

Quantitative Marine-Performance Evaluation of a Newfoundland Atlantic Salmon Strain for Bay d'Espoir Aquaculture

V.A. Pepper, R. Withler, T. Nicholls, and C. Collier

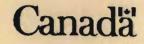
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Quantitative Marine-Performance Evaluation of a Newfoundland Atlantic Salmon Strain for Bay d'Espoir Aquaculture

by

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Use of brood stock from structured salmon breeding programs has been the goal of the Newfoundland Salmonid Growers Association (NSGA) since inception of the Bay d'Espoir aquaculture industry in the mid-1980s. A long-term goal of the association has been to use brood stocks that are adapted for superior performance under Bay d'Espoir aquaculture conditions.

The Department of Fisheries and Oceans (DFO) has had long-standing concerns regarding Atlantic salmon stocks applied to aquaculture businesses. These concerns have focused largely on potential consequences of genetic interactions of non-local strains of Atlantic salmon with wild populations. Concerns about the genetic integrity of wild salmon stocks reflect the views of the international scientific community and recommendations to fishery-resource managers that they should strive for aquaculture programs that are based on local populations as much as possible.

In 1988, the Newfoundland and Labrador Region of Fisheries and Oceans Canada worked out an arrangement with the NSGA by which the newly-developing industry could access Saint John River-origin Atlantic salmon eggs in support of objective evaluation of the aquaculture potential of Bay d'Espoir. Authorization for such imports from commercial Maritime hatcheries was to be contingent on industry participation in evaluation of a local Newfoundland salmon stock for Bay d'Espoir aquaculture development. As a consequence of this agreement, the Newfoundland and Labrador salmonid aquaculture industry became involved in evaluation of a multi-sea-year salmon stock from the Grand Codroy River (Pepper et al. 1998).

This report is an adjunct to Pepper et al. (2003) and documents a decade of performance of three year classes of Grand Cordoy-strain Atlantic salmon relative to that of the industry-standard strain of Saint John River salmon. Industry interest in the Grand Codroy strain culminated in incubation of 1.6 million eggs in 1997. Bay d'Espoir interest waned considerably during the next several months in light of industry scrutiny of relative growth rates and daily food consumption. Grand Cordoy-strain salmon that had been set aside as potential brood stock in the fall of 1998 were harvested in 1999, thereby ending the propagation strategy 10 years after it was started. By the time of the industry decision to terminate the GC initiative, DNA analyses had confirmed that the Newfoundland GC salmon stock was as genetically distinct from the local wild Bay d'Espoir salmon stocks of Conne River as the SJ stock. Performance comparisons between SJ- and GC-strain salmon inventories continued until December, 2000 when the last of the GC inventory was harvested. Performance-comparison analyses established that the GC strain, after three generations of propagation, was inferior to the industrystandard strain, thus supporting the industry's perceptions at the time of broodstock harvest in 1999. Industry's view of the decade of effort towards developing and evaluating GC strain salmon is that it was a costly learning experience.

RÉSUMÉ

Pepper, V.A., Withler, R, Nicholls, T. and Collier, C. 2004. Quantitative marineperformance evaluation of a Newfoundland Atlantic salmon strain for Bay d'Espoir aquaculture. Can Tech. Rep. Fish. Aquat. Sci. 2540: vii + 44 p.

Depuis le lancement de l'industrie de l'aquaculture dans la baie d'Espoir, au milieu des années 1980, la Newfoundland Salmonid Growers Association (NSGA) a toujours voulu utiliser des reproducteurs provenant de programmes structurés d'amélioration génétique du saumon. L'association cherche depuis longtemps à utiliser des géniteurs capables de donner un rendement supérieur, adaptés aux conditions aquacoles de la baie d'Espoir.

Le ministère des Pêches et des Océans (MPO) se préoccupe depuis longtemps de l'effet de l'utilisation des stocks de saumon atlantique sauvage en aquaculture. Il s'inquiète surtout des conséquences possibles des interactions génétiques entre les souches non indigènes de saumon atlantique et les populations sauvages. Ces inquiétudes au sujet de l'intégrité génétique de stocks de saumon sauvage sont répandues dans la communauté scientifique internationale qui recommande aux gestionnaires de la ressource halieutique de mettre sur pied des programmes d'aquaculture basés sur les populations locales, dans la mesure du possible.

En 1988, la Région de Terre-Neuve de Pêches et Océans Canada a conclu un accord avec la NSGA, lequel permettait à la nouvelle industrie aquacole d'utiliser les œufs du saumon atlantique indigène de la rivière Saint-Jean, en vue de faire une évaluation objective du potentiel aquacole de la baie d'Espoir. L'industrie avait reçu l'autorisation d'importer ces œufs d'écloseries commerciales des Maritimes, à condition de participer à l'évaluation d'un stock de saumon indigène de Terre-Neuve pour le développement aquacole dans la baie d'Espoir. Par suite de cet accord, l'industrie de la salmoniculture de Terre-Neuve et du Labrador a participé à un projet d'évaluation d'un stock de saumons pluribermarins de la rivière Grand Codroy (Pepper et coll., 1998).

Le présent rapport complète le rapport Pepper et al. (2003) et il documente le rendement de trois classes d'âge de la souche du saumon atlantique de la rivière Grand Codroy, pendant une décennie, par rapport au rendement de la souche de saumon de la rivière Saint-Jean, de norme industrielle. L'intérêt de l'industrie pour la souche de la rivière Grand Codroy a donné lieu à l'incubation de 1,6 million d'œufs en 1997. L'intérêt pour la souche de la baie d'Espoir a diminué fortement au cours des mois qui ont suivi, par suite de l'examen minutieux fait par l'industrie des taux de croissance relatifs et de la consommation alimentaire quotidienne. Les saumons de la rivière Grand Codroy qui avaient été mis de côté comme géniteurs potentiels à l'automne de 1998 ont été récoltés en 1999, ce qui a mis fin à la stratégie de propagation lancée dix ans plus tôt. Lorsque l'industrie a finalement décidé de mettre fin à l'initiative de la rivière Grand Codroy, des analyses d'ADN avaient confirmé que le stock de saumon de la Grand Codroy de Terre-Neuve était aussi distinct sur le plan génétique des stocks de saumon de la rivière Conne, indigènes de la baie d'Espoir, que le stock de la rivière Saint-Jean. Une comparaison du rendement des saumons de la souche de la rivière Saint-Lean et de la souche de la rivière Grand Codroy s'est poursuivie jusqu'en décembre 2000, lorsque le dernier individu de la Grand Codroy a été récolté. Des analyses comparatives de

rendement ont déterminé qu'après trois générations de propagation, la souche de la Grand Codroy était inférieure à la souche de norme industrielle, d'où confirmation des perceptions de l'industrie au moment de la récolte du stock reproducteur, en 1999. L'industrie estime que les efforts qu'elle a déployés pendant une décennie pour développer et évaluer une population de saumons de souche de la GC a été pour elle une expérience d'apprentissage coûteuse.

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INTRODUCTION

The commercial marine salmonid aquaculture industry in Newfoundland and Labrador is confined to the South/Southwest coast of the island part of the province (Fig. 1), largely due to this area's protection from the cold Labrador Current that permeates much of the island's coastal zone. Since embarking on development of a local salmon aquaculture industry in 1985, Newfoundland growers have made numerous attempts at alternative strategies in pursuit of improved industry viability in a cold-ocean environment.

Other salmon-aquaculture countries such as Norway, Chile, Scotland, and the United States have been using pedigree strains of Atlantic salmon (*Salmo salar*) and steelhead trout (*Oncorhynchus mykiss*). These pedigree strains have been developed specifically for a variety of traits including internal and external colour and growth. Such programs, now spanning several generations, have resulted in weight gains of 3.6% (Gjedrem 1983) and 4.3% (Gjerde 1986) per year for Atlantic salmon and rainbow trout respectively. For one of the strains of particular interest to salmon aquaculturists throughout the world, Gjedrem (1998) reported "A comparison of improved Atlantic salmon from the fourth generation of selection and a sample of wild fish from the river Namsen showed that the improved fish grow 77% faster than the wild. This makes a gain per generation of over 15%". While improvements in growth rate are of common interest among finfish farmers everywhere, breeding strategies to adapt aquaculture strains to the environment of the local Bay d'Espoir salmon-farming operations are equally important to the Newfoundland industry.

