Cryopreservation of milt from two endangered fishes: Atlantic Whitefish (*Coregonus huntsmani*) and inner Bay of Fundy Atlantic Salmon (*Salmo salar*)

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Abstract

Cryopreservation, the storage of tissues via freezing in liquid nitrogen, is an important component of the inner Bay of Fundy (iBoF) Atlantic Salmon (*Salmo salar*) Live Gene Banking (LGB) program. Cryopreservation has also been conducted as part of the conservation efforts for endangered Atlantic Whitefish (*Coregonus huntsmani*). Cryopreservation is a method to preserve tissue, in this case male gametes, for an indefinite amount of time. Gametes can then be thawed and used to fertilise female gametes and produce viable offspring. Cryopreservation could help to minimise the loss of genetic diversity and reduce the likelihood of extirpation of iBoF Salmon and the extinction of Atlantic Whitefish. However, development of effective cryopreservation protocols specifically for these endangered fishes has been challenging. The use of recently published cryopreservation protocols, and the development of new procedures, has yielded excellent post-thaw fertilisation success for Atlantic Salmon and moderate success for Atlantic Whitefish milt. The purpose of this document is threefold. First it describes the best techniques developed to date for the cryopreservation of Atlantic Whitefish and inner Bay of Fundy Atlantic Salmon male gametes. Second it outlines recommendations for future cryopreservation activities in the LGB. Third, it provides an inventory of fish from which milt has been cryopreserved.

Résumé

La cryopréservation, c'est-à-dire la conservation de tissus congelés dans de l'azote liquide, est un élément important du programme de stockage de gènes vivants de saumon atlantique (*Salmo salar*) de l'arrière-baie de Fundy. La cryopréservation est aussi utilisée dans le cadre des initiatives de conservation du corégone atlantique (*Coregonus huntsmani*), qui est en voie de disparition. Cette méthode permet de conserver des tissus, en l'occurrence des gamètes mâles, pendant un temps illimité. Les gamètes peuvent ensuite être décongelés pour servir à féconder des gamètes femelles et produire une progéniture viable. La cryopréservation pourrait donc contribuer à réduire la perte de diversité génétique et la probabilité de disparition du pays du saumon de l'arrière-baie de Fundy et de disparition de la planète du corégone atlantique. Toutefois, il s'est avéré difficile de concevoir des protocoles de cryopréservation efficaces dans le cas de ces poissons en voie de disparition. Le recours à des protocoles de cryopréservation récemment publiés et la mise en place de nouvelles procédures se sont soldés par d'excellents résultats dans la fécondation après décongélation pour ce qui est du saumon atlantique et à une réussite modérée dans le cas de la laitance de corégone atlantique. Le présent document a un triple objet : décrire les meilleures techniques conçues jusqu'ici pour cryopréserver les gamètes mâles de corégone atlantique et de saumon atlantique de l'arrière-baie de Fundy; formuler des recommandations au sujet des futures activités de cryopréservation dans le cadre du programme de stockage de gènes vivants et enfin dresser l'inventaire des poissons dont la laitance a été cryopréservée.

Introduction

Cryopreservation is the rapid freezing of tissue, typically in liquid nitrogen at -196°C. Prior to being frozen, tissue, usually male or female gametes, is mixed with a cryoprotectant. The cryoprotectant is typically composed of sugars and salts that prevent the destructive formation of ice crystals inside cells (Maxur 1970). This process may greatly increase the tissue's viability when thawed at a later date. Uses of cryopreservation vary from preservation of bull semen for commercial purposes (Gravance *et al.* 2009) to the conservation of gametes from individuals of endangered populations (Gwo *et al.* 1999).

The Live Gene Bank (LGB) is a captive breeding and rearing program for endangered inner Bay of Fundy (iBoF) Atlantic Salmon (*Salmo salar*) that was established in 1998 by the Department of Fisheries and Oceans (DFO; DFO 2010). The program involves the collection of representatives from remaining wild populations of iBoF Atlantic Salmon, the rearing of individuals in captivity to maturity, and the artificial spawning of adults. Offspring are either retained in captivity or released into the wild and recaptured at a later life stage for subsequent rearing to maturity and spawning. The goals of the program are to prevent the extirpation of this assemblage of populations, and to minimise the loss of genetic diversity and the loss of fitness due to time spent in captivity (DFO 2010, O'Reilly and Doyle 2007). Collections of Atlantic Whitefish from the one remaining wild population were obtained in 2000 through to 2005 from a lake on the Petite Rivière drainage, Nova Scotia. These fish were reared to maturity at the Mersey Biodiversity Facility and then spawned. Offspring of these founders are being used for experimental releases into the wild (DFO 2006) as well as for cryopreservation purposes.

Male gametes from genetically rare and important iBoF Atlantic Salmon have been cryopreserved every year since 2005. Male gametes from Atlantic Whitefish have been cryopreserved every year since 2006. Due to a lack of pedigree information for Atlantic Whitefish, genetically rare individuals could not be identified. Instead milt from as many as was possible Atlantic Whitefish founders and offspring produced by the first artificial spawnings was cryopreserved. Because the size of both captive populations of fish is finite, as is the number of matings carried out each year, it is expected that loss of genetic diversity will occur, especially if the conservation programs continue for many years (Lynch and O'Healy 2001). As the captive environment differs greatly from the wild environment, it is also expected that animals maintained in captivity for several generations will experience a reduced fitness when returned to the wild compared to fish with no history of captivity (Frankham 2008). Preserving milt from first captive generation salmon males (Sonesson *et al.* 2002) and using these gametes to fertilise eggs in later generations could help restore variation lost through genetic drift. In addition, the use of male gametes from these early (first or second generation) fish could help restore original allele frequency distributions that may have been altered through multiple generations of genetic drift and selection for captive conditions (O'Reilly and Doyle 2007). As well, in the event extinction of Atlantic Whitefish or extirpation of iBoF Atlantic Salmon occurs, cryopreserved gametes could be thawed and used to fertilise eggs from closely related species or populations to produce hybrid offspring. Multiple backcrosses using cryopreserved milt, could in theory, then be used to restore the original characteristics of the endangered species.

