Opportunities and challenges related to sperm cryopreservation in Atlantic salmon gene banks

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Abstract
Atlantic salmon are facing population declines and loss of productivity within populations due to anthropogenic impact factors and reduced survival at sea. Biobanking is an increasingly used tool to conserve the genetic integrity and diversity of populations threatened by extirpation. The aim of the current article is to discuss the opportunities and challenges that increased use of cryopreservation brings to biobanking activities, using the Norwegian Gene Bank (NGB) for Atlantic salmon as a model system. The NGB was established in 1985 and involves a traditional living gene bank, as well as “frozen gene bank” where paternal germplasm is stored as cryopreserved sperm. Cryopreservation is a method where cells or tissues are frozen in liquid nitrogen to temperatures where all biological processes are paused, thus allowing the cells to remain viable after later warming/thawing to temperatures above 0°C. Cryopreservation is therefore used in long-term preservation of genetic diversity and characteristics of wild populations. Until recently, implementation of large scale use of cryopreserved sperm in the live gene bank has been limited by a lack of protocols/capacity to preserve larger portions of sperms. More recent developments in cryopreservation methodologies, now enables preservation of samples sufficient for mass fertilization. Mass fertilization by cryopreserved sperm opens new opportunities to gene bank operations, including increased capacity to restore lost populations, mitigation of genetic changes in broodstock fish, as well as increased capacity at live gene bank facilities through the replacement of older males with frozen sperm. Knowledge demands regarding potential genetic damage to cryopreserved milt and potential epigenetic effects caused by the cryopreservation procedure should, however, be addressed.

KEYWORDS
Atlantic salmon, effective population size, fish conservation, frozen gene banks, live gene banks, population restoration

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INTRODUCTION

Due to widespread population declines in aquatic organisms, there is an urgent need to conserve and protect genetic variation of existing species (Clausen & York, 2008). In general, such conservation efforts occurs in situ, meaning that conservation efforts occurs within the natural distribution of the species or population, for example, through the protection of habitat and harvest regulations. Although the maintenance of biodiversity in situ is the preferred means of conservation, in some cases ex situ measures are necessary to ensure the continued viability of natural populations (Harvey & Trust, 1998). Ex situ conservation consists of methods or techniques where animals are maintained outside their natural habitat as broodstock, or through cryopreservation of germplasm. Both methods are generally referred to as “gene banks” and have the objective to preserve the genetic material of threatened and endangered species and populations in the short and long term. Gene banking of anadromous salmonids has been a widely used method in Norway, mainly due to the presence of the invasive ectoparasite, *Gyrodactylus salaris*, and its detrimental effects on infected Atlantic salmon populations (Johnsen & Jensen, 1986). The Norwegian Gene Bank (NGB) program for wild Atlantic salmon utilizes live gene banks (LGBs) as a temporary living reservoir of genetic material for the reestablishment of living stocks threatened by extirpation. Since the establishment of the first LGB station in 1989 (Gausen, 1993), 68 Atlantic salmon populations have been, or are currently, a part of the LGB program, 32 of which have successfully been re-established after the eradication of *G. salaris* (for a description of methods used to eradicate *G. salaris*, see Sandodden et al. (2018)). Furthermore, in an effort to preserve genetic material across the species' natural distribution in Norway, genetic material (sperm) from a total of 174 Atlantic salmon populations has been collected and cryopreserved in a so called “frozen gene bank” (Figure 1). Here, milt (seminal fluid of fish) is cryopreserved by cooling cells to very low temperatures (−196°C), which allows the cells to remain viable after later thawing to temperatures >0°C. Mainly due to their small size and relatively high resistance to freezing and thawing, cryopreservation of sperm cells is the most/best established technique for preservation of fish genomes and is an effective means to conserve genetic diversity ex situ (Martínez-Páramo et al., 2017). Due to more recent advances in sperm cryopreservation technology driven by a research project performed by Norwegian food- and assisted reproductive technology industry, the method is increasingly being used in the NGB. In this article, we discuss how increased use of sperm cryopreservation can improve genetic resource banking of fish, using the NGB as a model system. We begin by giving a description of the NGB strategy, before discussing some of the opportunities that increased use of sperm cryopreservation brings. We then give an overview of some of the main challenges associated with implementation of cryopreservation in gene bank and hatchery practices, and how the Norwegian gene banking program has addressed these challenges.

1.1 The national salmonid gene bank in Norway

The NGB was established by the Norwegian Directorate for Nature Management (now Norwegian Environmental Agency) in 1986, with the objective to contribute to a nationwide preservation of the genetic diversity and characteristics of natural Atlantic salmon populations (Gausen, 1993). During the early phases of the program,
the NGB was based exclusively on cryopreservation of milt in a frozen gene bank. The main objective was to preserve as much as possible of the natural genetic variation found in Norwegian Atlantic salmon populations (Figure 1) by creating a repository of genetic material from representative populations of Norwegian Atlantic salmon. The populations represented in the frozen gene bank thus includes both threatened and pristine populations, the latter ensuring that genetic diversity is preserved before there is substantial change or loss to wild populations. The cryopreservation protocol used involved the freezing of milt in “pellets” on dry ice (solid CO₂, −79°C) before being transferred to liquid nitrogen which was the final storage medium (Stoss & Refstie, 1983). The protocol only preserved a limited amount of milt of variable concentration, and the main use for the existing samples is therefore considered to be a genetic “repository” to be used exclusively for broodstock production (Gausen, 1993). “Live gene banks” for salmonid species were first established in Norway in 1989, and the main purpose was to provide a temporary living reservoir of genetic material for the re-establishment of living stocks threatened by extirpation (Gausen, 1993). The inclusion of populations in a LGB is a temporary measure used as a conservation strategy until the threat(s) that caused the initial population decline has been removed or stabilized, and the population can be restored. Although Atlantic salmon was initially the only salmonid included in the gene bank program, populations from about 33 anadromous brown trout (Salmo trutta) and 2 populations of anadromous Arctic charr (Salvelinus alpinus) have now also been incorporated into the program.

1.1.1 The gene bank strategy for Atlantic salmon in Norway

The basic gene bank strategy for Atlantic salmon in Norway is shown in Figure 2. The inclusion of a population into the LGB begins with the collection of founder individuals (F0) in the wild. Pairwise matings of F0 individuals produce the F1 generation that will become the broodstock in the LGB. The offspring of the F1 broodstock (F2) are then reintroduced to the natal river or raised to maturity to become F2 broodstock. The LGB is based on a principle of “eggs in–eggs out,” meaning that fertilized eggs is the only life stage that is transferred between the LGB and the wild. Eggs are disinfected, thus reducing the risk of transmission of horizontally transmitted disease. Disinfection of eggs is only one step in a biosecurity strategy of the NGB to minimize the risk of introduction, establishment, and transmission of pathogenic agents between and within aquatic animal populations.