Use of brood stock from structured salmon-breeding programs has been the goal of the Newfoundland Salmonid Growers Association (NSGA) since inception of the Bay d'Espoir industry in the mid-1980s. Fisheries and Oceans Canada concerns about this approach have been based largely on potential consequences of genetic interactions with indigenous wild populations. As stated by Hindar et al. (1991), there are direct genetic consequences if mature aquaculture fish come in contact with indigenous wild spawners. Genetic impacts can vary from complete gene pool mixing to no detectable effects on genetic population structure. There is an expanding body of literature that warrants caution regarding potential introduction of different genetic strains into water bodies containing locally-adapted, indigenous stocks (Phillip 1991; Evans and Willox 1991; Waples 1991; Campton 1995; Wang et al 2001; Utter and Epifanio 2002; McGinnity et al. 2003). What so far has escaped meaningful prediction are the long-term consequences of such genetic interactions under environmental conditions that vary markedly among natural watersheds (Ritter 1999). The recently-formulated National Code on Introductions and Transfers of Aquatic Organisms for Canada (http://www.dfompo.gc.ca/science/OAS/aquaculture/nationalcode/codedefault e.htm), states,

"Different stocks of aquatic organisms have clearly defined behavioral characteristics, many of which are genetically controlled. Interbreeding between divergent stocks of the same species, which could be separated spatially or temporally, may result in the reduction of, or changes to, particular traits that could alter the ability of an indigenous population to adapt to changing environmental conditions."

Such genetic concerns reflect the recommendation of Hindar et al. (1991) who suggested managers should strive for aquaculture programs that are based on local populations as much as possible. In consideration of the potential for genetic impacts on wild fish species, these authors also suggested rearing of reproductively-sterile fish for aquaculture should be encouraged through use of such procedures as induced triploidy.

BAY D'ESPOIR

The Bay d'Espoir region of Newfoundland has environmental conditions that may be utilized to industry advantage once the industry has strains specifically adapted to these conditions. Growth of the Bay d'Espoir salmonid aquaculture industry has been limited, at least in part, by difficulties in refining husbandry practices to capitalize on the environmental regime of the Bay d'Espoir estuarine fjord and by restricted access to aquaculture strains of Atlantic salmon and rainbow trout.

During the infancy of the Bay d'Espoir aquaculture industry, the salmonaquaculture development initiative was limited to "local"-origin wild stocks of Atlantic salmon. The early exploratory interval of 1985-88 saw net-pen rearing of Atlantic salmon in Bay d'Espoir based on trials with wild stock from Grey, Exploits, Conne and LaHave (Nova Scotia) rivers (Fig. 2). These stocks proved unsatisfactory due to early maturation and inferior resistance to harmful microorganisms. All of these wild stocks demonstrated such gross aquaculture deficiencies as to preclude the need for or, due to mortality, the opportunity to pursue quantitative relative-performance evaluations.

In 1988, the Newfoundland Region of Fisheries and Oceans Canada worked out an arrangement with the NSGA by which the newly-developing industry could access Saint John River-origin Atlantic salmon eggs in support of objective evaluation of the aquaculture potential of Bay d'Espoir. Authorization for such imports from commercial Maritime hatcheries was to be contingent on industry participation in evaluation of another local Newfoundland salmon stock for Bay d'Espoir aquaculture development. As a consequence of this agreement, the Newfoundland salmonid aquaculture industry became involved in evaluation of a multi-sea-year (MSY) salmon stock from the Grand Codroy River (Fig. 2). This particular salmon stock was chosen on the basis of the following: i) geneticists advised that, in the absence of information on genetic distance, it was best to choose the wild salmon population that was closest to Bay d'Espoir and had similar biological characteristics; ii) the GC stock was known to have a MSY component, a basic prerequisite for the industry; iii) the GC stock is an early-run stock similar to the Conne River stock of Bay d'Espoir; and, iv) the GC adult salmon spawning migration follows along the south coast of the island past the Bay d'Espoir fjord.

Since the late-1980's, the Bay d'Espoir aquaculture industry has been importing salmonid eggs from a variety of certified, disease-free sources in Canada and the United States. Imported eggs of Atlantic salmon and steelhead have been incubated in local Bay

d'Espoir hatchery facilities and resulting salmon grown successfully in the Bay d'Espoir environment. Though performance of these species has been encouraging, there is economic incentive for improvement. Survival from egg-to-market product has been an obvious candidate for improvement but additional culture factors such as growth, incidence of early maturation, and food conversion efficiency also have drawn industry attention.

In 1989, the Science Branch of Fisheries and Oceans Canada initiated an evaluation of Newfoundland's wild Grand Codroy (Fig. 2) Atlantic salmon stock as a potential source of brood for Newfoundland salmon farming. Previous efforts at local salmon strain evaluation had revealed that the province's predominantly-grilse Atlantic salmon stocks (early maturing, one sea-winter salmon) are not well suited to industry needs for a late-maturing stock. Initial trials with marine culture of salmon smolts of Grey, Exploits and Conne River stocks revealed poor performance and pronounced susceptibility to local pathogens (i.e. Vibrio sp. and Aeromonas salmonicida subspecies nova). In 1987, in response to a significant pathogen event, the Government of Newfoundland and Labrador issued a destruct order for all aquaculture salmon in Bay d'Espoir cages. In the interval between 1989 and 1992, Science Branch collaborated extensively with the fledgling industry to procure and evaluate the Grand Codroy (GC) River salmon stock for potential application to salmon farming in Bay d'Espoir. Concurrent evaluation of this "local" Newfoundland salmon stock for Bay d'Espoir aquaculture purposes was to be a condition of importation of the industry's then-preferred stock of Saint John River (SJ) strain (New Brunswick) salmon. Work with the GC stock began in 1989 and continued through 2000.

Concurrent with this initiative on broodstock selection, industry also participated in evaluation of technologies for production of all-female salmon and triploid (reproductively incapacitated) Atlantic salmon (Pepper 1991a, b; Pepper et al. 1996, 1998, 2003).

This technical report documents actions taken by both government and industry regarding evaluation of the Grand Codroy River Atlantic salmon stock towards commercial salmon aquaculture development in Bay d'Espoir, Newfoundland. Results of three generations of strain-performance evaluation are presented.

METHODS

COLLECTION OF WILD ATLANTIC SALMON GAMETES

GC spawner gametes were collected for the first time in 1989 from wild salmon brood stock for incubation and subsequent rearing of 1990 year-class juveniles at the Bay d'Espoir hatchery. Wild brood specimens were collected from the lower reaches of the Grand Codroy River during a one-week interval in September. Captured specimens were retained in cages in a local pond until stripping in late October. Even with significant helicopter support to the exercise, broodstock procurement was compromised by a low frequency of MSY individuals and markedly disproportionate gender representation in the MSY component of the spawning population, thereby limiting the effective population size of the brood stock. Effective population size was calculated (Falconer 1989) as:

$$N_e = \frac{4 x N_f x N_m}{N_f + N_m}$$

Resulting year classes from this foundation brood stock subsequently were spawned in captivity in 1993 and 1997.

All gametes procured in 1989 from wild GC salmon spawners were transferred on slush ice, either to the Bay d'Espoir hatchery via helicopter or to a wild stockenhancement incubation facility at Hughes Brook (Fig. 2) via truck in support of a stockcompensation program. Immediately after stripping, wild brood salmon were tagged and sent to a DFO Fish Health diagnostics lab for examination for fish pathogens.

The same fertilization protocol (a semi-factorial crossing of all males and females as per Pepper and Crim (1996)) was followed at both the Bay d'Espoir salmon hatchery and the Hughes Brook enhancement facility where the respective batches of eggs were incubated through the winter months. Incubation water temperature at the Bay d'Espoir hatchery was maintained at 8°C. These eggs hatched in January, 1990. Hughes Brook facility eggs were incubated at ambient water temperature (minimum of 0.5°C; average ~+2°C) and hatched in March. Resulting Hughes-Brook facility salmon fry were transported to the Grand Codroy River in June, 1990 and distributed to fluvial rearing habitat. GC Atlantic salmon fry at the Bay d'Espoir hatchery were retained in the hatchery through their entire freshwater phase and raised under identical conditions to the SJ strain juveniles.

