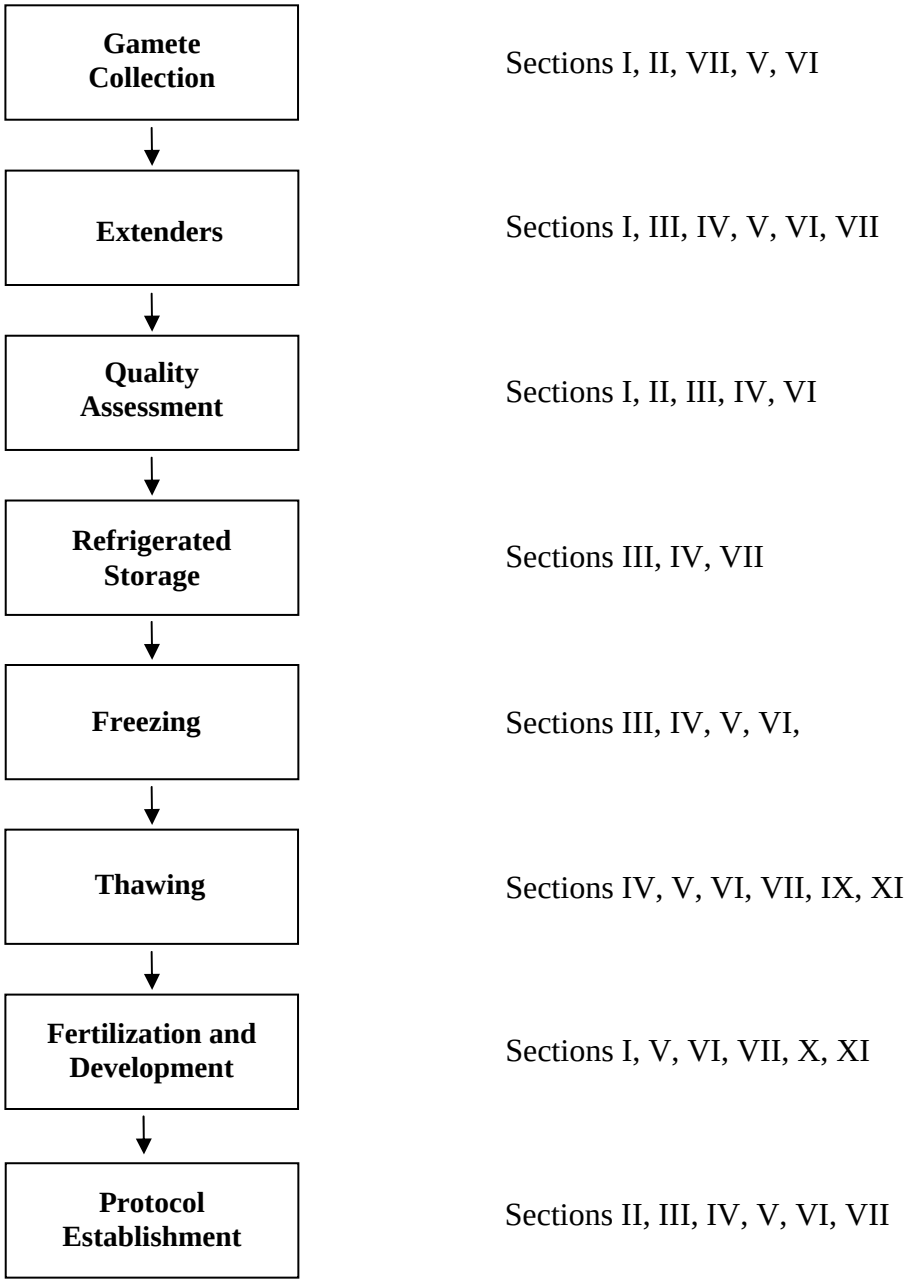

Figure 1. An outline of major steps in cryopreservation process and the corresponding sections in this volume in which the material is presented.