The development of successful cryopreservation techniques for Atlantic Whitefish and iBoF Atlantic Salmon has been challenging despite the reports of high rates of successful cryopreservation of male gametes from other populations of Atlantic Salmon, and from species related to Atlantic Whitefish (*e.g.*, Gwo *et al.* 1999, Lahnsteiner 2000, Piironen and Hyvarinen 1983). Initially, cryopreservation of Atlantic Salmon gametes met with little success: thawed cryopreserved sperm yielded very poor (0 to 5%) survival to eyed egg life stage relative to control (fresh) milt. In 2008 a new cryoprotectant and new freezing techniques to cryopreserve Atlantic Salmon milt yielded considerably higher rates of survival to the eyed egg stage. Unfortunately, no research has yet been published on the cryopreservation of Atlantic Whitefish milt. Methodology developed for other related fishes, in this case other whitefish species, is often not transferable and can yield poor fertilisation success.

The objectives of this document are to detail the best techniques developed to date for the cryopreservation of Atlantic Whitefish and iBoF Atlantic Salmon male gametes, outline recommendations for future cryopreservation activities, and to provide an inventory of all fish cryopreserved as of December 31, 2008.

Methods

Male gametes from iBoF Atlantic Salmon have been cryopreserved since 2005. However, from 2005 to 2007, the cryoprotectant used (Lahnsteiner 2000) yielded very low levels of survival to the eyed egg stage of eggs fertilised with thawed cryopreserved milt (data not shown). Only methods using the more successful cryoprotectant used in 2008 are reported here. All fish (Atlantic Salmon and Atlantic Whitefish) from which milt was cryopreserved in 2005 to 2008, regardless of the cryoprotectant used, are reported in Appendix I.

Cryopreservation of Atlantic Salmon male gametes predominantly occurred during their natural spawning time, which varies from early to late November at the Mactaquac and Coldbrook Biodiversity Facilities. Males were anesthetised in a mixture of MS-222 and water at a concentration of 40-50 mg/L. The ventral side of each male was dried to prevent activation of sperm by water, and milt was extruded using manual manipulation. Milt was immediately mixed with cryoprotectant in a dilution ratio of 1:3 (milt:cryoprotectant). A stock glucose extender (54.04 g glucose/L of distilled water and 1.7 g KCl/L of distilled water) was used to create the cryoprotectant, which was 10% methanol, 13.3% fresh egg yolk (free-range organic), and 76.7% glucose extender (as described in Jodun *et al.* 2006).

The mixture of milt and cryoprotectant was drawn into 0.5 ml straws (IMV Technologies, Catalogue Number 5569), leaving approximately 1 cm of space in the straw above and below the mixture to prevent cracking of the straw during freezing due to expansion of liquid. The end of the straw was stopped with a plastic sealing agent. Approximately ten straws were placed in visotubes (IMV Technologies, Catalogue Number 5561) held on canes (Gencor, Catalogue Number XC052). Each visotube was labelled with the unique identification number of the male whose gametes were contained within, and each cane was numbered. A record was kept of which males were held in which canes (see Appendix I).

The straws containing the mixture of milt and cryoprotectant in the visotubes held on the canes were then placed in a 'vapour Dewar' for 30 min. The vapour Dewar (PraxAir, Model MVE 10743027) contained approximately 10 cm of liquid nitrogen, and was thus mostly filled with nitrogen vapour. Straws cooled at a uniform rate of -22°C per minute in the vapour Dewar (Jodun *et al.* 2006) where they were exposed to nitrogen vapour rather than submersed in liquid nitrogen. After 30 minutes, the canes were removed from the vapour Dewar and immediately immersed in liquid nitrogen (-196°C) in the storage Dewar (Prax Air, Model MVE 10743027).

Cryopreservation of Atlantic Whitefish male gametes occurred during their natural spawning time in December at the Mersey Biodiversity Facility. All protocols were identical to those for the Atlantic Salmon with the following exceptions. A cryoprotectant, developed for Muksun (*Coregonus muksun*), composed of 0.3 M glucose, 20% glycerol, and 80% distilled water was used (Piironen and Hyvarinen 1983). Milt from more than one male was combined and mixed with the cryoprotectant in a 1:3 ratio, as insufficient volumes were obtained from single Whitefish males.

Test crosses were performed at all three Biodiversity Facilities in 2008 to evaluate the capability of the cryopreserved Atlantic Whitefish and Atlantic Salmon milt to produce viable eggs. Protocols were similar for both species. Eggs (approximately 1000) from individual females were split into two equal portions, one of which was fertilised with thawed cryopreserved milt and the other with fresh milt from the same male. Thawed cryopreserved milt from ten straws was used to fertilise one of the portions of eggs, and a volume of fresh milt equal to the volume of thawed cryopreserved milt was used to fertilise the second portion of eggs for each cross. Milt was thawed by placing the straws in a warm water bath (at ~40°C) for approximately seven seconds (Jodun *et al.* 2006). Ten of these crosses were conducted at the Coldbrook Biodiversity Facility, eight were conducted at the Mactaquac Biodiversity Facility, and five were conducted at the Mersey Biodiversity Facility. As noted above, milt from more than one Atlantic Whitefish male was combined, thus the crosses conducted using Atlantic Whitefish milt involved fertilising a female's eggs with milt from multiple males. Approximate rates of sperm motility were assessed throughout the test cross procedure. Survival through to the eyed egg stage was used as a metric to compare fresh and thawed-cryopreserved sperm. It represents a minimum estimate of fertilisation success as successfully fertilised eggs may have died during development or been infected with fungus prior to the eyed egg stage.