![Flow chart depicting the strategy of the Norwegian salmonid gene bank.](https://example.com-flow-chart)

**FIGURE 2** Flow chart depicting the strategy of the Norwegian salmonid gene bank. Milt and roe is collected from founder individuals (F0) in the wild and transferred to a hatchery in a live gene bank station. The F0 progeny become the F1 generation broodstock held at the live gene bank station. F1 broodstock produce eyed eggs and/or juveniles released in the wild for population restoration purposes or an F2 generation broodstock at the gene bank when necessary. The gene bank strategy is based on an “eggs in–eggs out” strategy, which means that disinfected eggs is the only life-stage transferred between the live gene bank and the wild. In cases where juveniles are released to the wild, a local hatchery is used to rear the disinfected eggs. The frozen gene bank contains cryopreserved sperm from wild F0 fish and from broodstock males from the live gene banks. These samples are sometimes used for the production of new broodstock families, or for the production of eggs or juveniles to be released in the wild.

The main objective of the LGBs is the restoration of threatened populations, and the practice can therefore be distinguished from conventional supportive breeding programs by the goal of the stocking. First of all, the LGB is a temporary measure and not an end in itself. The purpose is to achieve a rapid reintroduction of populations to a self-sustaining level where they are no longer in need of artificial propagation. Second, the goal is to avoid genetic losses or alterations of the genetic composition of population as a result of the gene bank operation or reintroduction. These goals thus differ from that of conventional stock enhancement programs, where the focus tends to be maximizing or maintaining fishery yields (Miller & Kapuscinski, 2003). Previous work has shown that supportive breeding may actually reduce the effective population size, increase inbreeding, and result in loss of genetic variation (Christie, Marine, French, Waples, & Blouin, 2012; Ryman & Laikre, 1991). Thus, captive breeding may negatively affect the long-term persistence and adaptability of captive populations on their return to the wild (Verspoor et al., 2007).

To minimize genetic losses and/or alterations, the NGB captive breeding program operates under rigorous
population management standards aimed at minimizing inbreeding, genetic drift, and domestication/selection. Four main standards of the gene bank strategy can be summarized as follows: to (a) obtain and maintain an adequate effective population size in the founder (F0) and broodstock (F1) generation to avoid inbreeding, minimize genetic drift, and to conserve evolutionary potential by adequately representing the genetic composition of the natural population (Charlesworth, 2009; Witzenberger & Hochkirch, 2011). (b) Reduce mean kinship within founder and broodstock individuals to further avoid inbreeding as well as unconscious selection and overrepresentation of some genotypes over others (Williams & Hoffman, 2009). Unconscious selection is also minimized by, (c) standardization of individual reproductive contribution and family sizes. Equal family sizes minimize reproductive variance between families, while preventing a reduction of \( N_e \) (Allendorf, 1993; Williams & Hoffman, 2009). This standardization occurs both in the production of F1 and F2 generation broodstock, as well as in the production of eyed eggs and juveniles to be released in the wild (Figure 2). Last, the potential for unwanted selection is low if survival is high, and an important strategy is therefore to, (d) minimize mortalities at all life stages during the captive phase. Vital to low mortalities in the gene bank is, among other factors, a biosecurity strategy that minimize the risk of disease leading to losses of genetic variation or entire stocks. The screening of all founder fish for relevant disease, stock specific water supplies, and strict disinfection routines are among some of the steps included in the biosecurity strategy used by the NGB.

The number of founder individuals collected per population is based on the principle of representing as much of the genetic variation as possible in the NGB. At the same time, the maximum number of collected founders is constrained by the census size of the natural population and most importantly, the number of families a LGB facility has the capacity to include at a given time. As a general rule, the NGB seeks to collect a minimum of 50 founder individuals in order to avoid inbreeding depression and to represent at least 95% of the original genetic composition of a population) in the short term (Frankel & Soulé, 1981). It is, however, strived to collect more than this and for larger populations the number of founders can exceed 200. Collection of F0 individuals span over a minimum of 3 years to collect several cohorts to further increase genetic diversity of founder fish (Mjølnerød et al., 1999). Founder collection is also combined with genetic screening for genetic introgression by farmed Atlantic salmon (Karlsson, Diserud, Moen, & Hindar, 2014; Karlsson, Moen, Lien, Glover, & Hindar, 2011) to eliminate farmed-wild hybrids in the founder gene pool. Kinship between all possible pairs of confirmed wild individuals are then estimated using molecular genetic markers single nucleotide polymorphism (SNPs) according to Wang (2011) to conduct pairwise matings based on a low kinship criterion. This criterion ensures that the ratio of \( N_c/N \) is maximized. A full pedigree also provides the information required to ensure that each founder fish (F0) is equally represented in the generations to be released in the wild. As a general rule, only one generation (F1) of broodstock are used, and in cases when the production of F2 generation is necessary, efforts are made to integrate new founders (F0) into the captive population.

## 2 OPPORTUNITIES RELATED TO INCREASED USE OF CRYOPRESERVATION WITHIN THE ATLANTIC SALMON GENE BANK

The population management standards utilized by the NGB requires the integration of many different considerations including population genetics, fish health, and biosecurity. Improvements in salmonid fish milt cryopreservation technology made within the last decade has facilitated addressing some of these considerations. A main development in the cryopreservation technology is a method that now enables the preservation of larger portions of milt. Since 2013, the NGB has been using a patent developed by Cryogenetics® called the SquarePack®, which cryopreserves 11 ml of diluted milt. This development was the result of a research collaboration between two artificial reproductive technology companies in Norway associated with the Norwegian food industry, with the intent of improving artificial reproductive technologies in the aquaculture industry. The research resulted in the creation of Cryogenetics, and the development of a new cryopreservation protocol for salmonid fish in addition to the patented SquarePack.

Previous work performed at the NGB has demonstrated that one SquarePack has the capability to fertilize minimum 4,000 eggs simultaneously in Atlantic salmon, compared to a standard straw, which can fertilize a few hundred eggs. The implementation of this method into gene bank practices has greatly facilitated the use of cryopreserved sperm in the production of eggs for population-restoration. In the following paragraphs, we describe some of the opportunities that the method brings to the NGB. These opportunities include increased capacity at LGBs through the replacement of older males with cryopreserved milt, increased control of fertilization success, as well as a means to minimize genetic changes in broodstock and offspring through
continuous supplementation of wild founder or early-generation broodstock milt to gene bank fish.