Likewise, this volume addresses multiple steps required for technology application and industry and programmatic development. This information is also distributed across the various sections of the volume (Figure 2, next page).

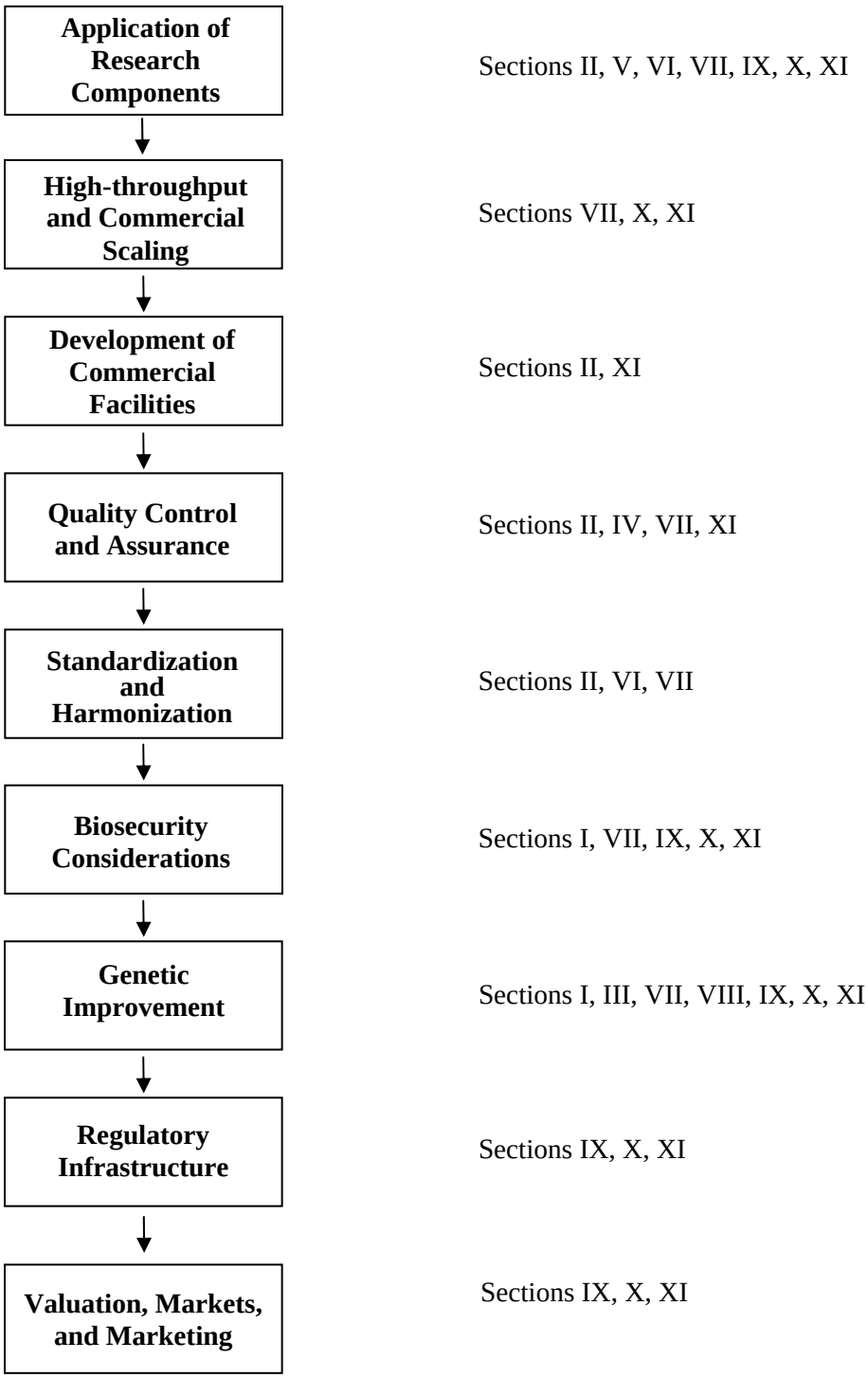

Figure 2. An outline of major steps in application and programmatic development and the corresponding sections in this volume in which the material is presented.