All cryopreserved gametes were stored in Dewars (PraxAir, Model MVE 10743027) maintained at the Bedford Institute of Oceanography, Dartmouth, Nova Scotia. Replenishment of liquid nitrogen is ongoing and will continue until all preserved milt is thawed to fertilise eggs.

Results

Male gametes from 256 Atlantic Salmon and approximately 35 Atlantic Whitefish have been cryopreserved as of December 2008 (Appendix I). In 2008 survival to the eyed egg stage of embryos fertilised with thawed cryopreserved Atlantic Salmon milt ranged from 0.3% to 93.6% and averaged 54.0% at the Coldbrook Biodiversity Facility (Table 1). Survival ranged from 1.2% to 16.0% and averaged 6.0% at the Mactaquac Biodiversity Facility (Table 2). Motility of Atlantic Salmon sperm before and after being mixed with the cryoprotectant was 100%, while post-thaw motility ranged from 0.0% to 70% for the Stewiacke River Atlantic Salmon, and from 0.0% to 5% for the Big Salmon River Atlantic Salmon. Survival to eyed egg stage of eggs fertilised with thawed cryopreserved Atlantic Whitefish milt averaged much lower than that of Atlantic Salmon, ranging from 0.0% to 2.1 and averaging 0.5% (Table 3). Motility of fresh Atlantic Whitefish sperm was 100% before being mixed with the cryoprotectant, but could not be detected after being mixed with the cryoprotectant. No post-thaw motility was observed.

Discussion

Cryopreservation techniques used to preserve iBoF Atlantic Salmon gametes in 2008 appear to be highly successful while methods used to preserve Atlantic Whitefish male gametes were moderately successful. Rates of survival of Atlantic Salmon embryos to the eyed egg life stage using thawed cryopreserved milt averaged 6.3% and 54.4% at the two Biodiversity Facilities. Thus, cryopreservation is a viable tool with which to preserve genetic diversity within the LGB in a manner that allows it to be accessed in the future. However, due to the limited percent survival of Atlantic Whitefish gametes produced using cryopreserved milt, improvements in techniques are needed.

Survival to eyed egg life stage of eggs fertilised with thawed cryopreserved milt was low (less than 5.0%) in years prior to 2008. The increased success of cryopreservation of Atlantic Salmon milt experienced in 2008 may be due to several factors. A cryoprotectant was used in 2008 that did not contain dimethyl sulfoxide (DMSO), present in previous cryoprotectants. Despite reports of high rates of fertilisation success (≤ 95%) using thawed Atlantic Salmon milt frozen with cryoprotectants containing DMSO (*e.g.*, Gwo *et al.* 1999, Lahnsteiner 2000), very low survival to eyed-egg stage was achieved for iBoF Atlantic Salmon (data not shown). DMSO can be toxic to animal cells (Sigma-Aldrich 2009) and likely Atlantic Salmon sperm as well, in that it allows other chemicals to readily pass through cell walls (USFDA 2009). It is possible that this potential toxicity, combined with a characteristic of sperm that is unique to iBoF Atlantic Salmon, may account for the lack of success obtained when using cryoprotectants containing DMSO. It is also possible that the method of cooling the milt and cryoprotectant mixture may account for the increased success in 2008, as prior to 2008 an alternate method (not described) was used that did not offer as much control over the rate of cooling. Other factors that could contribute to the increased survival to eyed egg stage in 2008 may include variation in egg and/or milt quality between years, and variation other environmental conditions in the Biodiversity Facilities.

The difference in fertilisation success between test crosses conducted using Big Salmon River Salmon at the Mactaquac Biodiversity Facility and Stewiacke River Salmon at the Coldbrook Biodiversity Facility is noteworthy. It is possible that differences in sperm characteristics between salmon from the two rivers may account for the differences in fertilisation success. For example, milt from Big Salmon River Salmon may be more vulnerable to damage during the cryopreservation process than that from Stewiacke River salmon. As well, it is possible that the timing of cryopreservation relative to the spawning season may have influenced the observed fertilisation success. Cryopreservation of Big Salmon River male gametes occurred toward the end of the spawning season and involved the use of milt from males that had been previously spawned, whereas cryopreservation of Stewiacke River male gametes occurred during the spawning season and involved the use of milt from males that had not been previously spawned. A decreased incidence of blood and urine was observed in the Stewiacke River milt compared to the Big Salmon River milt (data not shown), suggesting that repeat spawning may decrease the quality of milt obtained.

The low rate of survival to the eyed egg stage of eggs fertilised with thawed cryopreserved Atlantic Whitefish male gametes compared to that obtained using Atlantic Salmon male gametes is likely due to the fact that the cryoprotectant used was developed for another species, *Coregonus muksun*. Cryoprotectants are often species specific as sperm characteristics vary among species (Lahnsteiner 2000). Alternate cryoprotectants should be tested.

Long-term storage of cryopreserved gametes currently relies on levels of liquid nitrogen being manually replenished making samples are vulnerable to destruction through human error.

Based on the above considerations the following recommendations are made:

1. The methanol-based cryoprotectant used in 2008 to cryopreserve Atlantic Salmon male gametes should continue to be used, along with the Dewar method of cooling.
2. Milt from as many founders and first or second generation LGB males as is possible should be preserved before they die or are released.
3. Cryopreservation should occur during the first half of the spawning season and use milt from males that have not been spawned that year, if possible.
4. New cryoprotectants should be tested on Atlantic Whitefish.