STRAIN PERFORMANCE

GC strain Atlantic salmon smolts and a parallel group of SJ strain smolts from the Bay d'Espoir salmon hatchery were transferred to estuarine cages via tank truck in the late spring/early summer of 1991, 1995 and 1999. Truck loading, transfer and unloading into the estuarine cages took about two to five hours. Salmon in the estuarine cages were fed to satiation three times daily (0700, 1300 and 1800 hours) with a commercial dry diet (Shur-Gain #2.5 pellet, 40-70 g; #3.5 pellet, 70-200 g; #5.0 pellet, 200-750 g; #6.5 pellet, 750-1500 g). An Aquaculture Veterinarian visited the site regularly to monitor fish health and administer fish-pathogen treatments as appropriate over the years of the study. The year classes of stock comparisons are represented in Table 1.

The SJ Atlantic salmon strain was imported to Bay d'Espoir annually as eyed eggs from certified commercial Maritime hatcheries beginning in 1988. As these annual importations of eyed Atlantic salmon eggs into Newfoundland were private transactions between aquaculture businesses, there is no record of how many brood salmon were used to obtain the imported eggs. Both strains raised at the Bay d'Espoir hatchery were maintained separately. Brood stock produced from these year classes was used subsequently to propagate each strain and supplement continuing egg importations from mainland hatcheries in support of industry production requirements. Throughout the duration of these GC strain evaluations, the SJ Atlantic salmon strain, considered to be the industry standard throughout the Northwest Atlantic in the early 1990s, was used as the reference or control group. For the 1990 year class of performance evaluation of the GC-strain salmon, marine rearing (1991) was conducted as a double-blind test in which GC and SJ smolt groups were identified to the farmers only as cages A, B, C, and D to avoid any unconscious husbandry bias by site workers.

	Stock Origin							
	Gran	d Codroy		Saint John				
		Brood	Stock					
Year	Stage	Females	Males	Imported				
1989	Wild adults	13 ^a	5*	Eggs: 100,000				
1990	Hatchery rearing							
1991	1 st marine year			Eggs: 100,000				
1992	2 nd marine year			Eggs: 100,000				
1993	Propagation	30	17	Eggs: 150,000				
1994	Hatchery rearing			Fry: 300,000				
1995	1 st marine year			Parr: 25,000				
1996	2 nd marine year			Fry: 270,000 Parr: 380,000				
1997	Propagation	. 170	100	Eggs: 300,000 Parr: 250,000				
1998	Hatchery rearing							
1999	l st marine year							
2000	2 nd marine year							
2001	Termination							

Table 1. Propagation history of Grand Codroy and Saint John River Atlantic salmon strains for Bay d'Espoir aquaculture.

^a12 females and 2 males were multi-sea year salmon.

As with the first year class of Bay d'Espoir salmon aquaculture trials, continuity of salmon culture methods during the interval of the 1994 and 1998 year classes of performance-evaluation was compromised by incidents of vibriosis and atypical

furunculosis. In response, the Bay d'Espoir salmonid farmers implemented a vaccination program in 1995. Starting in that year, both strains of salmon received an intraperitoneal injection with 0.2 ml. Lipogen Tripple (AquaHealth) toward the end of their hatchery phase (i.e., November) in preparation for transfer to marine cages the following spring.

MONITORING AND INDUSTRY PRACTICES

For all three year classes of these comparisons, only one cage of each strain was sampled each month during the summer marine-rearing interval. For the 1990 year class of GC/SJ Atlantic salmon strain evaluation, all monitoring was conducted by industry personnel. Batch weights were determined periodically through the marine rearing interval in 1991. Inventory records were provided by industry personnel for the 1991 marine operations but not for the 1992 rearing season. These data were not sufficiently comprehensive to support the intended strain-performance analyses. Starting in 1995, both government and industry collaborated on strain sampling and data collection. Food was withheld for intervals of 24-36 h before sampling. Samples were obtained by drawing up the nets or introducing a small amount of food at the surface to attract fish to the upper level of the cage. A hand seine or long-handled dipnet was used to obtain the required specimens (i.e. 25 specimens per cage per sample in 1995, 35 specimens per sample starting in 1996). Monthly sampling was limited to this small-sample size in an attempt to minimize handling stress and negative impacts on growth performance. Specimen handling at times of elevated temperature or mortality was avoided to minimize anthropogenic stressors. When environmental conditions allowed, fish sampled were anaesthetized with TMS, weighed on site with an electronic balance (to the nearest gram), and measured to the nearest 0.1 cm. Specimens were allowed to recover from the anaesthetic before being returned to their respective cages.

Monitoring of environmental variables (temperature, dissolved oxygen, salinity) was conducted during the second and third marine-rearing intervals. All cages were examined regularly by SCUBA divers. Salmon mortalities were counted and removed from the cages daily during the summer and about once per week during the winter. Dead fish, removed from each of the aquaculture cages, were incinerated at provincially-approved incinerator sites in the area of the aquaculture operations. It was only in the latter years of the experiment that current-monitoring equipment was available to the project. Starting in 1997, InterOcean S4 and Aanderaa RCM7 current meter mooring arrays were positioned at strategic locations within the fjord. Fixed-mooring data from these units was supplemented with Acoustic Doppler Current Profiling (Workhorse Sentinel, RD Instruments) in the vicinity of the salmon cages in 2000.

Throughout much of the decade of these performance comparisons, marine-cage culture in Bay d'Espoir was a learning experience from which husbandry practices were refined and adapted to the estuarine-fjord environment of Bay d'Espoir. For the 1990 year class, salmon smolt were placed in cages in the Ship Cove area and towed to Roti Bay where they were retained through the entire marine-rearing interval (i.e. 1991 and 1992). In support of feeding and monitoring at the site during the winter months, a hole was cut in the ice ($\sim 2.3 \text{ m x } 2.3 \text{ m}$) above each net once sufficient ice had formed over

the farm site to allow safe access by attendants. A small enclosure (i.e., a translucent plastic cover) was built over these openings in the ice to encourage a hothouse effect and thereby maintain access to air for salmon swim-bladder regulation.

For the 1994 year class of the GC/SJ evaluations, smolts were placed in cages in Ship Cove in 1995 and then towed to Roti Bay. These salmon were retained in cages in Roti Bay through the first summer and winter, then towed to Deer Cove for their second marine summer. Refinements to industry practices in the mid-to-late 1990s resulted in site rotation and fallowing between summer and winter operations. At this point, Roti Bay became designated solely as an overwintering site. For the 1998 year class of the GC/SJ Atlantic salmon strain evaluations, smolts were placed in cages towards the head of Bay d'Espoir (~3 km from Ship Cove) and towed to May Cove for their first summer. That fall (1999) the salmon cages were towed to Roti Bay where they were retained for the winter months.

As of the mid-1990s, following the 1^{st} winter of salmon rearing in Roti Bay, it was standard Bay d'Espoir industry practice to relocate Atlantic salmon away from this brackish site to a full-salinity marine site further out the bay. On completion of the winter period in Roti Bay, the salmon were removed from their overwintering nets and placed in 70 m circumference circular cages fitted with 6m-deep nets of 57 mm stretch mesh. Cages then were relocated to a site in Little Passage (Deer Cove, Fig. 1), to which they were towed by long-liner. The 16.9 km transfer of cages took place most often in June and required ~6 h of towing.

In late autumn, after completion of the second summer of rearing in the Bay d'Espoir marine cages, all salmon for each of the year classes of the experiment were graded into four categories (maturing, non-maturing and <3 kg., non-maturing and 3 to <3.5 kg, and non-maturing and ≥3.5 kg.). All maturing salmon, plus salmon less than 3.5 kg were eliminated from the Bay d'Espoir livestock inventory at the time of harvest in the fall of their 2nd year in the marine cages leaving only the largest fish of the year class as brood stock for the following year. Typically, a brood stock of about 500 non-maturing individuals was set aside at the time of harvest in support of strain propagation the following autumn. A second cull was applied to each brood stock prior to final broodstock maturation in support of mass-selection breeding. Industry interest in the GC strain peaked in 1997 with incubation of 1.6 million eggs and allocation of GC brood stock for egg production in 1998.