Despite the problems described above, cryopreservation has been successfully applied, at least in sperm, in numerous aquatic species. In many cases research groups have reinvented

protocols or developed alternative methods to yield comparable results. Fortunately, to facilitate the transition in aquatic species to commercial-scale application there are multi-million dollar industries already in place for cryopreservation of livestock semen which can provide methodologies, equipment, and insights. Cryopreservation of fish gametes gradually increased in the four decades since Blaxter (1953) reported the freezing of sperm to hybridize spring and fall spawning herring. Within the past 15 yr, the number of studies has expanded to the point where considerable uncertainty exists even in the number of aquatic species for which sperm has been cryopreserved (reported for example as between 50 and 200 species) and a current estimate is needed. Cryopreservation technology has enhanced hatchery and aquaculture operations by providing flexibility in spawning of females, greater control in breeding programs, and the ability to store favorable genes for extended periods. In addition, concern for native fish populations has resulted in examining sperm cryopreservation as a way to preserve genetic material and transfer genes between wild and hatchery populations.

A review on cryopreservation of fish sperm in the Introduction of the first edition of this book summarized 185 reports (including abstracts, conference proceedings, technical reports, book chapters, and 138 peer-reviewed journal articles) published between 1953 and 1996. It was found that research on sperm cryopreservation had at that point been described in print for at least 83 fish species from 35 families. The majority of publications were on economically important species, focused primarily on the salmonids, cyprinids and catfishes. These studies addressed freshwater (49%), marine (31%), brackish (7%) and anadromous (13%) fishes, or when viewed by categories, commercial and sport fisheries (51%), cultured ornamental and food fishes (39%), wild (non-sport) fishes (7%) and threatened and endangered species (3%). These basic trends likely hold true today, but there has been a great increase in the global nature of this work and expansion into amphibians and invertebrates. It would be very useful for cross-sector stakeholders to gather and census the numbers, types, and activity levels of global efforts in aquatic species. *Indeed, it is likely that an international society and journal could now be developed in the area of aquatic germplasm and genetics.* A basic illustration of this growth can be seen in the number of peer-reviewed publications addressing cryopreservation, which since the year 2000 is equal or larger to the number of all publications prior to that time (Figure 3).

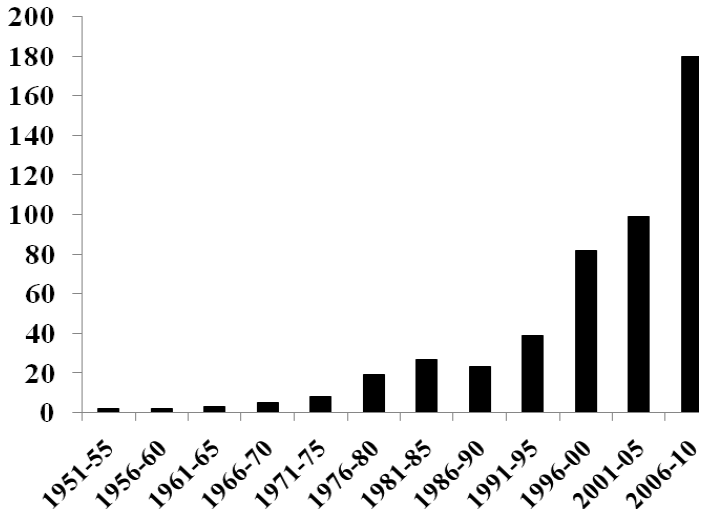

Figure 3. Six decades of peer-reviewed publications addressing fish sperm cryopreservation.