5. Test crosses to evaluate fertilisation success of thawed cryopreserved milt should continue at all three Biodiversity Facilities.
6. Permanent and secure long-term options for storage of cryopreserved samples, including maintenance at two or more sites, should be investigated.

Finally, while the described cryopreservation techniques produce male gametes that are able to successfully fertilise eggs, it is not certain whether there are other impacts of cryopreservation on embryo development. It is possible that DNA is damaged or altered (through epigenetic effects for example) in the process, in such a way that the growth, survival or morphology of offspring is affected. Hayes *et al.* (2005) found that captive steelhead trout (*Oncorhynchus mykiss*) produced using cryopreserved milt had smaller body sizes than offspring produced using fresh milt, in trials with low food availability. As well, offspring produced using cryopreserved milt exhibited higher levels of the stress hormone cortisol when exposed to stress (Hayes *et al.* 2005). To our knowledge, no similar studies have been conducted on Atlantic Salmon, and none on any salmonid in wild native habitat, where environmental conditions are expected to be less benign. We recommend the testing of survival and growth of the offspring produced using cryopreserved milt as well as their next generation offspring to evaluate the possibility of any intergenerational genetic or epigenetic effects of cryopreservation.

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Table 1. Survival to eyed egg life stage of ten test crosses conducted using fresh (F) and cryopreserved (C) milt from Stewiacke River Atlantic Salmon males crossed with Stewiacke River females. Each test cross was conducted using the milt and eggs from one male and one female. Crosses occurred on November 6 2008 at the Coldbrook Biodiversity Facility, and mortalities were assessed no later than January 29 2009. Average percent survival was calculated excluding cross # 7, as the lack of fertilisation success using the fresh milt indicated that the female gametes may have not be viable.

Test cross	Total number of eggs from female	Number of surviving fertilised eggs	Number of mortalities	Percent survival
1F	445	302	143	67.9
1C	288	2	286	0.7
2F	647	579	68	89.5
2C	513	289	224	56.3
3F	399	327	72	81.9
3C	462	273	189	59.1
4F	524	519	5	99.0
4C	497	465	32	93.6
5F	354	351	3	99.2
5C	492	386	106	78.5
6F	931	840	91	90.2
6C	825	635	190	77.0
7F	188	0	188	0.0
7C	312	1	311	0.3
8F	525	521	4	99.2
8C	401	123	278	30.7
9F	519	496	23	95.6
9C	513	275	238	53.6
10F	545	529	16	97.1
10C	545	218	327	40.0
Average F				91.1
Average C				54.4

Table 2. Survival to eyed egg life stage of eight test crosses conducted using fresh (F) and cryopreserved (C) milt from Big Salmon River Atlantic Salmon males crossed with Big Salmon River females. Each test cross was conducted using the milt and eggs from one male and one female. Crosses occurred on December 2 2008 at the Mactaquac Biodiversity Facility and mortalities were assessed no later than February 19 2009. Information provided by Graham Chafe, DFO, Mactaquac Biodiversity Facility.

Test cross	Total number of eggs from female	Number of surviving fertilised eggs	Number of mortalities	Percent survival
1F	1571	1097	474	69.8
1C	1304	111	1193	8.5
2F	1992	1060	932	53.2
2C	1726	39	1687	2.3
3F	1250	1013	237	81.0
3C	1215	194	1021	16.0
4F	1278	983	295	76.9
4C	1328	66	1262	5.0
5F	1015	242	773	23.9
5C	517	6	511	1.2
6F	1462	1167	295	79.8
6C	1565	87	1478	5.6
7F	1952	1783	169	91.3
7C	1769	164	1605	9.3
8F	2086	303	1783	14.5
8C	1206	29	1177	2.4
Average F				61.3
Average C				6.3

Table 3. Survival to eyed egg life stage of five test crosses conducted using fresh (F) and cryopreserved (C) milt from Atlantic Whitefish males crossed with Atlantic Whitefish females. Each test cross was conducted using the eggs from one female and the milt from multiple (<6) males. Crosses occurred on December 4 2008 at the Mersey Biodiversity Facility and mortalities were assessed no later than March 10 2009.

Test cross	Total number of eggs from female	Number of surviving fertilised eggs	Number of mortalities	Percent survival
1F	1241	647	594	52.1
1C	1174	0	1174	0.0
2F	738	504	234	68.3
2C	711	1	710	0.1
3F	1044	536	508	51.3
3C	1216	25	1191	2.1
4F	491	210	281	42.8
4C	524	0	524	0.0
5F	689	367	322	53.3
5C	599	2	597	0.3
Average F				53.6
Average C				0.5

Appendix

Table A. Inventory and storage locations in Dewars of all Atlantic Salmon and Atlantic Whitefish whose gametes have been cryopreserved from 2005 to 2008. Pit or Carlin Tag indicates the unique (within river) identifier given to all Atlantic Salmon in the LGB. No unique identifier was available for cryopreserved Atlantic Whitefish and so the generation (first generation, G1, for all fish) and number of males cryopreserved is provided.