The last of the strain-comparison samples of Atlantic salmon at the marine farm (Deer Cove, Little Passage) was completed in September, 2000 after which the cages were relocated to the fish processing plant (Ship Cove, Fig. 1) towards the head of the estuary. Maturing salmon were culled from the cages at the time of processing by seining the freshwater layer of the cage. The GC strain was harvested on December 12, 2000. The SJ strain was harvested on November 23, 2000. For both strains, whole weight and fork-length data were obtained from 35 grilse. Samples of non-maturing salmon (i.e. market fish) were taken from 100 SJ strain and 116 GC strain salmon.

GENETICS

In consideration of a potential long-term breeding program for the Bay d'Espoir aquaculture industry, we documented the level of genetic diversity at microsatellite DNA loci in the Newfoundland domestic GC and SJ Atlantic salmon strains. Identification of the genetic profile of wild Atlantic salmon of the Conne River, that empties into the Bay d'Espoir estuary, also was undertaken as a base from which to judge potential future genetic interactions between the wild stock and aquaculture strains.

Approximately 160 adult fish of each of the GC and SJ Atlantic salmon strains reared in net pens in Bay d'Espoir were sampled for genetic analysis during spawning in November 1997 (i.e. end of the 1994 year class performance evaluation). An opercularpunch tissue sample was obtained from each of the aquaculture fish sampled. Tissue samples were placed in vials in 95% ethanol. DNA was extracted from the operculumtissue samples at the Pacific Biological Station (PBS molecular genetics laboratory, DFO, Nanaimo) and subjected to analysis at four microsatellite DNA loci (Ssa14, Ssa197, Ssa202, and Ssa289). The different genetic variants (alleles) that occur at each microsatellite locus differ in size, thereby allowing DNA fragments to be separated by size by gel electrophoresis. PCR (polymerase chain reaction) was conducted to amplify the alleles from the DNA of each fish for each of four microsatellite loci. The amplified DNA products were run on non-denaturing polyacrylamide gels, and scored for size using 20 base pair molecular-weight size-standard ladders. Allele frequencies calculated for the GC and SJ samples were compared with frequencies observed in samples of wild Atlantic salmon from the local Conne River (Conne River, Twillick Brook, Bernard Brook) reported in Beacham and Dempson (1998).

The program GENEPOP (Raymond and Rousset 1995) was used to calculate allele frequencies and observed heterozygosity values (H_0 = percentage of fish that carry two different alleles averaged over all loci) and to determine if the observed genotypic distributions for the samples were in Hardy-Weinberg equilibrium. The program PHYLIP (Felsenstein 1993) was used to calculate pairwise Cavalli-Sforza-Edwards genetic distances between the SJ, GC, Conne River, Twillick Brook and Bernard Brook samples.

On the basis of initial DNA work with the 1997 brood stocks, in 1998 the NSGA embarked on a program of pedigree breeding for its "domesticated" salmon strains. In May of that year the association set aside salmon of each of the GC (1994 year class fish that did not spawn in 1997) and SJ Atlantic salmon strains (1995 year class) as brood stock for that fall. Passive Integrated Transponder (PIT) tags (AVID Canada) were applied to the dorsal musculature at a point mid-way between the dorsal fin and the lateral line at the mid-length of the dorsal fin. Tissue samples (i.e. opercular punch) were taken from 130 brood specimens of each of the two salmon strains and again were placed in vials of 95% ethanol that were marked with the PIT tag number. DNA extracted from tissue samples at the PBS was subjected to microsatellite DNA analysis at six loci

(Ots107, Ssa14, Ssa85, Ssa171, Ssa197 and Ssa202). The amplified DNA products were run on denaturing polyacrylamide gels on an ABI 377 automated DNA sequencer, with alleles sized by comparisons with internal size standards run in each lane.

Genotype data were analyzed using the program of Blouin et al. (1996) to calculate the level of allele sharing between each pair of individuals calculated by the method of Queller and Goodnight (1989). PHYLIP was used to arrange the 130 individuals in a neighbour-joining dendrogram in which the most closely related individuals (as indicated by high levels of allele sharing) were clustered together. Each of these clusters within both the SJ and GC Atlantic salmon strains was then identified as a 'family group', although the exact relationships among individuals within each group could not be determined.

All of the SJ-strain brood salmon with PIT tags were sacrificed immediately after stripping in November, 1998. PIT tags were retrieved for reuse. GC-strain brood salmon were retained for reconditioning and possible use as brood again in future years. Broodstock were stripped into individually-labeled (specimen number) gamete containers and taken to the St. Alban's incubation facility. All brood specimen identities were referenced by family and mating was conducted so as to avoid intra-family-group crosses.

PERFORMANCE-COMPARISON ANALYSES

The suite of performance indicators for these aquaculture experiments included growth, survival and food conversion ratio (FCR; both economic and biological versions). Data collected during the experiment were examined for overall patterns of strain performance. Comparison statistics were developed as per Pepper et al. 2003.

Analyses centered on mean weight and biomass of the two strains at the beginning and again at the end of the experiment. Instantaneous growth rates (Ricker 1975) between sampling intervals were used to calculate daily mean weight for each strain, which, combined with records of daily mortality and amount of food dispensed, allowed interpretation of daily biomass, growth and feed conversion. Estimates of increase in biomass from start to end were based on biomass calculations using start and end weights and start and end inventory. Comparison statistics and confidence intervals were calculated using STATISTICATM V6 (StatSoft Inc.).

Analyses of strain performance for the 1995 and 1998 year classes of the experiment were confined to intervals for which there were comparison data for both daily inventory/feeding and weight samples.

RESULTS

COLLECTION OF WILD ATLANTIC SALMON GAMETES

Atlantic salmon brood specimens were collected from the Grand Codroy River from September 14-17, 1989. A serious bias in the sex ratio of the GC spawning escapement resulted in expenditure of considerable effort to balance gender representation of the brood stock for the aquaculture endeavour. In spite of three additional days of helicopter support to the field effort to locate additional MSY males, effective population size of the initial aquaculture brood (14) was disappointing. In all, 72 Atlantic salmon of the GC spawning escapement were captured and held until scale analyses could be completed to determine age-at-first maturation. All but four grilse specimens were released on completion of scale analyses. Scales of specimens from which gametes were procured in October confirmed 12 MSY females and 2 MSY males. Stripping also included one grilse female and three grilse males. All 18 of these brood salmon were submitted for fish health screening and were found to be free of certifiable pathogens as per Schedule II of the Canadian Fish Health Protection Regulations. The Bay d'Espoir Hatchery received 35,900 viable salmon eggs. The Hughes Brook facility received ~47,000 eggs as part of a wild-stock compensation strategy for the Grand Codroy River.

Morphometric characteristics of the foundation GC brood stock collected initially in 1989, and corresponding data for the 1994 and 1998 year class brood stocks are as per Table 2. Due to the biased sex ratio of the foundation brood stock, effective population size for the foundation population was only 14.4 individuals. Weight-length regression statistics for the foundation stock and subsequent broodstocks are presented in Table 3.

	Maria	Strain						
Brood Year	Mean of Parameter ^a -	Saint Joh	n River ^b	Grand Codroy River				
	1 al allietel	Females	Males	Females	Males			
1989	Weight			4.24	3.97			
1909	Fork Length			73.9	73.3			
1993	Weight	6.7	7.0	5.2	5.7			
1775	Fork Length	77.6	82.3	72.1	77.3			
1007	Weight	6.3	6.3	6.0	6.5			
1997	Fork Length	75.3	78.1	76.3	82.0			

Table 2. Broodstock characteristics for Grand Codroy and Saint John River Atlantic salmon strains.

^aWeight in kg. and fork length in cm.

^bBroodstock characteristics of initial (1989) SJ strain salmon unknown.

Egg incubation during the winter of 1989/90 proceeded without incident at the Hughes Brook incubation facility and the Bay d'Espoir hatchery. Of the ~47,000 eggs delivered to the Hughes Brook incubation facility, ~14,000 fry (~30% egg-to-fry survival) were distributed back to suitable rearing habitat of the Grand Codroy River. Fry returned to the river in 1990 represent ~17% of the total eggs transferred from the Grand Codroy River in 1989.