As the gaps in communication within the international community become ever smaller, increased exchange of information will accelerate research and application in cryopreservation. We should be aware that application will bring the potential for rapid changes in other fields. For example, cryopreservation can assist development and distribution of improved lines, including those produced by gene transfer. Therefore the availability of cryopreservation could accelerate application and distribution of genetically modified organisms. It is not unreasonable to assume that new product forms will emerge and current regulations may not be adequate. For example, consider the ease of transport of millions of transgenic oyster embryos in a few frozen straws compared to the transport of even a few hundred broodstock oysters. We have also seen a great increase in interest from members of the biomedical fish community since 2000. Large genetic screening projects utilize mutagenesis strategies that can yield thousands of new research lines, and this coupled with transgenesis and other technologies has produced a conservatively estimated current inventory of some 20,000 research lines maintained as live populations in zebrafish *Danio rerio* alone.

Given that cryopreservation is not perfected as yet, we should give consideration to the use of low-quality samples including non-motile sperm. Techniques such as intracytoplasmic injection (ICSI) of sperm can allow fertilization that would otherwise not be possible. There is, moreover, reason to suggest (while it is perhaps surprising to do so in a book on cryopreservation) that most germplasm repositories based on cryogenic storage in liquid nitrogen will eventually fail. Technical problems, accidents, loss of key personnel, political pressures, and changes in priorities can result in loss of cryogenic repositories. These problems would be compounded in developing countries where the expense of liquid nitrogen could inhibit repository maintenance. Efforts should be made to identify methods that complement cryogenic storage. Development of techniques such as ICSI for use in aquatic species could thus not only reclaim damaged sperm, but would also open the door to use of other less-costly methods of storage such as freeze-drying.

Fortunately, as indicated above, the published resources in aquatic species and for cryopreservation in general have expanded greatly since 2000. There are currently at least 30 high-quality reference works available in book form (most appearing in the past 10 yr) that address cryobiology, cryopreservation technology, and spermatology (Table 2, next page). In addition, there are now at least 60 reviews in a variety of forms specifically addressing relevant topics in aquatic species (Table 3). This provides a wealth of information for students and practitioners.

The benefits of cryopreservation as they are typically currently viewed include at least five aspects of improvement for existing industries (some indicated above) and for creation of new industries. First, cryopreservation can be used to improve existing hatchery operations by providing sperm on demand and simplifying the timing of induced spawning. Second, 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. Third, valuable genetic lineages such as endangered species, research models, or improved farmed strains can be protected by storage of frozen sperm. Fourth, cryopreservation opens the door for rapid genetic improvement. Frozen sperm can be used in breeding programs to create improved lines and shape the genetic resources available for aquaculture. Finally, cryopreserved sperm of aquatic species will at some point become an entirely new industry itself.

Table 2. Books addressing topics relevant to cryobiology and cryopreservation including those addressing aquatic species.