2.1 Increased capacity at LGBs by the replacement of live males with cryopreserved sperm

Due to control and prevention of infectious disease and transmittance of pathogens from one genetic unit to another, different stocks at LGB facilities are isolated in separate tanks, with separate water supplies throughout the life cycle. Water supply and availability of tanks is thus a limiting factor on the number of populations that can be represented within a LGB facility. Furthermore, Atlantic salmon broodfish may attain sizes up to 30 kg resulting in a considerable requirement of tank volume and water supply. This limits the maximum number of broodfish that can be kept per stock, thus limiting the number of eggs that can be produced, and potentially the effective population size ($N_e$). Reducing the number of individual males to increase the number of producing females is not feasible as a 1:1 sex ratio in the broodstock is required to achieve 1:1 crosses which maximizes $N_e$ (Wright, 1940). Cryopreservation of milt, however, enables the reduction of male biomass without affecting sex-ratios or reducing $N_e$. This can be achieved by collecting milt early in the lifecycle and thereby replace older males with cryopreserved milt during the production of new broodstock or eggs for restocking of purposes.

The replacement of older males by cryopreserved milt is feasible in a gene bank environment due to some characteristic traits of salmonid and Atlantic salmon male reproductive biology. First, Atlantic salmon males tend to sexually mature at a younger age than females (Barson et al., 2015). For example, in 12 populations present in one of the LGB facilities in Norway between year 2000 and 2012, 74% of the males on average sexually matured by age 2 compared to 44% of the females, and 80% of males were mature at age 3 compared to 50% of the females. The earlier maturation by males ensures that milt can be collected in advance to, or concurrent with the availability of eggs from females. To avoid selection for younger maturing fish, those males that do not mature by age 3 will have be kept alive until they sexually mature and milt can be collected. Imperative to the strategy of only using younger males, however, is that young individuals produce sufficient quantities of sperm cells to cover the future need for the paternal germplasm of that individual. Previous studies suggest an inverse relationship between salmonid size and age and sperm concentration (Poole & Dillane, 1998; Yamamoto, Maruta, Suzuki, & Kitanishi, 2015), so that younger and smaller males produce a higher number of sperm cells per unit volume of milt. An inverse relationship between size/age and sperm concentration have also been supported by investigations of sperm density of milt from broodstock F1 generation males in a LGB facility (Figure 3a,b). The higher sperm concentration produced by younger males seems to compensate somewhat for their lower volumes of milt produced compared to older males as seen by a positive relationship between fish size/age and milt volume (Figure 3c). Experiences made by the NGB has shown that a 3 year-old-male on average produces enough milt to obtain four SquarePacks during a reproduction season. Because one SquarePack holds enough cells to fertilize a minimum of 4,000 eggs, cryopreserved milt from a 3-year-old fish has the capacity to produce more than 12,000 fertilized eggs. This number is an adequate number to use as standardization of family size during three different reproductive cycles, which a female generally contributes during her reproductive lifespan at a LGB facility (i.e., 4,000 eggs per reproductive cycle). Four SquarePacks of cryopreserved milt will therefore cover the entire future need for the germplasm of one male.

The increased tank capacity following the removal of older live males can be used to either include more individuals in the broodstock (hence increase egg production and $N_e$), or alternatively to include more populations in the facility. The possibility to include more individuals in the captive stock by replacing males older than 3 years by cryopreserved sperm and the resulting effect on $N_e$ and biomass based on growth and mortality rates in the LGBs is showed in Figures 4 and 5, respectively. The strategy shown in this Figure 4 doubles $N_e$ without requiring more tank space. Cryopreservation, therefore, is a means to greatly enhance the capacity of existing LGBs without having to resort to site expansion, or the establishment of new facilities.

2.2 Continuous and predictable milt supply over time and space

A major advantage of using cryopreserved sperm in gene bank practices is that cryopreservation facilitates the transportation of genetic material over time as well as space. Cryopreserved milt can be stored almost indefinitely without substantial effect on cell viability or quality (Stoss & Refstie, 1983). This means that cryopreserved sperm can be used over different generations and locations and facilitates operations such as the supplementation of genetic material from wild founder individuals (F0) to broodstock collections. Cryopreservation of milt also ensure that milt can be stored and used when eggs
are available. This is advantageous when variation in timing of male maturity leads to uneven sex-ratios during a reproductive season, or in worst case scenarios where incidents of increased male mortality has occurred. Since uneven sex-ratios causes an unequal reproductive contribution between individuals, the use of cryopreserved milt can counteract the potential for unwanted selection in response to some males contributing more than others.

2.3 Mitigation of genetic changes in the captive population by integration of frozen milt

Genetic adaptation in captivity will be positively related to the number of generations in captivity, intensity of selection, genetic diversity, and effective population size (Frankham, 2008). As a general rule, only F1 generation broodfish are utilized for the production of progeny to be released in the wild. If the initiation or duration of the reintroduction activity surpasses the reproductive lifespan of F1 fish, however, a second (F2) or even third (F3) generation broodstock may need to be produced. In these instances, it is of particular importance to incorporate special measures to minimize genetic changes in response to unconscious selection and/or in response to genetic drift (Fraser, 2008). Genetic changes due to unconscious selection and genetic drift can be minimized by the supplementation of genetic material from earlier generations to the broodstock material. As such, cryopreserved sperm obtained from males in the founder (F0) or early (e.g., F1) generations of captivity could be

FIGURE 3 Scatterplot of (a) male length and (b) male age, versus sperm concentration (billion cells · ml⁻¹) from 229 broodstock males from the Norwegian live gene bank for Atlantic salmon (pooled populations). (c) Scatterplot of male length and sperm volume in 285 broodstock males from the Vosso, Lærdal, and Fusta population at a live gene bank facility. Males were manually stripped. The regression lines represents the significant correlations between length (cm) and sperm concentration ($F = 1231.227, p \leq .001, r^2 = .35$), age and sperm concentration ($F = 841.227, p \leq .001, r^2 = .27$), and length and sperm volume ($F = 1001.224, p \leq .001, r^2 = .30$)
used to fertilize female eggs in subsequent generations. In the case of cryopreservation of male broodstock sperm, the collection should occur early in the lifespan to reduce the duration of time that the males are exposed to potential selection pressures in captivity. By reducing the number of generations and duration of time the males are kept in LGBs, the risk of domestication and subsequent losses in genetic variation will thus be reduced (Sonesson, Goddard, & Meuwissen, 2002).