Regression Coefficients Brood $\mathbf{F}^{\mathbf{b}}$ Year Gender No. Intercept Slope Strain r 1989 Saint John 43 0.98 Male -2.4971 +3.3179 882.05 1993 +3.2720 Female 29 -2.3476 0.98 694.02 Male 34 -1.8754 +3.0181 0.92 174.43 1997 Female 5 -2.0336 +3.1281 0.99 2135.83 Male +3.0868 0.97 5 -2.1815 60.76 1989 Female 13 -1.4955 +2.74070.83 24.15 Male 39 -1.9086 +2.97990.98 707.89 Grand 1993 Codroy Female 36 -1.8032 +2.96160.96 421.59

19

16

-1.5213

-1.1103

+2.7861

+2.5732

0.94

0.92

130.37

80.59

Table 3. Broodstock weight-length regression^a parameters for Grand Codroy and Saint John River Atlantic salmon brood stocks through three year classes of Bay d'Espoir aquaculture experimentation.

^aLog₍₁₀₎ Weight (g.) = Intercept + Slope * (Log₍₁₀₎ Fork Length (cm.)).

Male

Female

^bAll log-log regressions significant at p < 0.004.

1997

PERFORMANCE COMPARISON ANALYSES

Industry records on relative performance of the two strains during the hatchery phase of the life cycle are not available. Mean smolt weight at the time of transfer to marine cages is as per Table 4.

Fig. 3a illustrates mortality figures for the two strains for the 1990 year class in 1991. Growth through the entire marine-rearing interval for the year class is illustrated in Fig. 3b. Industry observations during the first season of marine culture of this year class (i.e. 1991) were recorded by alphabetic characterization (i.e. cages A through D). The GC strain (cages C & D) seemed to be less susceptible to local Bay d'Espoir pathogens than the SJ strain (cages A & B). Mortality attributable to pathogens was 19.6% for the SJ strain in contrast to 15.6% for the GC strain. Industry personnel recorded also that growth and food conversion through the first summer in the marine cages was better for the SJ salmon than the GC salmon. As of the time of harvest in 1992, the most obvious

difference in marine performance between the two salmon strains was in the relative frequency of salmon in the grilse and largest-size categories.

		-	•			
	Mean		95% Confidence Interval			
Strain	Year Class	Weight (g)	Low	High		
	1991	110	industry, batch-w	veight mean		
GC	1994	52.1	47.0	57.3		
	1998	76.4	69.5	83.3		
	1991	120	industry, batch-w	veight mean		
SJ	1994	52.3	48.2	56.3		
	1998	88.5	82.2	94.8		

Table 4. Smolt mean weight at time of transfer to marine cages.

Size distribution of the two populations at the end of the comparative-performance evaluation experiment in 1992 was as per Table 5.

Table 5. Size categories and harvest-weight (whole) distribution for the 1990 year class of Grand Codroy and Saint John River Atlantic salmon.

		1992 Harvest Category						
Strain	Grilse	1-2 kg.	2-3 kg.	3-4 kg	>4 kg	Total		
Grand Codroy	25%	23%	24%	17%	11%	5538		
Saint John	8%	5%	7%	27%	53%	4654		

Size distribution of the 1994 year class for the two strains of the comparativeperformance evaluation experiment in 1996 was as per Table 6.

Table 6. Size categories and harvest-weight (whole) distribution for the 1994 year class of Grand Codroy and Saint John River Atlantic salmon.

			Grading Results						
Strain	Cage	Grilse		Small		Large		Totals	
		#	%	#	%	#	%		
	1	1163	15%	946	12%	5871	74%	7980	
GCR	2	878	14%	937	15%	4321	70%	6136	
	Total	2041	14%	1883	13%	10192	72%	14116	
				_					
	1	1083	14%	2659	33%	4249	53%	7991	
SJR	2	836	14%	1157	20%	3838	66%	5831	
	Total	1919	14%	3816	28%	8087	59%	13822	

Time intervals for analyses of strain performance for the 1994 and 1998 year classes of the experiment are as per Table 7.

Year Class	Strain	Start Date	End Date
1994	Grand Codroy	June 21, 1995 June 21, 1995	November 26, 1996 November 29, 1996
	Saint John	June 21, 1993	november 29, 1990
1998	Grand Codroy	June 23, 1999	December 12, 2000
1790	Saint John	June 23, 1999	November 23, 2000

Table 7. Interval of data collection for the 1994 and 1998 year classes of the Atlantic salmon strain-performance evaluations.

Monthly monitoring of salmon through the summers of 1995, 1996, 1999 and 2000 was interrupted by mortality peaks that took place typically in July (Fig. 4). Specimen handling at such times was avoided to minimize anthropogenic stressors. Feed and biomass data for the 1994 and 1998 year classes of the experiment are illustrated in Fig. 5. The 1996 harvest revealed no difference in mean weight between the two strains (t = 1.80, p = 0.70). Variance observed between the two strains for the 1996 harvest-weight samples was similar (Levene F = 2.41, p = 0.12). In contrast, the harvest meanweight data for 2000 revealed a marked difference between strains (t = 7.83, p < 0.0001). Again, variances were observed to be similar between strains (Levene F = 1.07, p = 0.30). Comparison of weight-at-harvest statistics for the two strains for the 1994 and 1998 year classes of the evaluation is illustrated in Fig. 6. The distribution of harvest weights for the two most recent harvest years is illustrated in Fig. 7. FCR statistics for the 1994 and 1998 year classes (Fig. 8) of these experiments suggested significant strain differences in biomass accumulated relative to food consumed for the 1998 year class.

Of potential significance to performance of the 1998 year class is the observation of salmon regurgitating food in August of both 1999 and 2000. This resulted in a fatty scum on the surface of the water and on the cage-support structures, making the cages extremely slippery and hampering cage access by personnel. Though surface water temperatures in August occasionally reached 20°C, temperature at 5 m rarely exceeded 16°C. For all of our sampling intervals, weather conditions at time-of-sampling were sufficiently calm to allow meaningful use of the electronic balance. Hence, weather and sea-state conditions at the time of the observations were mild. There were no obvious reasons for what looked like an occasional inability of the salmon digestive system to process the feed.

Throughout the decade of these performance evaluations, both strains had a significant incidence of maturation, thus rendering varying amounts of production unfit for market (Table 8). This was especially pronounced for the 1990 and 1998 year classes of GC salmon. Industry perception of performance of the 1998 year class of GC salmon was sufficiently negative throughout the first summer of marine rearing that the salmon business decided to harvest all of the GC salmon set aside as potential brood stock in the fall of 1999. Smolts of the 1998 year class, introduced to the marine cages in the spring of 1999, were carried through the 18 month marine-rearing interval.

	Year Class						
Strain	1990	1994	1998				
Grand Codroy	25	14	52				
Saint John	8	14	44				

Table 8. Percent maturation recorded for the three year classes of the performance-evaluation experiment.

Data for the second year of marine performance of the 1990 year class are not available. Analyses of estimated biomass of the two strains at the beginning and again at the end of the performance-evaluations of the 1994 and 1998 year classes confirmed that there was correspondence between the magnitude of the mean and the variance and therefore, challenges of heteroscedasticity (minimum Levene F = 50.9, p < 0.0001). This is illustrated graphically in Fig. 9. For purposes of estimating gain in biomass between the start and end of the performance interval, biomass estimates were converted to natural logs. This was successful in rendering variances homogeneous only for the 1994 year class GC samples (Levene F=0.98, p=0.32). With this deficiency clearly in mind regarding strict statistical interpretation, log differences were converted back to linear values (Sokal and Rohlf 1997) before calculation of confidence intervals on FCRs (Fig. 8). An overview of the FCR(_{economic}) strain-performance calculations is presented in Table 9.

-	Year	Duration	Mean We	eight (g)	Nur	nber				FC	CR
Strain	Class	(days)	End	Start ^a	End	Start	G*	Z	R	Bio	Eco
	1990	527	4410.0	110.0	5338	9740	0.0070	0.0011	0.0059		
GCR	1994	524	2469.4	72.7	15859	25578	0.0067	0.0009	0.0058	1.5	1.7
	1998	538	4124.1	111.8	4212	7908	0.0067	0.0012	0.0055	2.8	5.5
	1990	527		130.0	4654	9740		0.0014			
SJR	1994	527	2309.8	75.5	14657	21942	0.0065	0.0008	0.0057	1.4	1.6
	1998	519	4752.2	115.0	5151	7518	0.0072	0.0007	0.0064	2.1	2.6

Table 9. Overview of three year classes of salmon-strain performance.