Title	Citation
Life and Death at Low Temperatures	Luyet and Gehenio 1940
Biological Effects of Freezing and Supercooling	Smith 1961
Cryobiology	Meryman 1966
Current Trends in Cryobiology	Smith 1970
The Frozen Cell	Wolstenholme and O'Connor 1970
The Freezing of Mammalian Embryos	Elliott and Whelan 1977
Low Temperature Preservation in Medicine and Biology	Ashwood-Smith and Farrant 1980
ATCC Preservation Methods: Freezing and Freeze-Drying	Simione and Brown 1991
Fish Evolution and Systematics: Evidence from Spermatozoa	Jamieson 1991
Advances in Low-Temperature Biology	Steponkus 1993
Cryopreservation and Freeze-Drying Protocols	Day and McLellan 1995
Reproductive Tissue Banking: Scientific Principles	Karow and Critser 1997
Action Before Extinction	Harvey et al. 1998
Cryopreservation in Aquatic Species (first edition)	Tiersch and Mazik 2000
Cryobanking the Genetic Resource: Wildlife Conservation for the Future?	Watson and Holt 2001
Low Temperature and Cryogenic Refrigeration	Kakac et al. 2003
Life in the Frozen State	Fuller et al. 2004
The Sperm Cell	De Jonge and Barratt 2006
Spermatology	Roldan and Gomendio 2007
Advances in Biopreservation	Baust and Baust 2007
Cryopreservation and Freeze-Drying Protocols, 2nd edition	Day and Stacey 2007
Vitrification in Assisted Reproduction	Tucker and Liebermann 2007
Theory and Techniques of Fish Spermatozoa and Embryos Cryopreservation	Chen 2007
The Fish Oocyte	Babin et al. 2007
Fish Spermatology	Alavi et al. 2008
The Effects of Low Temperature on Biological Systems	Grout and Morris 2009
Sperm Banking: Theory and Practice	Pacey and Tomlinson 2009
Sperm Biology	Birkhead et al. 2009
Methods in Reproductive Aquaculture Marine and Freshwater Species	Cabrita et al. 2009
Reproductive Biology and Phylogeny of Fishes, Volumes A and B	Jamieson 2009
Fundamentals of Cryobiology	Zhmakin 2009
Fertility Cryopreservation	Chian and Quinn 2010
WHO Laboratory Manual for the Examination and Processing of Human Semen	World Health Organization 2010

Table 3. Book chapters, review articles, workshop proceedings ("W Proceeding"), and conference proceedings ("C Proceeding") addressing topics relevant to cryobiology and cryopreservation of aquatic species.

Short Title	Type	Citation
Cryopreservation of fish spermatozoa and ova	Article	Horton and Ott 1976
Cryogenic preservation of fish and mammalian spermatozoa	Article	Mounib 1978
Cryopreservation of the sperm of some freshwater teleosts	Article	Stein and Bayrle 1978
Some data on gametes preservation and artificial insemination in teleost fish	C Proceeding	Billard 1978
Reproduction and artificial insemination in teleost fish	C Proceeding	Billard 1980
Preservation of gametes of freshwater fish	C Proceeding	Erdahl and Graham 1980
A review of the biology, handling and storage of salmonid spermatozoa	Article	Scott and Baynes 1980
Cryogenic storage of gametes of carps and catfishes	Article	Withler 1980
Cryopreservation of spermatozoa of freshwater fishes of Asia	Article	Withler 1981
Cryobiology and the storage of teleost gametes	C Proceeding	Harvey 1982
Cryopreservation of fish sperm	Chapter	Kopeika and Novikov 1983*
Fish gamete preservation and spermatozoan physiology	Chapter	Stoss 1983
Cryopreservation and fertility of fish, poultry and mammalian spermatozoa	C Proceeding	Graham et al. 1984
Some factors affecting the preservation of salmonid spermatozoa	Article	Erdahl et al. 1984
Artificial insemination and gamete management in fish	Article	Billard 1988
Artificial insemination and the preservation of semen	Chapter	Watson 1990
Live preservation of fish gametes	Chapter	Leung and Jamieson 1991
Fish sperm cryopreservation in Taiwan	Article	Chao 1991
Conservation and preservation of genetic variation in aquatic organisms	Chapter	McAndrew et al. 1992
Cryopreservation of aquatic gametes and embryos	C Proceeding	Rana 1995a
Cryopreservation of fish spermatozoa	Chapter	Rana 1995b
Preservation of gametes	Chapter	Rana 1995c
Cryopreservation of fish spermatozoa: effect of cooling methods	C Proceeding	Rana and Gilmour 1996
Cryopreservation of fish semen	C Proceeding	Maisse 1996
Cryopreservation of finfish and shellfish sperms	Article	Chao 1996
Cryopreservation of embryos in the oyster and clam	Article	Chao et al. 1997
Cryoconservation du sperme et des embryons de poissons	Article	Maisse et al. 1998
Cryopreservation and aquaculture: a case study with penaeid shrimp larvae	Article	Subremoniam and Arun 1999
Cryopreservation in Aquatic Species	Book	Tiersch and Mazik (editors) 2000
Cryopreservation of gametes in aquatic species	Special issue	Lahnsteiner (editor) 2000
Techniques of genetic resource banking in fish	Chapter	Billard and Zhang 2001