2.4 Frozen gene banks and the potential for restoration of lost populations and/or original genomes

Due to the many and complex threats faced by anadromous salmonids today, numerous Atlantic salmon, as well as anadromous brown trout and Arctic charr populations are experiencing population losses and are vulnerable to extirpation (Anon., 2019a, 2019b; Svenning, Falkegård, & Hanssen, 2012). This is particularly true for small populations that are more vulnerable to stochastic events such as catastrophic weather episodes, anthropogenic activities, as well as genetic introgression by escaped farmed Atlantic salmon (Karlsson, Diserud, Fiske, & Hindar, 2016). Genetic introgression by domesticated Atlantic salmon occurs throughout the species’ Norwegian distributional range, causing potentially irrevocable genetic changes in locally adapted populations (Diserud et al., 2019; Diserud, Hindar, Karlsson, Glover, & Skaala, 2017; Glover et al., 2019). Thus, Atlantic salmon in Norway is currently experiencing a nationwide decline in genetic variation, both due to population declines and population losses, and due to interbreeding with farmed
Atlantic salmon. Because the use of cryopreservation allows for long-term repository of important genetic material, frozen gene banks can be a valuable tool to preserve the genetic resources of wild salmonid populations. In addition to being an important repository, cryopreservation can with the help of reproductive biotechnologies and LGBs play an important role in population restoration by regenerating original wild populations from cryopreserved sperm. If a sufficiently large material of founder milt has been collected, cryopreserved sperm from an extirpated or critically endangered populations can be used to fertilize eggs from a genetically related population, for example, population in close geographic proximity (King et al., 2007), followed by repeated cycles of backcrosses with founder milt to phase out the non-native/local/endemic maternal genome. Alternatively, embryos can be produced with all-paternal inheritance using androgenesis (Komen & Thorgaard, 2007; Parsons & Thorgaard, 1984). This method involves the use of unfertilized eggs from a donor population, which are then irradiated to inactivate their genetic material. The eggs can then be fertilized using cryopreserved sperm, resulting in an egg that consist of DNA solely derived from the male, thus the original extirpated population (Fraser, 2008). High mortality rates observed among androgenetic specimens, however, is still a limiting factor for large scale-applications of androgenesis (Ocalewicz et al., 2019), such as population restoration purposes.

In addition to restoration of lost populations, cryopreservation may also aid in the restoration of original genomes in cases where genetic alterations have occurred in response to genetic selection in response to hatcheries, fisheries, or introgression due to escaped farmed congeners (Fraser, 2008; Karlsson et al., 2016). Imperative to such a use of cryobanked material, however, is that collection of sperm is completed before irrevocable changes occur in the locally adapted populations.

3 | CRYOPRESERVATION IN THE GENE BANK CONCEPT—CHALLENGES AND SOLUTIONS

The successful implementation of cryopreservation in gene bank operations requires that inherent challenges with the method can be addressed in a way that is in accordance with the gene bank objectives. In the following section, we will describe some of the main challenges listed by the fish cryopreservation literature, and how they relate to the gene bank operation. We then describe how the NGB has addressed some of these challenges and outline future work and knowledge demands.

3.1 Obtaining sufficient and predictable sperm quality and quantity in/from cryopreserved milt

The successful implementation of cryopreserved sperm in hatchery practices of gene banks requires that the material being preserved is of an acceptable quality/viability, and quantity (Judycka, Nynca, & Ciereszko, 2019). Acceptable quantities of viable cells ensures high fertilization success of the number of eggs required for the purpose of population restoration, or production of new broodstock generations.

The quality of cryopreserved sperm may be affected by a number of factors including collection and handling procedures prior to preservation (Kommisrud et al., 2020), as well as the cryopreservation protocol being used. Cryopreservation may produce damage in cells, which affects plasma membrane, mitochondria, and chromatin structure with potential implications to fertilization success (Cabrita et al., 2010; Figueroa et al., 2016; Mayer, 2019). Until recently, published protocols for cryopreservation of salmonid milt showed relatively low and variable fertilization rates post cryopreservation. For example, Figueroa et al. (2015) recorded 46.2 ± 4.4% fertility rate with sperm from Atlantic salmon that has undergone vitrification, which was significantly lower than that achieved by fresh sperm. In addition to sperm collection procedures and cryopreservation protocols, variation in sperm characteristics including quality, quantity, and viability may occur due to individual factors such as age and health. Sperm samples may also vary within individuals in response to season during collection, temperature, feed, and pH contaminations (e.g., Rurangwa, Kime, Ollevier, Nash, 2004). Protocols ensuring high and predictable fertilization success are therefore vital for the successful implementation of sperm cryopreservation in hatchery practices.

More recent cryopreservation protocols show an increased fertility rate compared to earlier methods. For example, Figueroa et al. (2016, 2018) demonstrated fertilization rates of 81.6% ± 1.9% and 80.3% ± 3%, respectively compared to about 93% in fresh milt controls. The cryopreservation protocol developed by Cryogenetics® and used by the NGB was tested by Kommisrud et al. (2020) and demonstrated a high and predictable fertilization rate of 73.9 ± 1.7% rate using cryopreserved milt of Atlantic salmon compared to 81.1 ± 1.2% for fresh milt controls (Kommisrud et al., 2020). Similar results were obtained for cryopreserved milt from Arctic charr in which no difference in fertility rate was detected between cryopreserved milt performed by Cryogenetics and fresh milt (Grevle, Ritter, & Sunde, 2015). High fertilization rates by cryopreserved sperm, however, are contingent on proper handling, storage, and shipping prior
to the cryopreservation procedure. Protocols concerning sperm collection, storage, and transportation prior to preservation has therefore been developed and implemented in the NGB. An important factor contributing to successful fertilization with cryogenically frozen sperm of salmonids spp. is ambient temperature and proper handling during storage and shipment prior to cryopreservation. Previous work demonstrates that storage under temperatures around 5–6°C for less than 3 days ensures that fertilization success is not compromised prior to preservation (Kommisrud et al., 2020). In NGB, collected samples are therefore placed on ice immediately after collection, and shipped to a cryopreservation laboratory after a maximum of 2 days following collection. The successful implementation of cryopreserved sperm in the hatchery practices and gene banks also requires that a sufficient number of cells can be preserved, ensuring that the necessary quantities of eggs are successfully fertilized per crossing (Judycka et al., 2019). Plastic straws are the most widespread method used to contain sperm of livestock species for cryopreservation purposes (Judycka et al., 2019). However, plastic straws, as well as the “pellets” (Stoss & Refstie, 1983) previously utilized by the NGB, are in many instances inadequate for use in most finfish species because of the low numbers of egg a portion of milt can fertilize. Typically, one straw or an ampulla of pellets fertilize a few hundred salmonid eggs, which is inadequate for most anadromous salmonids like Atlantic salmon that produce thousands of eggs during a reproductive cycle (Klemetsen et al., 2003). Depending on the size and carrying capacity of a river system, several thousands of eggs per female may be utilized during the reintroduction of lost or near extirpated populations. This makes the use of cryopreserved milt from straws or pellets for reintroduction purposes inadequate. A fertilization capacity of a minimum of 4,000 eggs by the SquarePack®, however, enables the use of cryopreserved material for this purpose.