^aStart weight as recorded here is taken as the mean weight on the date for which the first of the daily feeding records was available.

ENVIRONMENTAL

Typical historical temperature and salinity fluctuations in the upper-water column for the industry's main overwintering site in Roti Bay for the two-year interval of May, 1987 through April, 1989 are illustrated in Fig. 10. Winter thermal characteristics for the site during the latter part of this study are tabulated in Appendix 1 and summarized in Table 10. The lowest recorded water temperature within the duration of this study was in April, 2000 at 1.5°C. The greatest variability in daily temperature was recorded in February. Mean daily temperature "stabilized" at ~+2°C during much of March and April. Other than for periodic feeding of a maintenance ration, the fish were left undisturbed (i.e. not sampled) during the winter months.

Duration	Minimum Daily Temperature		Mean Daily (°(Standard Deviation Temperature		
	<2.0	<2.0	<3.0	<4.0	<5.0	>0.35
# days	60	12	66	115	151	38
% of days	28	5	31	53	69	18

Table 10. Thermal characteristics at 9 m for salmon cages in Roti Bay during the winter of 1999-2000.

Located at approximately 47.749°N, -55.880′W, May Cove is about one half the distance along the longest axis of the Bay d'Espoir fjord. Summer temperatures at the sites referenced in this report are categorized in Table 11. Positioned within Little Passage, at approximately 47.674°N, -55.927′W, Deer Cove is about 35 km from the head of Bay d'Espoir. Although well removed from the main freshwater discharge to Bay d'Espoir, there is some surface runoff in the area. Even at this location, typical estuarine layering is evident in the upper water column. The low-salinity lens at all of the farm sites varied considerably with wind direction but typically exceeded 1 m (Fig. 11).

Table 11. Hourly temperature recordings for Bay d'Espoir salmon farms and frequency of observed water temperatures relative to optimal thermal regimes for Atlantic salmon (Dwyer and Piper 1987).

		Water Temperature at 8 m ^a .										
Site Location	Monitoring	< 13	.0°C	13.0-1	16.0°C	>16.0°C						
	Interval	Number	%	Number	%	Number	%					
	metval	Records	Records	Records	Records	Records	Records					
Roti Bay	May 16 to Nov. 21	3043	79.24	409	10.7	120	3.13					
Deer Cove	June 25 to Sept. 30/00	2396 ^b	66.2	657	18.2	566	15.6					
May Cove	June 14 to Nov. 22/00	2221	57.84	1160	30.21	459	11.95					

^aDepth in the water column occupied by most of the salmon most of the time. ^bRepresents data from two thermographs on two different cages.

Spot measurements of current velocity at various locations within the fjord revealed great geographic variation among farm sites. For much of the area, current speed during the summer in proximity to salmon farms was in the range of 10-19 cm·sec⁻¹. Winter holding sites typically had lower current velocity, often in the range of 4-5 cm·sec⁻¹ (Joe Craig, DFO, St. John's, pers. comm.). In marked contrast, examination of the velocity sections of Little Passage (area of the Deer Cove farm) revealed much greater velocities, often of a magnitude of 100 cm·sec⁻¹. The Deer Cove

site, at which salmon of this experiment were located during the summer of 2000, was subject to currents that ranged between these extremes.

GENETICS

Both the SJ and GC aquaculture strains examined from the 1994 year class in the marine cages generally possessed alleles at the four microsatellite loci that were present in the wild Conne River samples (Beacham and Dempson 1998). However, allele frequencies differed significantly both between the domestic and wild strains, and between the two domestic strains (Table 12).

Cavalli-Sforza-Edwards pairwise distances averaged 0.009 among the three Conne River sample groups. The average genetic distance between the Conne River samples and the SJ and the GC strains was 0.036 and 0.054, respectively. The SJ and GC samples were separated by a genetic distance of 0.047.

 H_0 levels at the four microsatellite loci (Ssa14, Ssa197, Ssa202, and Ssa289) in the domestic strains were within the range of those observed in the wild populations, except for the high levels of H_0 recorded at Ssa197 (95%) and Ssa202 (94%) in the GC strain (Table 12).

At Ssa14, all samples possessed alleles 138 and 142, with allele 138 predominating in the Conne River samples and allele 142 predominating in the SJ and GC samples (Table 13). An additional allele (140) was present at low frequency in the SJ and GC strains, and allele 144 was observed in a single SJ fish. The genotype distributions at all four loci were in Hardy-Weinberg equilibrium in both the SJ and GC strains, except that there was an excess of heterozygotes at Ssa202 in the GC strain.

At Ssa197, 17 alleles were observed in the five samples (Table 12) examined in this study. Allelic profiles of the two domestic strains were quite distinct from the Conne River populations and from each other. Allele 152 was a common allele in the Conne River samples, but absent from both the SJ and GC strains. Alleles 168 and 172 were at much lower frequencies in the GC strain than in the Conne River or SJ samples, whereas alleles 176 and 180 were most prevalent in the GC strain. Alleles in the 192 - 202 base pair (bp) size range were present at low frequencies in the domestic but not the wild populations, whereas the reverse was true for alleles greater than 202 bp in length.

At Ssa202, there was less allelic distinction between the domestic and wild samples, but the GC strain possessed two alleles (249 and 265) that were absent or rare in the other populations. Relative to the Conne River wild populations, the SJ strain possessed high frequencies and the GC strain low frequencies of alleles 289 and 293.

Ssa14	N	138	140	142	144					
Conne	143	0.538	0.003	0.458	0.000	_				
Twillick	115	0.691	0.000	0.309	0.000					
Bernard	99	0.662	0.000	0.338	0.000					
St. John	162	0.333	0.028	0.636	0.003					
Gr. Codroy	154	0.344	0.026	0.630	0.000					
Ssa197	N	142	152	156	160	164	168	172	176	180
Conne	145	0.003	0.134	0.055	0.041	0.162	0.134	0.259	0.107	0.041
Twillick	121	0.000	0.174	0.037	0.054	0.116	0.169	0.219	0.079	0.012
Bernard	104	0.005	0.202	0.038	0.067	0.115	0.139	0.197	0.063	0.043
St. John	159	0.000	0.000	0.009	0.119	0.173	0.242	0.214	0.072	0.041
Gr. Codroy	153	0.039	0.000	0.072	0.023	0.239	0.003	0.114	0.160	0.154
		184	188	192	197	202	217	222	227	
Conne		0.021	0.007	0.000	0.000	0.000	0.000	0.031	0.003	
Twillick		0.054	0.008	0.000	0.000	0.000	0.008	0.062	0.008	
Bernard		0.077	0.005	0.000	0.000	0.000	0.000	0.038	0.010	
St. John		0.025	0.016	0.013	0.009	0.066	0.000	0.000	0.000	
Gr. Codroy		0.098	0.003	0.095	0.000	0.000	0.000	0.000	0.000	
Ssa202	N	237	241	245	249	253	257	261	265	269
Conne	113	0.000	0.004	0.000	0.000	0.000	0.004	0.018	0.013	0.004
Twillick	104	0.005	0.072	0.005	0.000	0.000	0.000	0.024	0.014	0.000
Bernard	89	0.034	0.118	0.000	0.000	0.000	0.000	0.034	0.017	0.006
St. John	158	0.000	0.000	0.013	0.006	0.000	0.003	0.063	0.006	0.041
Gr. Codroy	153	0.026	0.000	0.000	0.127	0.003	0.000	0.007	0.134	0.059
		273	277	281	285	289	293	298	303	308
Conne		0.071	0.115	0.208	0.177	0.155	0.168	0.022	0.022	0.018
Twillick		0.019	0.091	0.202	0.212	0.159	0.163	0.024	0.010	0.000
Bernard		0.028	0.045	0.219	0.185	0.163	0.129	0.022	0.000	0.000
St. John		0.041	0.057	0.089	0.177	0.206	0.247	0.022	0.028	0.000
Gr. Codroy		0.059	0.121	0.092	0.154	0.092	0.098	0.029	0.000	0.000
Ssa289	N	110	112	114	116					
Conne	142	0.975	0.007	0.018	0.000	_				
Twillick	115	0.948	0.013	0.039	0.000					
Bernard	99	0.949	0.005	0.045	0.000					
St. John	158	0.870	0.000	0.085	0.044					
Gr. Codroy	153	0.993	0.000	0.000	0.007					

Table 12. Allele frequencies at four microsatellite DNA loci in five Atlantic salmon strains. Data for the Conne River, Twillick Brook and Bernard Brook wild populations are from Beacham and Dempson (1998).