Pit or Carlin Tag	Cryopreservation Date	Biodiversity Facility	River of Origin	Cane Number	Position in Cane (Top or Bottom)	Canister	Dewar
Atlantic Salmon							
RDHZ58369	18-Nov-05	Mactaquac	Upper Salmon	n/a	n/a	1	A
RDHZ58371	18-Nov-05	Mactaquac	Upper Salmon	n/a	n/a	1	A
AA84251	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
D845	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
D286	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
A774	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
A770	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
A661	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
AA84503	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
F6692	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
A785	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
4315404942	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
D299	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
A646	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
A736	21-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
AA84190	22-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
AA84547	22-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
43153C2A6E	22-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
F6867	22-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
F6691	22-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
AA84518	22-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
435F6A6B76	22-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
A801	22-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
430E401E77	22-Nov-05	Mactaquac	Big Salmon	n/a	n/a	1	A
4237782970	16-Nov-06	Coldbrook	Stewiacke	7	T	2	A
4233012A62	16-Nov-06	Coldbrook	Stewiacke	13	B	2	A
4233007314	16-Nov-06	Coldbrook	Stewiacke	15	B	3	A
42353A3A68	16-Nov-06	Coldbrook	Stewiacke	15	T	3	A
4232790614	16-Nov-06	Coldbrook	Stewiacke	16	T	3	A
430E632C5A	16-Nov-06	Coldbrook	Stewiacke	10	B	2	A
431544610B	16-Nov-06	Coldbrook	Stewiacke	8	T	2	A
42327A3117	16-Nov-06	Coldbrook	Stewiacke	7	B	2	A
430E77064B	16-Nov-06	Coldbrook	Stewiacke	12	B	2	A
43155E3F38	16-Nov-06	Coldbrook	Stewiacke	13	T	2	A
431042424C	16-Nov-06	Coldbrook	Stewiacke	14	T	3	A
430E651133	16-Nov-06	Coldbrook	Stewiacke	16	B	3	A
421D7A2B2D	16-Nov-06	Coldbrook	Stewiacke	10	T	2	A

Pit or Carlin Tag	Cryopreservation Date	Biodiversity Facility	River of Origin	Cane Number	Position in Cane (Top or Bottom)	Canister	Dewar
4225417E13	16-Nov-06	Coldbrook	Stewiacke	11	T	2	A
421D541C76	16-Nov-06	Coldbrook	Stewiacke	12	T	2	A
435F7A7738	16-Nov-06	Coldbrook	Stewiacke	14	B	3	A
4229120D28	16-Nov-06	Coldbrook	Stewiacke	17	N/A	3	A
430E493A2D	16-Nov-06	Coldbrook	Stewiacke	11	B	2	A
A45177	16-Nov-06	Coldbrook	Stewiacke	6	B	1	A
454B3C182B	16-Nov-06	Coldbrook	Stewiacke	2	B	1	A
454B440D21	16-Nov-06	Coldbrook	Stewiacke	3	T	1	A
A45246	16-Nov-06	Coldbrook	Stewiacke	3	B	1	A
A45248	16-Nov-06	Coldbrook	Stewiacke	4	B	1	A
43600A3B17	16-Nov-06	Coldbrook	Stewiacke	1	N/A	1	A
A45183	16-Nov-06	Coldbrook	Stewiacke	9	T	2	A
A45166	16-Nov-06	Coldbrook	Stewiacke	8	B	2	A
454B486772	16-Nov-06	Coldbrook	Stewiacke	4	T	1	A
454B486772	16-Nov-06	Coldbrook	Stewiacke	5	T	1	A
454B555D5E	16-Nov-06	Coldbrook	Stewiacke	5	B	1	A
454827092	16-Nov-06	Coldbrook	Stewiacke	6	T	1	A
454A240923	16-Nov-06	Coldbrook	Stewiacke	2	T	1	A
4225707D68	16-Nov-06	Coldbrook	Stewiacke	9	B	2	A
4310664825	22-Nov-06	Mersey	Stewiacke	M2	T	4	A
421E21187D	22-Nov-06	Mersey	Stewiacke	M1	T	4	A
42257E2F64	22-Nov-06	Mersey	Stewiacke	M5	B	4	A
421E0C1825	22-Nov-06	Mersey	Stewiacke	M5	T	4	A
4225704A1B	22-Nov-06	Mersey	Stewiacke	M6	T	4	A
435F66053F	22-Nov-06	Mersey	Stewiacke	M8	B	4	B?
4311331A67	22-Nov-06	Mersey	Stewiacke	M1	B	4	A
430E671154	22-Nov-06	Mersey	Stewiacke	M2	B	4	A
435F71606F	22-Nov-06	Mersey	Stewiacke	M3	B	4	A
430E691C17	22-Nov-06	Mersey	Stewiacke	M3	T	4	A
431146471D	22-Nov-06	Mersey	Stewiacke	M4	B	4	A
4315574F7F	22-Nov-06	Mersey	Stewiacke	M4	T	4	A
430E445E02	22-Nov-06	Mersey	Stewiacke	M6	B	4	A
435F775463	22-Nov-06	Mersey	Stewiacke	M7	B	4	B?
435F587F0E	22-Nov-06	Mersey	Stewiacke	M7	T	4	B?
430E553068	22-Nov-06	Mersey	Stewiacke	M8	T	4	B?
486E173D20	16-Nov-07	Coldbrook	Stewiacke	20	B	2	B
486C103C0B	16-Nov-07	Coldbrook	Stewiacke	29	B	2	B
486A35596C	16-Nov-07	Coldbrook	Stewiacke	35	T	3	B
486A3F095B	16-Nov-07	Coldbrook	Stewiacke	38	B	3	B
42290A5E03	16-Nov-07	Coldbrook	Stewiacke	21	T	2	B
421C477A59	16-Nov-07	Coldbrook	Stewiacke	27	T	2	B
421E043203	16-Nov-07	Coldbrook	Stewiacke	28	T	2	B
435F5D172A	16-Nov-07	Coldbrook	Stewiacke	28	B	2	B
A45164	16-Nov-07	Coldbrook	Stewiacke	18	T	2	B
435F74500A	16-Nov-07	Coldbrook	Stewiacke	19	T	2	B
4311083570	16-Nov-07	Coldbrook	Stewiacke	22	B	2	B
4315550727	16-Nov-07	Coldbrook	Stewiacke	22	T	2	B
4311440A7C	16-Nov-07	Coldbrook	Stewiacke	23	B	2	B