Standardization and evaluation procedures further results in a high predictability in the outcome of results (fertilization). Standardization is important because sperm density may vary between and within individuals and consequently result in variation in fertilization success. Standardization procedures is performed at the central lab of Cryogenetics® and consist of analyses based on photometry to determine cell density of collected samples. Cell density is determined in order to standardize the number of sperm cells per ml by dilution using a proprietary fish sperm extender. This ensures that sperm-to-egg ratio is consistent in all samples, allowing for a more predictable outcome when cryopreserved sperm is used. Prior to cryopreservation, the samples are also evaluated and rated for the presence and degree of contamination as well as the degree of motility, which is determined by microscopy. Evaluation and standardization of milt contributes to a high and predictable fertilization success which is critical in terms of obtaining a production of progeny necessary for the gene bank operation, but also in terms of avoiding potential selection effects in response to the cryopreservation procedures itself. When all males achieve similar fertilization success, there is a low risk of certain males contributing more to the production of progeny than others, which would result in unequal family sizes (Allendorf, 1993).

### 3.2 Genetic damages to cryopreserved milt

An absolute requirement for the use of cryopreserved sperm is that the freezing, cryobanking, and thawing processes does not alter the information carried by the cellular genome. Such changes may lead to development abnormalities and losses of genetic information in the progeny (Figueroa et al., 2016; Pérez-Cerezales et al., 2009). Previous work in the earlier phases of cryopreservation development demonstrated that the cryopreservation process may produce reactive oxygen species (ROS) which are highly deleterious for DNA (Labbe, Martoriat, & Gerard, 2001). The consequences can be dramatic as ROS may induce both strand breaks and base modifications in DNA, which in term alters the genome of progeny. The presence of DNA alternations due to cryopreservation, however, appears to be species specific as a result of extreme dissimilarities in gamete biology and structure among species (Fickel, Wagener, & Ludwig, 2007). For example, cryopreservation was shown to increase the vulnerability of sperm DNA to in vitro denaturation in the case of human sperm (Spanò et al., 1999), but no such effect was found on boar spermatozoa (Hamamah, Royère, Nicolle, Paquignon, & Lansac, 1990). Results from more recent investigations on the effect of cryopreservation on DNA integrity in salmonid fish show some inconclusive results. For example, Labbe and Maise (2001) found slight modification of DNA as a result of cryopreservation of rainbow trout sperm, whereas Martínez-Páramo et al. (2009) showed that cryopreserved sperm from brown trout provided offspring with a similar genetic profile to unfrozen milt. Cabrita, Robles, Rebordinos, Sarasquete, and Herráez (2005) reported a high level of DNA integrity after thawing, with only 3–5% fragmented DNA for both fresh and frozen semen (Figueroa et al., 2016). Due to the variation in gamete structural qualities and response to cryopreservation, species specific investigations should be performed to assess potential negative effects of cryopreservation protocols in use. Current knowledge regarding the performance of salmonid offspring from cryopreserved sperm, however, implies that the genetic
benefits of applying the method outweighs the potential risk associated with the process (Young, Frenyea, Wheeler, & Thorgaard, 2009).

### 3.3 Genetic or epigenetic effects in offspring derived from cryopreservation

A previous study performed on rainbow trout (*Onorhynchus mykiss*) showed that cryopreserved sperm from males derived from cryopreserved sperm themselves had 8% higher fertilization success (89.6%) compared to cryopreserved sperm from males derived from fresh sperm (81.7%) (Babiak, Glogowski, Dobosz, Kuzminski, & Goryczko, 2002). When eggs where fertilized with fresh sperm from the same males, however, no difference was found. As the individuals in the study were full-sibs and reared under the same conditions, the differences in fertilization success could not be attributed to physiological, or genetic factors. Instead, it was suggested that cryopreservation may promote selection of spermatozoa resistant to the freezing procedure, and that more tolerant sperm cells to freezing is a trait carried over to offspring. Later work has been able to confirm the existence of a link between the phenotype of sperm-cells and the phenotype of the resulting offspring in Atlantic salmon (Immler, Hotzy, Alavioon, Petersson, & Arnqvist, 2014), as well as in other species (Marshall, 2015). If this co-variance is based on genetic-factors, then selection on the level of sperm cells could lead to a variation in the genetic profile of offspring obtained with cryopreserved sperm compared to offspring obtained with fresh sperm (Cabrita et al., 2010). Sperm phenotype is, however, predominantly determined by testicular gene expression, and thus the diploid genome of the male (Eddy, 2002). As such, relevant genetic variation within sperm-cells from a single male is limited (Pitnick, Hosken, & Birkhead, 2008), thus the potential for selection. Sperm phenotype may also be influenced by nongenetic factors such as the paternal phenotype and/or the paternal environment, and this environmentally induced variation in sperm phenotype can affect the phenotype of offspring (Marshall, 2015). Therefore, a cryopreservation procedure undoubtedly has the potential to have an environmentally induced effect on sperm properties, which may lead to epigenetic effects carried over to offspring.

Potential unwanted selection or epigenetic effects in response to cryopreservation of sperm cells may be reduced by applying a cryopreservation protocol that is associated with limited harm to cells and consequently a high and consistent fertilization success, such as the cryopreservation protocol utilized by the NGB. Because most epigenetic are believed to be erased with each new generation (Weinhold, 2006), however, its presence may not be of great consequence to the gene bank objective. More studies are nevertheless required to determine whether epigenetic effects due to cryopreservation are widespread, and the consequence of such effects on offspring.