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Strain	Family Group												
Stram	1	2	3	4	5	6	7	8	9	10	11	12	13
Grand Codroy	11	19	21	8	6	12	4	20	8	6	5	7	3
St. John	7	4	16	11	5	15	6	10	7	23	20	6	-

Table 13. Number of Atlantic salmon in each family group of the 1998 GC and SJ River brood strains.

At Ssa289, allele 110 was the most common allele in all five samples, and was close to fixation in the GC sample. The Conne River samples possessed alleles 112 and 114 at low frequencies, the SJ strain alleles 114 and 116. Allele 116 was observed in just two GC individuals. Results of the initial DNA profiling in 1997 gave no indication of reduced genetic variability due to founder effects, genetic drift or inbreeding in the SJR or GCR domestic strains.

Analysis of variation at the six microsatellite DNA loci in the 1998 GC and SJ brood samples indicated that the SJ strain was based on a total of 12 family groups. Thirteen family groups were identified in the Grand Codroy strain salmon brood stock used by the Bay d'Espoir salmon farming industry. This DNA-based perspective is consistent with expectations based on initial brood extraction from the Grand Codroy River in 1989.

Spawning of SJ strain salmon in 1998 resulted in pedigree representation from all 12 families of females and nine of the 12 families of SJR males. A total of ~260,000 SJ eggs from known interfamily crosses were placed in the St. Alban's incubators in 1998. The Grand Codroy brood stock, reconditioned from brood stripped in 1997, consisted of only seven fish (one male and six females) from six families. Stripping of the Grand Codroy bood salmon in 1998 resulted in incubation of 116,000 eggs, all produced from one male family and five different female families.

In the absence of a research-hatchery facility in which to maintain separate family lines, fry of the 1999 year-class had to be ponded within the existing production hatchery without reference to breeding history. In light of inferior family representation among GC-strain spawners in 1998, the industry decision to terminate the GC strain in 1999, lack of facilities in which to conduct pedigree breeding, and a serious cash-flow issue being faced by the industry at the time, all work on a structured breeding program for Bay d'Espoir salmon aquaculture was abandoned.

DISCUSSION

Any planned extraction of salmon from indigenous populations is replete with obligations to stock replenishment and local-community concerns (Pepper and Crim 1996). A condition imposed by local-community conservation interests on extraction of

Grand Codroy Atlantic salmon eggs from the natural spawning stock in support of this aquaculture initiative was that Grand Codroy salmon fry derived from the 1989 egg take be returned to the river in sufficient numbers to compensate for losses to natural egg deposition. Natural egg-to-fry survival in Newfoundland rivers has been documented previously (Sturge 1968) as ranging from 10 to 30%, with a mean value of about 20%. Accordingly, the salmon production-compensation strategy for the Grand Codroy River was to return salmon fry back to the river at a level of ~20% of the eggs taken. Of the roughly 83,000 eggs transferred from Grand Codroy spawners in 1989, some 14,000 fry (17%) were returned to the river in June, 1990 and released to classic parr-rearing habitat. This is within the range for natural riverine egg-to-fry survival in Newfoundland and is thought to conform with conservation targets for the 1989 salmon-spawning population.

Though gametes in support of both the aquaculture endeavour and the wild-stock compensation strategy were obtained on the expected schedule, the 1989 stock-extraction effort was not entirely successful. The disproportionate spawner sex ratio characteristic of Newfoundland rivers (Dalley et al. 1983) greatly compromised the effective population size of the GC foundation population that was deficient relative to normal standards (Tave 1986). The small number of fish used to found the Grand Codroy strain made inbreeding a significant challenge to the overall initiative. This deficiency dictated the semi-factorial breeding strategy of the first-year fertilizations and also led to an attempt towards a pedigree-breeding program. Microsatellite analyses were considered to be the best approach then available to address this concern. This is discussed in greater detail below.

It is inevitable that strain-performance evaluations, conducted on three consecutive year classes and spanning an interval of greater than a decade, would cover a range of environmental conditions and evolving husbandry practices. Data provided by industry personnel for the 1990 year class were not sufficient to support the intended analyses of year-class performance and resulted in more extensive government-industry collaboration in monitoring 1994 and 1998 year-class performance. On the basis of harvest data, it is apparent there was a greater incidence of "runts" among the Grand Codroy fish and that many of the fish in this category were maturing (i.e., grilse of Table 5). In addition to maturation, apparent differences in performance between the two strains for the 1990 year class were in mean weight at harvest and response to the Bay d'Espoir pathogen spectrum. Though the GC broodstock-development experiment produced some interesting results, the experience generated little incentive among the salmon farmers to continue working with this strain.

Relative-performance indicators for the 1994 year class of the experiment suggested the SJ and GC strains were similar in their production characteristics. While this result was sufficient to stimulate industry investment to procure and incubate 1.6 million GC eggs in 1997, subsequent performance observations in 1998 were disappointing to the farmers. Overall, results suggest superior performance of the SJ strain relative to GC-origin salmon. This is not that surprising considering that the SJ strain has been subjected to domestication for several generations and was three or four generations removed from the wild even at the time of early-industry trials in New Brunswick (Sutterlin et al. 1981). When viewed in a comparative sense, growth of the 1998 GC strain year class (i.e., 3rd generation removed from the wild) in Bay d'Espoir during the 595 days between start and end dates, actually was somewhat better than for the 579 days of the 4th generation SJ salmon evaluation in the Bay of Fundy (Fig. 12). Food-consumption data for the early Bay of Fundy experiment (Fig. 13), when compared with similar data for the 1998 year class GC salmon in Bay d'Espoir (Fig. 5) suggest the GC strain in Bay d'Espoir was performing reasonably well. However, this interpretation must be tempered with the caution of both geographic and husbandry differences between the early Bay of Fundy operations and those of the present Bay d'Espoir experiments.

As referenced by Sutterlin et al. (1981), water-current velocities at the Deer Island site ranged from 5 to 19 cm·sec⁻¹. Current velocity in the Deer Cove area of Little Passage, at which the 1998 year class was located during its last marine summer, often exceeded current velocities observed in the Bay of Fundy. A further dichotomy between husbandry conditions of the 4th generation SJ strain in New Brunswick and 3rd generation (i.e. 1998 year class) GC strain performance in Bay d'Espoir is in quality of diet.

Atlantic salmon diet formulations generally have improved greatly since the early days of salmon farming in Atlantic Canada (Lall 1991). There have been many improvements in salmon diet formulations (Hillestad et al. 1998; Sveier et al. 1999) resulting in a strong downward (improvement) trend in FCR. Much of this improvement is attributable to the use of extruded feeds (improved technical quality), feed containing a higher level of digestible energy, better formulations and improved feed management (pers. comm., Keith Were, Nutritionist/Technical Manager, Skretting, St. Andrews). The moist-pellet formulation used for the early Bay of Fundy experiment (Sutterlin et al. 1981, their Table 1) is not directly comparable with the dry-diet, commercial-pellet results of the Bay d'Espoir experiments. Progression in diet formulation from a locallyprepared, semi-moist composition to the commercial dry-pellet standard now used likely could be expected to elicit greater feeding efficiency of the Bay d'Espoir farmed salmon. This however is confounded by the observation that, at least during part of the summer operations, Bay d'Espoir salmon were observed regurgitating the salmon feed. Given that water-column thermal characteristics at the site provided salmon with access to conditions well within their physiological optima (Dwyer and Piper 1987), and that conditions at the time of sampling were relatively calm, there is no obvious reason why the fish were rejecting the feed. The fact that there was a fatty scum on the water in proximity to the cages, that this scum coated much of the water-level cage structure, and that the incidence of this phenomenon corresponded with observations of salmon vomiting, suggests low palatability of feed during these summer intervals. When viewed in the context of observed differences between the early Bay of Fundy operations and those of Bay d'Espoir, it is apparent that early Bay of Fundy results are not directly comparable with these 3rd generation GC performance indicators.