Pit or Carlin Tag	Cryopreservation Date	Biodiversity Facility	River of Origin	Cane Number	Position in Cane (Top or Bottom)	Canister	Dewar
D600	16-Nov-07	Coldbrook	Stewiacke	24	B	2	B
430E553551	16-Nov-07	Coldbrook	Stewiacke	25	B	2	B
435F795166	16-Nov-07	Coldbrook	Stewiacke	26	T	2	B
431537666F	16-Nov-07	Coldbrook	Stewiacke	29	T	2	B
431165084B	16-Nov-07	Coldbrook	Stewiacke	30	T	3	B
D849	16-Nov-07	Coldbrook	Stewiacke	33	B	3	B
4315570E25	16-Nov-07	Coldbrook	Stewiacke	35	B	3	B
436010413D	16-Nov-07	Coldbrook	Stewiacke	36	T	3	B
D534	16-Nov-07	Coldbrook	Stewiacke	37	T	3	B
43115B0747	16-Nov-07	Coldbrook	Stewiacke	37	B	3	B
D663	16-Nov-07	Coldbrook	Stewiacke	38	T	3	B
D882	16-Nov-07	Coldbrook	Stewiacke	39	T	3	B
454B57620F	16-Nov-07	Coldbrook	Stewiacke	18	B	2	B
43110C1B17	16-Nov-07	Coldbrook	Stewiacke	19	B	2	B
43132C226F	16-Nov-07	Coldbrook	Stewiacke	20	T	2	B
4360134643	16-Nov-07	Coldbrook	Stewiacke	21	B	2	B
4549337745	16-Nov-07	Coldbrook	Stewiacke	24	T	2	B
454A270C63	16-Nov-07	Coldbrook	Stewiacke	25	T	2	B
454957073B	16-Nov-07	Coldbrook	Stewiacke	26	B	2	B
45494B5A74	16-Nov-07	Coldbrook	Stewiacke	30	B	3	B
4549363B78	16-Nov-07	Coldbrook	Stewiacke	31	T	3	B
4549086473	16-Nov-07	Coldbrook	Stewiacke	31	B	3	B
4548740618	16-Nov-07	Coldbrook	Stewiacke	32	T	3	B
454964232A	16-Nov-07	Coldbrook	Stewiacke	32	B	3	B
430E74563F	16-Nov-07	Coldbrook	Stewiacke	33	T	3	B
454A1D1533	16-Nov-07	Coldbrook	Stewiacke	34	T	3	B
454B3F2906	16-Nov-07	Coldbrook	Stewiacke	34	B	3	B
454B617176	16-Nov-07	Coldbrook	Stewiacke	36	B	3	B
B693	5-Dec-07	Mactaquac	Big Salmon	40	T	3	B
B719	5-Dec-07	Mactaquac	Big Salmon	45	T	4	B
470C1D5412	5-Dec-07	Mactaquac	Big Salmon	56	B	4	B
B604	5-Dec-07	Mactaquac	Big Salmon	42	B	3	B
B883	5-Dec-07	Mactaquac	Big Salmon	41	B	3	B
B874	5-Dec-07	Mactaquac	Big Salmon	43	B	3	B
B557	5-Dec-07	Mactaquac	Big Salmon	44	B	3	B
454B570A33	5-Dec-07	Mactaquac	Big Salmon	60	B	4	B
B574	5-Dec-07	Mactaquac	Big Salmon	54	T	4	B
460E264E56	5-Dec-07	Mactaquac	Big Salmon	54	B	4	B
4233324478	5-Dec-07	Mactaquac	Big Salmon	52	T	4	B
F8174	5-Dec-07	Mactaquac	Big Salmon	53	B	4	B
460D684070	5-Dec-07	Mactaquac	Big Salmon	49	T	4	B
4549675747	5-Dec-07	Mactaquac	Big Salmon	50	B	4	B
4549555262	5-Dec-07	Mactaquac	Big Salmon	62	B	5	B
AA84541	5-Dec-07	Mactaquac	Big Salmon	59	T	4	B
42377C6377	5-Dec-07	Mactaquac	Big Salmon	48	B	4	B
4235452810	5-Dec-07	Mactaquac	Big Salmon	42	T	3	B
F8169	5-Dec-07	Mactaquac	Big Salmon	51	B	4	B
F8153	5-Dec-07	Mactaquac	Big Salmon	49	B	4	B