### 3.4 Database development and management

Adequate labeling and tracking of cryopreserved milt and the creation of robust databases capable of handling biological information concerning samples is a prerequisite for the successful implementation of cryopreservation techniques in hatchery and gene banking operations (Judycka et al., 2019; Tiersch, 2008). Any lacking or inaccurate documentation can affect the final result and lead to disturbances of genetic variation having detrimental consequences to the achievement of the gene banking objectives. Within the NGB, a protocol for tracking of individual pedigrees, relatedness, results from genetic and pathogen screening, as well as biological indices such as morphological and demographic parameters at the time of collections, needs to be documented in addition to data on sperm quality evaluations and cryopreservation protocols. Furthermore, systems need to be in place to track the use of multiple samples, as samples from a single male may be used for more than one purpose (e.g., production of F1 and F2 broodstock, frozen gene bank, pathogen screening, and scientific studies).

The NGB utilizes a specially constructed database system built for the purpose of managing the genetic resources at the NGB. The database is based on a software called Innova developed for the food industry, and is continuously developing through close communication of gene bank administrators and software developers. At present, the system contains an overview of all fish that has historically been or is presently in the gene bank program. All available information on founder fish, including results from genetic analyses and health monitoring, capture location, date of gamete collection and more, are included in the system. Pedigrees, morphological, and demographic parameters are registered for all individual broodstock fish, which are individually tagged by electronic tags (PIT tags) and measured annually. During collection of eggs and milt, all fish are screened for the PIT so that all samples can be labeled by the maternal or paternal ID as well as a unique barcode ID for the egg or milt sample through the use of a computer controlled printing system (Figure 6).
3.5 | Storage and logistics

Final storage of samples in liquid-phase nitrogen (−196°C) in a storage dewar is a standard method for cryopreserved samples (Martínez-Páramo et al., 2017). This ensures all biological and biochemical processes are inactivated so that samples can be stored indefinitely (Stoss & Refstie, 1983). During storage, efficient data administration and labeling are essential components to ensure sample identification and traceability (Judycka et al., 2019; Martínez-Páramo et al., 2017). As up to 3,700 Squarepacks or 140,000 straws can be stored in one dewar flask, sample sorting, and archiving is another critical component during storage.

Samples from the NGB are stored at Cryogenetics storage facilities located in two locations in Norway (Figure 1). This is a “user-pays” agreement where the necessary storage space and supervision are services provided by Cryogenetics. Here, unique identification numbers ensure that each sample is easily located and retrieved. Each sample is associated with an address locating the dewar flask and the placement within the dewar flask. Additionally, split-sample storage, in which separate locations containing duplicate samples is performed for added security. This ensures that if an unexpected event such as natural disasters or technical failure occurs in one location, a backup samples is always available.

4 | FUTURE WORK AND KNOWLEDGE DEMANDS

Challenges associated with the cryopreservation of sperm of sufficient and predictable quantity, and quality, has largely been addressed through the implementation of more recently developed cryopreservation protocols. Limited research, however, have been conducted on potential genetic and epigenetic consequences of implementation of large-scale cryopreservation of Atlantic salmon sperm in hatchery practices. As such, future research should focus on evaluating potential DNA damage and DNA methylation as a result of the protocol in use.

5 | CONCLUSIVE REMARKS

Cryopreservation of salmonid sperm enables the long-term preservation of valuable genetic diversity. Contrary to LGBs, the genetic material is not exposed to selection while stored and can be transferred across time and space. The method thus has the potential to restore lost populations, or to enhance those threatened by extirpation and or genetic degradation. Experiences made by the NGB, however, is that the full potential of the method has not previously been reached due to the limitations in the quantities of milt that could be preserved. A more recent method now enables the preservation of portions that can fertilize a predictable and high number of eggs, providing several advantages and opportunities in gene banks. These opportunities consist of mass fertilization of eggs for the restoration of lost or near extirpated populations, increased capacity at LGBs through the replacement of older males with cryopreserved milt, and mitigation of genetic changes in live broodstock through the supplementation of wild founder milt.

The implementation of cryopreservation in daily operations of gene banks is contingent on the development and standardization of efficient cryopreservation protocols to obtain (a) a fertilization success sufficient for gene bank practices and (b) reduce variation in fertilization success among individuals. There is still a lot of
research conducted to test different cryoprotectants, extenders, and the rate of time for freezing in order to establish cryopreservation protocols for Atlantic salmon (Judycka et al., 2019; Martínez-Páramo et al., 2017). The development of such protocols, as well as its implementation in daily gene bank activities requires the use of stringent evaluation of sperm quality to assess how such protocols affects factors that determine fertilization success. Sperm motility, as well as sperm density, will in large part affect fertilization capacity, and these parameters need to be evaluated in order to objectively assess (a) the effectiveness of the cryopreservation protocol in use and (b) the usefulness of individual batches of collected milt in terms of the usefulness for cryopreservation. Standardization of cell density in cryopreserved samples is another vital tool ensuring high and predictable fertilization success.

The use of a tested protocol developed by a commercial provider ensures that desired results in terms of mass fertilization is achieved, and that these results are consistent. The commercial provider used by the NGB is one of the few commercial providers of technology and services for the preservation of salmonid milt. However, an increasing number of studies working on standardization of cryopreservation technologies of salmonid sperm has recently been initiated (Judycka et al., 2019), and so more providers may be available in the future. The experiences made by the NGB in cooperation with Cryogenetics® demonstrates that the technology available is adequate to meet the requirement of gene bank operations.

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CONFLICT OF INTEREST
The authors declare no conflicts of interests.

AUTHOR CONTRIBUTIONS
Arne Sivertsen and Bjørn Bjørn conceptualized the manuscript and Kristin Bøe led the writing and developed figures. Steffen Wolla, Marthe Tanguyld Bårdsen, and Anveig Nordtug Wist offered critical insight and edits to the manuscript.

DATA AVAILABILITY STATEMENT
The data presented in this study are available from the authors upon reasonable request.

ETHICS STATEMENT
All activities conducted in the LGB program are approved by internal animal welfare guidelines and Norwegian legislation on animal research and animal welfare.