Short Title	Type	Citation
Cryopreservation of finfish and shellfish gametes and embryos	Article	Chao and Liao 2001
Cryopreservation in aquarium fishes	Article	Tiersch 2001
Main improvements in semen and embryo cryopreservation for fish and fowl	W Proceeding	Blesbois and Labbe 2003
Biosecurity and regulatory considerations for aquatic species	Chapter	Tiersch and Jenkins 2003
Cryopreservation of gametes and embryos of aquatic species	Chapter	Zhang 2004
Cryopreservation of semen of the *Salmonidae*	Chapter	Lahnsteiner 2004
Aspectos generales de la crioconservacion espermatica en peces teleosteos	Article	Medina-Robles et al. 2005
Extenders and cryoprotectants on fish spermatozoa cryopreservation	Article	Muchlisin 2005
Semen cryopreservation in catfish species	Article	Viveiros 2005
Cryobanking of fish somatic cells	Article	Mauger et al. 2006
Evaluation of the damage in fish spermatozoa cryopreservation	Article	Li et al. 2006
Cryopreservation of fish sperm	Chapter	Kopeika et al. 2007
Sperm cryopreservation in fish and shellfish	C Proceeding	Tiersch et al. 2007
Low-temperature preservation of fish gonad cells and oocytes	Chapter	Zhang et al. 2007
Theory and Techniques of Fish Spermatozoa and Embryos Cryopreservation	Book	Chen (editor) 2007**
On the biology of fish sperm	W Proceeding	Rosenthal (editor) 2008
Strategies for commercialization of cryopreserved fish semen	Article	Tiersch 2008
Fish sperm cryopreservation in France	C Proceeding	Haffray et al. 2008
Role of bacteria in the chilled storage and cryopreservation of sperm	Chapter	Nimrat and Vuthiphandchai 2008
Variability of sperm quality after cryopreservation in fish	Chapter	Kopeika and Kopeika 2008
Methods in Reproductive Aquaculture Marine and Freshwater Species	Book	Cabrita et al. (editors) 2009
Sperm quality and cryopreservation of Brazilian freshwater fish species	Article	Viveiros and Godinho 2009
Criopreservacion de gametos y embriones	Chapter	Herraez 2009
Prospects and development in fish sperm and embryo cryopreservation	Chapter	Robles et al. 2009a
Germplasm cryobanking in aquarium model species	Article	Robles et al. 2009b
Live preservation of fish gametes	Chapter	Gwo et al. 2009
Current status of sperm cryopreservation in biomedical research fish models	Article	Yang and Tiersch 2009
Sperm proteins in teleostean and chondrostean fishes	Article	Li et al. 2009
Criopreservacion para la conservacion y produccion de organismos marinos	W Proceeding	Proyecto FONDEF 2009
Cryopreservation of fish gametes and embryos	Article	Diwan et al. 2010
On the biology of fish gametes	W Proceeding	Rosenthal et al. (editors) 2010

* In Russian; **In Chinese

Future development of a germplasm industry will require integrated practices for sample collection, refrigerated storage, freezing, thawing, rules for use and disposal, transfer agreements, and database development. Indeed the development of this new industry continues to be constrained by factors including the technical requirements for scaling-up to commercial operations during the transition from research, and the absence of uniform quality control practices, industry standards, marketing and price structures, and appropriate biosecurity safeguards (e.g., Figure 2).