Pit or Carlin Tag	Cryopreservation Date	Biodiversity Facility	River of Origin	Cane Number	Position in Cane (Top or Bottom)	Canister	Dewar
435F7D580F	5-Dec-07	Mactaquac	Big Salmon	61	B	5	B
F8160	5-Dec-07	Mactaquac	Big Salmon	59	B	4	B
B568	5-Dec-07	Mactaquac	Big Salmon	51	T	4	B
4234094B23	5-Dec-07	Mactaquac	Big Salmon	53	T	4	B
430E553765	5-Dec-07	Mactaquac	Big Salmon	58	B	4	B
421D7E0073	5-Dec-07	Mactaquac	Big Salmon	46	T	4	B
4549690071	5-Dec-07	Mactaquac	Big Salmon	52	B	4	B
460D623270	5-Dec-07	Mactaquac	Big Salmon	41	T	3	B
45493F263A	5-Dec-07	Mactaquac	Big Salmon	47	T	4	B
45494E347E	5-Dec-07	Mactaquac	Big Salmon	48	T	4	B
454B29122D	5-Dec-07	Mactaquac	Big Salmon	60	T	4	B
AA84219	5-Dec-07	Mactaquac	Big Salmon	55	T	4	B
B592	5-Dec-07	Mactaquac	Big Salmon	46	B	4	B
B610	5-Dec-07	Mactaquac	Big Salmon	57	B	4	B
B563	5-Dec-07	Mactaquac	Big Salmon	40	B	3	B
A807	5-Dec-07	Mactaquac	Big Salmon	47	B	4	B
A672	5-Dec-07	Mactaquac	Big Salmon	57	T	4	B
454A024B18	5-Dec-07	Mactaquac	Big Salmon	43	T	3	B
46184F1964	5-Dec-07	Mactaquac	Big Salmon	44	T	3	B
421D240808	5-Dec-07	Mactaquac	Big Salmon	55	B	4	B
454B434B09	5-Dec-07	Mactaquac	Big Salmon	62	T	5	B
422E1C3677	5-Dec-07	Mactaquac	Big Salmon	56	T	4	B
430E4F080F	5-Dec-07	Mactaquac	Big Salmon	50	T	4	B
F8188	5-Dec-07	Mactaquac	Big Salmon	61	T	5	B
F8185	5-Dec-07	Mactaquac	Big Salmon	58	T	4	B
RDHZ58553	5-Dec-07	Mactaquac	Upper Salmon	45	B	4	B
421C1D625C	06-Nov-08	Coldbrook	Stewiacke	72	N/A	1	C
43110C1B17	06-Nov-08	Coldbrook	Stewiacke	73	N/A	1	C
4549491E70	06-Nov-08	Coldbrook	Stewiacke	74	N/A	1	C
486A624231	06-Nov-08	Coldbrook	Stewiacke	75	B	1	C
454B57620F	06-Nov-08	Coldbrook	Stewiacke	75	T	1	C
4874584B18	06-Nov-08	Coldbrook	Stewiacke	76	B	1	C
486A1D535E	06-Nov-08	Coldbrook	Stewiacke	77	T	1	C
4549363B78	06-Nov-08	Coldbrook	Stewiacke	77	B	1	C
454A092E7F	06-Nov-08	Coldbrook	Stewiacke	78	B	1	C
431042424C	06-Nov-08	Coldbrook	Stewiacke	78	T	1	C
47041D6A0D	06-Nov-08	Coldbrook	Gaspereau	64	B	6	B
486B0C5930	06-Nov-08	Coldbrook	Gaspereau	65	B	6	B
422565697B	06-Nov-08	Coldbrook	Gaspereau	65	T	6	B
4B08672344	06-Nov-08	Coldbrook	Gaspereau	66	B	6	B
4708771552	06-Nov-08	Coldbrook	Gaspereau	66	T	6	B
486B0E7B60	06-Nov-08	Coldbrook	Gaspereau	67	B	6	B
48761F2F11	06-Nov-08	Coldbrook	Gaspereau	68	B	6	B
47081A3D2E	06-Nov-08	Coldbrook	Gaspereau	67	T	6	B
470E050926	06-Nov-08	Coldbrook	Gaspereau	69	B	6	B
4708090E5B	06-Nov-08	Coldbrook	Gaspereau	68	T	6	B
48756C7609	06-Nov-08	Coldbrook	Gaspereau	69	T	6	B
470E155F63	06-Nov-08	Coldbrook	Gaspereau	70	B	6	B

Pit or Carlin Tag	Cryopreservation Date	Biodiversity Facility	River of Origin	Cane Number	Position in Cane (Top or Bottom)	Canister	Dewar
48760C420B	06-Nov-08	Coldbrook	Gaspereau	70	T	6	B
4708744D60	06-Nov-08	Coldbrook	Gaspereau	71	B	6	B
4311633138	06-Nov-08	Coldbrook	Stewiacke	76	T	1	C
43600A0615	06-Nov-08	Coldbrook	Stewiacke	79	T	1	C
486E196250	06-Nov-08	Coldbrook	Stewiacke	79	B	1	C
486B235149	06-Nov-08	Coldbrook	Stewiacke	80	B	1	C
4548740618	06-Nov-08	Coldbrook	Stewiacke	80	T	1	C
486E173D20	06-Nov-08	Coldbrook	Stewiacke	81	B	1	C
4876553075	06-Nov-08	Coldbrook	Stewiacke	81	T	1	C
486D6A5140	06-Nov-08	Coldbrook	Stewiacke	82	B	1	C
43112D2422	06-Nov-08	Coldbrook	Stewiacke	82	T	1	C
4876175E61	06-Nov-08	Coldbrook	Stewiacke	83	B	1	C
48745D2726	06-Nov-08	Coldbrook	Stewiacke	83	T	1	C
4360057134	06-Nov-08	Coldbrook	Stewiacke	84	T	1	C
454B3A1D79	06-Nov-08	Coldbrook	Stewiacke	84	B	1	C
4876202009	06-Nov-08	Coldbrook	Stewiacke	85	B	1	C
486B2A2948	06-Nov-08	Coldbrook	Stewiacke	85	T	1	C
4311232A76	06-Nov-08	Coldbrook	Stewiacke	86	B	1	C
48761F5753	06-Nov-08	Coldbrook	Stewiacke	86	T	1	C
4874680746	06-Nov-08	Coldbrook	Stewiacke	87	B	1	C
45494B5A74	06-Nov-08	Coldbrook	Stewiacke	87	T	1	C
430E6A2E2D	06-Nov-08	Coldbrook	Stewiacke	88	B	1	C
486A652F4B	06-Nov-08	Coldbrook	Stewiacke	88	T	1	C
431049417F	06-Nov-08	Coldbrook	Stewiacke	89	B	1	C
435F74446E	06-Nov-08	Coldbrook	Stewiacke	89	T	1	C
435F750019	06-Nov-08	Coldbrook	Stewiacke	90	B	1	C
467C014A5F	02-Dec-08	Mactaquac	Big Salmon	91	n/a	2	C
B675	02-Dec-08	Mactaquac	Big Salmon	92	n/a	2	C
431058734E	02-Dec-08	Mactaquac	Big Salmon	93	n/a	2	C
486B1B2355	02-Dec-08	Mactaquac	Big Salmon	94	n/a	2	C
46182F1F3C	02-Dec-08	Mactaquac	Big Salmon	95	n/a	2	C
430E6A3421	02-Dec-08	Mactaquac	Big Salmon	96	n/a	2	C
486A3A0B77	02-Dec-08	Mactaquac	Big Salmon	97	n/a	2	C
460E382220	02-Dec-08	Mactaquac	Big Salmon	98	n/a	2	C
454B411038	02-Dec-08	Mactaquac	Point Wolf	103	T	3	C
48641F712F	02-Dec-08	Mactaquac	Big Salmon	103	B	3	C
48743B6742	02-Dec-08	Mactaquac	Big Salmon	102	B	3	C
461844034C	02-Dec-08	Mactaquac	Big Salmon	101	T	3	C
467D180D60	02-Dec-08	Mactaquac	Big Salmon	102	T	3	C
467D037344	02-Dec-08	Mactaquac	Big Salmon	99	T	3	C
486A5A575A	02-Dec-08	Mactaquac	Big Salmon	100	T	3	C
B732	02-Dec-08	Mactaquac	Big Salmon	99	B	3	C
AA84109	02-Dec-08	Mactaquac	Big Salmon	100	B	3	C
467C3A4151	02-Dec-08	Mactaquac	Big Salmon	101	B	3	C
B505	02-Dec-08	Mactaquac	Big Salmon	105	B	3	C
486A7E3F7D	02-Dec-08	Mactaquac	Big Salmon	105	T	3	C
470C254757	02-Dec-08	Mactaquac	Big Salmon	104	B	3	C
454B594961	02-Dec-08	Mactaquac	Big Salmon	104	T	3	C