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REFERENCES
Allendorf, F. (1993). Delay of adaptation to captive breeding by equalizing family size. Conservation Biology, 7, 416–419.
Anon. (2019a). Klassifisering av tilstanden til 430 norske sjøørretbestander. Temarapport fra Vitenskapelig råd for lakseforvaltning nr 7.
Anon. (2019b). Status for norske laksebestander i 2019. Rapport fra Vitenskapelig råd for lakseforvaltning nr 12.
Babiak, I., Głogowski, J., Dobosz, S., Kuzminski, H., & Goryczko, K. (2002). Semen from rainbow trout produced using cryopreserved spermatozoa is more suitable for cryopreservation. Journal of Fish Biology, 60, 561–570.
Barson, N. J., Aykanat, T., Hindar, K., Baranski, M., Bolstad, G. H., Fiske, P., … Primmer, C. R. (2015). Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. Nature, 528, 405–408.
Cabrita, E., Robles, V., Rebordinos, L., Sarasquete, C., & Herrera, M. P. (2005). Evaluation of DNA damage in rainbow trout (Oncorhynchus mykiss) and gilthead sea bream (Sparus aurata) cryopreserved sperm. Cryobiology, 50, 144–153.
Christie, M. R., Marine, M. L., French, R. A., Waples, R. S., & Blouin, M. S. (2012). Effective size of a wild salmonid population is greatly reduced by hatchery supplementation. Heredity, 109, 254–260.
Clayton, R. K., & York, R. (2008). Global biodiversity decline of marine and freshwater fish: A cross-national analysis of economic, demographic, and ecological influences. Social Science Research, 37, 1310–1320.
Diserud, O. H., Fiske, P., Siegrov, H., Urdal, K., Aronsen, T., Lo, H., … Hindar, K. (2019). Escaped farmed Atlantic salmon in Norwegian rivers during 1989–2013. ICES Journal of Marine Science, 76, 1140–1150.
Diserud OH, Hindar K, Karlsson S, Glover K, Skaala Ø. (2017). Genetisk påvirkning av nvt opprettelses på ville laksebestander - status 2017. NINA rapport 1337. Norsk Institutt for Naturlforsking.
Eddy, E. M. (2002). Male germ cell gene expression. Recent Progress in Hormone Research, 57, 103–129.
Fickel, J., Wagener, A., & Ludwig, A. (2007). Semen cryopreservation and the conservation of endangered species. *European Journal of Wildlife Research*, 53, 81–89.

Figuerola, E., Farias, J. G., Lee-Estevéz, M., Valdebenito, I., Risopatrón, J., Magnotti, C., ... Oliveira, R. P. S. (2018). Sperm cryopreservation with supplementation of α-tocopherol and ascorbic acid in freezing media increase sperm function and fertility rate in Atlantic salmon (Salmo salar). *Aquaculture*, 493, 1–8.

Figuerola, E., Merino, O., Risopatrón, J., Isachenko, V., Sánchez, R., Effer, B., ... Valdebenito, I. (2015). Effect of seminal plasma on Atlantic salmon (Salmo salar) sperm vitrification. *Theriogenology*, 83, 238–245.

Figuerola, E., Valdebenito, I., Merino, O., Ubilla, A., Risopatrón, J., & Farias, J. G. (2016). Cryopreservation of Atlantic salmon *Salmo salar* sperm: Effects on sperm physiology. *Journal of Fish Biology*, 89, 1537–1550.

Frankel, O. H., & Soulé, M. E. (1981). *Conservation and evolution*. Cambridge: Cambridge University Press.

Frankham, R. (2008). Genetic adaptation to captivity in species conservation programs. *Molecular Ecology*, 17, 325–333.

Fraser, D. J. (2008). How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evolutionary Applications*, 1, 535–586.

Gausen, D. (1993). The Norwegian Gene Bank Programme for Atlantic salmon (Salmo salar). In *Genetic Conservation of Salmonid Fishes* (pp. 181–187). New York: Plenum Press.

Glover, K. A., Urdal, K., Næsje, T., Skoglund, H., Floro-Larsen, B., Otterå, H., ... Wennvik, V. (2019). Domesticated escapes on the run: The second-generation monitoring programme reports the numbers and proportions of farmed Atlantic salmon in Norwegian rivers annually. *ICES Journal of Marine Science*, 76, 1151–1161.

Grevle, I., Ritter, M., Sundt, J. (2015). *Conrad extraction of sperm from Arctic char (Salvelinus alpinus) increases reproduction efficiency in aquaculture*. In P. Terentjev (Ed.) 8th International Charr Symposium (Book of Abstract). Tromsø.

Hamamah, S., Royère, D., Nicolas, J.-C., Paquignon, M., & Lansac, J. (1990). Effects of freezing-thawing on the spermatozoon nucleus: A comparative chromatin cytophotometric study in the porcine and human species. *Reproduction, Nutrition, Development*, 30, 59–64.

Harvey, B. J., & Trust, W. F. (1998). *Action before extinction*. An International Conference on Conservation of Fish Genetic Diversity. Victoria, BC: World Fisheries Trust.

Immler, S., Hotzy, C., Alavioon, G., Petersson, E., & Arnaqvist, G. (2014). Sperm variation within a single ejaculate affects offspring development in Atlantic salmon. *Biology Letters*, 10, 20131040.

Johnsen, B. O., & Jensen, A. J. (1986). Infestations of Atlantic salmon, Salmo salar, by Gyrodactilus salaris in Norwegian rivers. *Journal of Fish Biology*, 29, 233–241.

Judycka, S., Nynja, J., & Ciereszko, A. (2019). Opportunities and challenges related to the implementation of sperm cryopreservation into breeding of salmonid fishes. *Theriogenology*, 132, 12–21.

Karlsson, S., Diserud, O. H., Fiske, P., & Hindar, K. (2016). Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. *ICES Journal of Marine Science*, 73, 2488–2498.

Karlsson, S., Diserud, O. H., Moen, T., & Hindar, K. (2014). A standardized method for quantifying unidirectional genetic introgression. *Ecology and Evolution*, 4, 3256–3263.

Karlsson, S., Moen, T., Lien, S., Glover, K. A., & Hindar, K. (2011). Generic genetic differences between farmed and wild Atlantic salmon identified from a 7K SNP-chip. *Molecular Ecology Resources*, 11, 247–253.

King, T. L., Verspoor, E., Spidle, A. P., Gross, R., Phillips, R. B., Koljonen, M.-L., ... Morrison, C. L. (2007). Biodiversity and population structure. In E. Verspoor, L. Stradmeyer, & J. L. Nielsen (Eds.), *The Atlantic salmon: Genetics, conservation and management* (pp. 115–160). Oxford: Blackwell Publishing Ltd.

Klemetsen, A., Amundsen, P.-A., Dempson, J. B., Jonsson, B., Jonsson, N., O’Connell, M. F., & Mortensen, E. (2003). Atlantic salmon Salmo salar L., brown trout Salmo trutta L. and Arctic charr Salvelinus alpinus (L.): A review of aspects of their life histories. *Ecology of Freshwater Fish*, 12, 1–59.

Komen, H., & Thorgaard, G. H. (2007). Androgenesis, gynogenesis and the production of clones in fishes: A review. *Aquaculture*, 269, 150–173.