However, moving forward in the future will involve more than increasing the scale and types of our activities; we will need to change the way we view these technologies and their utility and value forms. For example, cryopreservation is a technology that provides new ways to generate, maintain and distribute genetic resources. These resources represent a bankable form of wealth. For example, as indicated above, within aquatic biomedical models, genetic resources (e.g., newly characterized mutations or phenotypes) are discovered, catalogued, studied, and integrated into "omic" platforms with each step along this pathway increasing the informational value. Practical utility provides additional value when this genetic information can be manipulated and studied in living organisms (e.g., fish). Thus because of tremendous research effort, the genetic resources associated with biomedical model fishes are increasing rapidly in information value, but are increasingly limited in utility value because of the constraints imposed by maintaining these ever-expanding genetic resources as live populations.

Germplasm is another form of wealth that can be viewed simply as the gametes necessary to perform matings, or more expansively as an exchange currency allowing creation, maintenance and transport of the informational and utility values of genetic resources. If we view this as an economic system, the ability to accumulate, store, and catalog germplasm represents a readily transferable form of wealth that is bankable. In our current system we are over-invested in informational value, constrained in utility value, and essentially without investment in exchange currency. This equation can be balanced by taking advantage of the opportunities offered by establishment of large, interactive germplasm repositories to bank genetic resources. To fully open new mechanisms for accruing value from genetic resources, germplasm banking must be on a scale of thousands or tens of thousands of samples This can only be accomplished by development of high-throughput cryopreservation approaches integrating biological variables, cryobiological principles, equipment and facility development, process control for sample handling, inventory and databasing, quality control and assessment, standardization and establishment of industrial standards, and institution of biosecurity systems – in short, more than simply freezing a few sperm samples. This would also involve a conceptual shift from an informational (theoretical) bias to an expansion of utility value by recognizing the essentially unexploited value of germplasm.

As a practical example, the substantial genetic improvement in global dairy herds has been accomplished almost entirely through the use of cryopreserved sperm to enable selective breeding of bulls to serve as a means to improve milk yields in their daughters. This has produced a multi-billion dollar global market for germplasm where the genetic resources (germplasm) are worth more than the individual bulls they originated from. Genetic information (e.g., data on milk production) is converted into utility value (more efficient dairy herds) through cryopreserved germplasm (the exchange currency). Discussions within aquatic species communities are needed to facilitate the transfer of conceptual approaches that have succeeded in organisms such as livestock by looking for linkages across current needs and opportunities in facilities, equipment, and protocols. High-throughput should be scalable to the needs of

individual laboratories and should strive to establish a central pathway that can accommodate all current levels and methods of application, while simultaneously funneling these activities into a standardized approach that incorporates new technologies such as microfluidics and micro-devices (e.g., see new chapter by Park et al.). This process should also take advantage of industrial methods supported by commercial vendors of specialized equipment, supplies and reagents, and industrial-level service providers for cryopreservation, storage and quality control.

Matters such as these have been actively considered in fields outside of aquatic sciences. In fact, books such as *Improving Cattle by the Millions: NAAB and the Development and Worldwide Application of Artificial Insemination* (Herman 1981) should be required reading for all who wish to apply cryopreservation to aquatic species. That book provides a history of the National Association of Animal Breeders (NAAB) and their activities with dairy and beef cattle throughout the 20th century. It is interesting to note (as done in the first edition of this book) that early in the century, arguments against the use of artificial insemination in cattle included:

1) Reaction to the word "artificial" which lead to claims that such work was against the laws of God or nature.
2) The fear that it would produce abnormal offspring.
3) The concern that it would alter sex ratios.
4) The concern that it would spread disease.
5) The concern that mistakes would result in contaminated bloodlines.
6) The concern that it required too much time and effort.
7) The concern that it would cost too much.

It is perhaps significant that as we continue to move into the 21st century we find these same objections directed against the application of cryopreservation to aquatic species. Given that these and other arguments were refuted or easily remedied, it is clear that there is more than technique that can be transferred from livestock to aquatic species.

Acknowledgements

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