Pit or Carlin Tag	Cryopreservation Date	Biodiversity Facility	River of Origin	Cane Number	Position in Cane (Top or Bottom)	Canister	Dewar
487528361D	02-Dec-08	Mactaquac	Big Salmon	106	T	3	C
B861	02-Dec-08	Mactaquac	Big Salmon	106	B	3	C
AA84539	02-Dec-08	Mactaquac	Big Salmon	108	T	3	C
486C075C62	02-Dec-08	Mactaquac	Big Salmon	111	B	3	C
486C114164	02-Dec-08	Mactaquac	Big Salmon	109	B	3	C
4233026634	02-Dec-08	Mactaquac	Big Salmon	109	T	3	C
467C7D7D6E	02-Dec-08	Mactaquac	Big Salmon	108	B	3	C
A625	02-Dec-08	Mactaquac	Big Salmon	112	T	3	C
4875746141	02-Dec-08	Mactaquac	Big Salmon	112	B	3	C
4315556347	02-Dec-08	Mactaquac	Big Salmon	113	T	3	C
4618582E07	02-Dec-08	Mactaquac	Big Salmon	113	B	3	C
482433001D	02-Dec-08	Mactaquac	Point Wolf	114	T	3	C
470A446529	02-Dec-08	Mactaquac	Big Salmon	114	B	3	C
486E08547B	02-Dec-08	Mactaquac	Big Salmon	115	T	3	C
470A307B29	02-Dec-08	Mactaquac	Big Salmon	115	B	3	C
470A68617F	02-Dec-08	Mactaquac	Big Salmon	116	T	3	C
470C367F73	02-Dec-08	Mactaquac	Big Salmon	116	B	3	C
467C6A6771	02-Dec-08	Mactaquac	Big Salmon	117	n/a	3	C
470C673F13	02-Dec-08	Mactaquac	Big Salmon	118	T	3	C
4311073558	02-Dec-08	Mactaquac	Big Salmon	118	B	3	C
486E1E7C39	02-Dec-08	Mactaquac	Big Salmon	119	T	3	C
460E602C12	02-Dec-08	Mactaquac	Big Salmon	119	B	3	C
Atlantic Whitefish							
G1, ≤5 males	20-Dec-06	Mersey	Petite Rivière	MWF	several canes	3	A
G1, ≤5 males	13-Feb-07	Dalhousie	Petite Rivière	DALWF	1 cane	2	B
G1, ≤5 males	14-Dec-07	Mersey	Petite Rivière	WF071	n/a	6	B
G1, ≤5 males	14-Dec-07	Mersey	Petite Rivière	WF072	n/a	6	B
G1, ≤5 males	14-Dec-07	Mersey	Petite Rivière	WF073	n/a	6	B
G1, ≤5 males	14-Dec-07	Mersey	Petite Rivière	WF074	n/a	6	B
G1, ≤5 males	14-Dec-07	Mersey	Petite Rivière	WF075	n/a	6	B
G1, ≤5 males	14-Dec-07	Mersey	Petite Rivière	WF076	n/a	6	B
G1, ≤5 males	04-Dec-08	Mersey	Petite Rivière	120	n/a	3	C
G1, ≤5 males	04-Dec-08	Mersey	Petite Rivière	121	n/a	3	C
G1, ≤5 males	04-Dec-08	Mersey	Petite Rivière	122	n/a	3	C
G1, ≤5 males	04-Dec-08	Mersey	Petite Rivière	123	n/a	3	C
G1, ≤5 males	04-Dec-08	Mersey	Petite Rivière	124	n/a	3	C
G1, ≤5 males	04-Dec-08	Mersey	Petite Rivière	125	n/a	3	C