Kommisrud, E., Myrosmilen, F. D., Stenseth, E.-B., Zeremichael, T. T., Hofman, N., Grevle, I., & Sundt, J. (2020). Viability, motility, ATP content and fertilizing potential of sperm from Atlantic salmon (Salmo salar L.) in milk stored before cryopreservation. *Theriogenology*, 151, 58–65.

Labbe, C., & Maise, G. (2001). Characteristics and freezing tolerance of brown trout spermatozoa according to rearing water salinity. *Aquaculture*, 201, 287–299.

Labbe, C., Martoriati, A., & Gerard, M. (2001). Effect of sperm cryopreservation on sperm DNA stability and progeny development in rainbow trout. *Molecular Reproduction and Development*, 60, 397–404.

Marshall, D. J. (2015). Environmentally induced (co)variance in sperm and offspring phenotypes as a source of epigenetic effects. *Journal of Experimental Biology*, 218, 107–113.

Martínez-Páramo, S., Horváth, A., Labbé, C., Zhang, T., Robles, V., Herráez, P., ... Cabrita, E. (2017). Cryobanking of aquatic species. *Aquaculture*, 472, 156–177.

Martínez-Páramo, S., Pérez-Cerezales, S., Gómez-Romano, F., Blanco, G., Sánchez, J. A., & Herráez, M. P. (2009). Cryobanking as tool for conservation of biodiversity: Effect of brown trout sperm cryopreservation on the male genetic potential. *Theriogenology*, 71, 594–604.

Mayer, I. (2019). The role of reproductive sciences in the preservation and breeding of commercial and threatened teleost fishes. In P. Comizzoli, J. L. Brown, & W. V. Holt (Eds.), *Reproductive sciences in animal conservation. Advances in experimental medicine and biology* (pp. 187–224). New York: Springer.

Miller, L. M., & Kapuscinski, A. R. (2003). Genetic guidelines for hatchery supplementation programs. In E. M. Hallerman (Ed.), *Population genetics: Principles and applications for fisheries scientists* (pp. 329–355). Bethesda, MD: American Fisheries Society.

Mjølnerod, I. B., Refseth, U. H., & Hindar, K. (1999). Spatial association of genetically similar Atlantic salmon juveniles and sex bias in spatial patterns in a river. *Journal of Fish Biology*, 55, 1–8.

Ocalewicz, K., Gurgul, A., Pawłina-Tyszko, K., Szmatała, T., Jasielczuk, I., Bugno-Poniewierska, M., & Dobosz, S. (2019). Induced androgenetic development in rainbow trout and transcriptome analysis of irradiated eggs. *Scientific Reports*, 9, 8084.

Parsons, J. E., & Thorgaard, G. H. (1984). Induced androgenesis in rainbow trout. *Reproductive Biology*, 231, 407–412.
Pérez-Cerezales, S., Martínez-Páramo, S., Cabrita, E., Martínez-Pastor, F., de Paz, P., & Herráez, M. P. (2009). Evaluation of oxidative DNA damage promoted by storage in sperm from sex-reversed rainbow trout. Theriogenology, 71, 605–613.

Pitnick, S., Hosken, D. J., & Birkhead, T. R. (2008). Sperm morphological diversity. In T. R. Birkhead, D. J. Hosken, & S. Pitnick (Eds.), Sperm biology: An evolutionary perspective (pp. 75–149). Burlington, MA: Elsevier Academic Press.

Poole, W. R., & Dillane, M. G. (1998). Estimation of sperm concentration of wild and reconditioned brown trout, Salmo trutta L. Aquaculture Research, 29, 439–445.

Rurangwa, E., Kime, D., Ollevier, F., & Nash, J. (2004). The measurement of sperm motility and factors affecting sperm quality in cultured fish. Aquaculture, 234, 1–28.

Ryman, N., & Laikre, L. (1991). Effects of supportive breeding on the genetically effective population size. Conservation Biology, 5, 325–329.

Sandodden, R., Brazier, M., Sandvik, M., Moen, A., Wist, A. N., Adolfsen, P., & Barnes, M. (2018). Eradication of Gyrodactylus salaris infested Atlantic salmon (Salmo salar) in the Rauma River, Norway, using rotenone. Management of Biological Invasions, 9, 67–77.

Sonesson, A. K., Goddard, M. E., & Meuwissen, T. H. E. (2002). The use of frozen semen to minimize inbreeding in small populations. Genetical Research, 80, 27–30.

Spanò, M., Cordelli, E., Leter, G., Lombardo, F., Lenzi, A., & Gandini, L. (1999). Nuclear chromatin variations in human spermatozoa undergoing swim-up and cryopreservation evaluated by the flow cytometric sperm chromatin structure assay. Molecular Human Reproduction, 5, 29–37.

Stoss, J., & Refstie, T. (1983). Short-term storage and cryopreservation of milt from Atlantic salmon and sea trout. Aquaculture, 30, 229–236.

Svenning, M-A., Falkegård, M., Hanssen, Ø. K.. (2012). Sjørøya i Nord-Norge - en fallende dronning? NINA Rapport 780.

Tiersch, T. R. (2008). Strategies for commercialization of cryopreserved fish semen. Revista Brasileira de Zootecnia, 37, 15–19.

Verspoor, E., Stradmeyer, L., Nielsen, J. L., & editors. (2007). The Atlantic salmon: Genetics, conservation, and management. Oxford: Blackwell Publishing Ltd.

Wang, J. (2011). Coancestry: A program for simulating, estimating and analysing relatedness and inbreeding coefficients. Molecular Ecology Resources, 11, 141–145.

Weinhold, B. (2006). Epigenetics: The science of change. Environmental Health Perspectives, 114, A160–A167.

Williams, S. E., & Hoffman, E. A. (2009). Minimizing genetic adaptation in captive breeding programs: A review. Biological Conservation, 142, 2388–2400.

Witzenberger, K. A., & Hochkirch, A. (2011). Ex situ conservation genetics: A review of molecular studies on the genetic consequences of captive breeding programmes for endangered animal species. Biodiversity and Conservation, 20, 1843–1861.

Wright, S. (1940). Breeding structure of populations in relation to speciation. The American Naturalist, 74, 232–248.

Yamamoto, T., Maruta, H., Suzuki, T., & Kitanishi, S. (2015). Sperm traits dependent on body size in masu salmon Oncorhynchus masou. Fisheries Science, 81, 815–820.

Young, W. P., Freneya, K., Wheeler, P. A., & Thorgaard, G. H. (2009). No increase in developmental deformities or fluctuating asymmetry in rainbow trout (Oncorhynchus mykiss) produced with cryopreserved sperm. Aquaculture, 289, 13–18.

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