The most obvious differences in the present Bay d'Espoir performance evaluations are presented in Table 8. The observed discrepancy in maturation between the two strains caused concern to the Bay d'Espoir salmon farming industry in that the grilsing rate for Grand Cordoy fish translated into a >\$1.00 per kg increase (an industry calculation) in cost of production relative to SJ salmon that had an overall lower grilsing rate. This calculation is based on the value of marketable salmon produced relative to the cost of the food consumed by the strain for the year class (labour costs being equivalent for the two strains). GC strain maturation contributed much of the apparent diminished biomass progression of Fig. 5c. It is apparent that the SJ strain generally was superior with respect to growth, maturation, and food conversion. Regarding susceptibility to disease, it was apparent for the 1990 year class of the experiment that the two salmon populations responded differently in both timing and magnitude of mortality attributable to the Bay d'Espoir pathogens. At that time, GC salmon seemed to be better adjusted to the local pathogens. However, relative response of the 1994 and 1998 year classes to pathogen events switched around in favor of the SJ strain.

In the fall of 1996, on completion of performance evaluation of the 1994 year class of GC salmon, the Newfoundland salmon-farming community interpreted that the GC strain had performed sufficiently well that the initiative might yet prove significant to local-industry development. Accordingly, at the time of grading for market in late 1996, industry set aside 1,000 GC-strain salmon as potential brood stock for 1997. On completion of the marine-rearing interval for this 1994 year class, the two strains appeared to have much the same production potential and, given that most of the performance indicators were similar, identical incidence of maturation for strains of the 1994 year class (Table 8) proved a deciding factor in continuing the experiment. The 1.6 million eggs procured from the GC brood stock in 1997 provided the smolts on which performance evaluation of the 1998 year class was based. Samples procured during spawning in November 1997 for genetic analysis were intended to form the basis of a systematic, pedigree-breeding program for the Bay d'Espoir industry.

These industry initiatives towards establishing a systematic Atlantic salmon breeding program failed largely due to lack of sufficient capital for hatchery facilities to support pedigree and family breeding strategies and to marginal effective population size of the one-time, wild-broodstock collection. Industry perception of performance for the 1998 year class of Grand Codroy salmon throughout the first summer of marine culture was sufficiently deficient that the salmon business decided to harvest all of the Grand Codroy salmon set aside as potential brood stock in the fall of 1998. This GC brood stock was harvested in 1999. Based on mean smolt weights at time of transfer to marine cages (Table 4) and the mean end weights of Table 9, it is apparent there was actually erosion in growth performance over the three generations of the experiment. The 1990 year class produced the largest smolt and largest harvest salmon of all three generations. Perhaps what is most readily apparent is the correspondence between the size of the smolt produced and the size of the salmon harvested. These observations suggest the potential for marine-growth performance is established during the hatchery phase. For purposes of comparison with published literature (Gjedrem 1983, 1988; Gjerde 1986), strictly on the basis of final mean weight at harvest, Grand Codroy strain growth diminished by 2.2% per generation over the course of the three generations while SJ strain growth increased by 3.6% over the two generations (1994 and 1998 year classes) for which data are available.

GENETICS

A microsatellite locus is non-coding DNA consisting of a simple DNA sequence between 1 and 6 bp in length, repeated many times. Microsatellite loci evolve very quickly, with new alleles containing different copy numbers of the basic 'core' DNA repeat. Thus, the many alleles at these highly polymorphic loci differ from each other in size, and can be identified when run out on a gel that separates DNA fragments on the basis of size. The high level of polymorphism at these loci allows even individuals from the same population to be distinguished from each other throughout their lifetime, and progeny in mixed-family groups to be identified by parentage. Each fish has two copies of all genetic information (one inherited from each parent). Therefore, at each microsatellite locus, each fish has two alleles that may be the same or different sizes. If a fish has two copies of the same allele, it is called 'homozygous'; a fish that has one copy each of two different alleles is called 'heterozygous'. The proportion of fish that are heterozygous (called heterozygosity) at each microsatellite locus in a population or strain is one measure of the genetic variability present in the strain. Because microsatellite loci are highly polymorphic, and the different alleles do not harm or benefit the individuals that carry them, they are considered the best genetic markers to monitor genetic diversity and conduct pedigree analysis in a hatchery strain.

Results of initial genetic profiling of the domesticated strains of this report gave no indication of reduced genetic variability due to founder effects, genetic drift or inbreeding in the SJ or GC domestic strains. However, the small number of fish used to found the GC strain in 1989 (and likely also the individual brood years of the SJ strain) suggested inbreeding in future year classes could be expected. Moreover, the low effective number of breeders from which eggs were procured likely limited genetic variation within the strains for traits of importance in aquaculture, with the result that breeding to alter performance traits (growth, proportion of upper mode parr, etc.) would produce limited response. The genetic analysis also indicated that neither aquaculture strain was phylogenetically closely related to the wild Conne River populations described by Beacham and Dempson (1998).

The SJ and GC River domestic strain samples of 1997 displayed numbers of alleles, genotype frequency distributions and levels of heterozygosity at four microsatellite loci similar to those observed in wild populations of Atlantic salmon in other studies (McConnell et al. 1995, McConnell et al. 1997, Beacham and Dempson 1998). Microsatellite allele frequency data are not available for samples of wild fish from the Saint John and Grand Codroy rivers. However, the allele frequencies observed in the SJ and GC strains were significantly different from those characterizing the local Conne River populations, and likely reflected the genetic profiles of the wild populations from which they were derived.

Genetic distances indicated that the GC strain was as different from the Conne River populations as was the SJ strain, and the two domestic strains were as genetically distant from each other as they were from the Conne River populations. This is not surprising given the strong geographic structuring of microsatellite variation in Canadian populations of Atlantic salmon, including the genetic distinctiveness of Bay of Fundy populations such as the St. John River, and the possibility of a 'European influence' on Newfoundland populations (Davidson et al. 1990) such as the Conne and Grand Codroy rivers. The large genetic distance between the Grand Codroy and Conne River populations indicates that not all wild anadromous populations of Atlantic salmon in Newfoundland are more closely related to each other than to other populations in eastern Canada. Thus, the choice of a Newfoundland strain for aquaculture development does not ensure that it will be closely related to all wild populations throughout the province. However, even Newfoundland populations from different phylogenetic lineages may share local adaptations (for traits such temperature tolerance, disease resistance, etc) that predispose them to survival in the region.

Genotype distributions at all four loci of the 1997 sample, that tended to be in Hardy-Weinberg equilibrium in both the SJ and GC strains, suggest that samples taken for each of the domestic strains were representative samples of the population (i.e. not derived from a greatly restricted number of families). As expected, given the semifactorial mating scheme employed, mating occurred at random with respect to microsatellite genotypes within each strain. The excess of heterozygotes at Ssa202 in the GC strain likely was due to random sampling error due to the small number of founding fish.

Microsatellite markers such as those employed in this and other studies on Atlantic salmon can be used to establish a pedigree system in the domestic strain(s) maintained for use in aquaculture in Newfoundland. More than six highly polymorphic loci would be required to allow identification of a high proportion of progeny to fullsibling family (i.e. the progency resulting from a single male and a single female parent). Thus, application of the six-loci analyses of the 1998 samples allowed identification only of 'family groups' that likely consisted primarily of full- and half-sibling progeny. The relatively high level of genetic diversity displayed by the GC samples was surprising because the strain was founded in 1989 with gametes from only 13 female and 5 male salmon. Thus, the 13 family groups identified for the hatchery-reared Grand Codroy strain brood stock appear to represent descendants of the original 13 females, each crossed with males from multiple families.

In spite of the small numbers of fish used to found the GC domesticated strain, the number of alleles and percentage of heterozygotes observed at each locus in the GC sample was comparable to those in samples from wild Newfoundland and Nova Scotia populations. The GC strain was close to fixation for the most common allele at Ssa289, but this also may be a common feature of wild Newfoundland populations. The frequency of the Ssa289 common allele was approximately 0.95 in the Conne River populations, and a sample of 39 fish from the Gander River was monomorphic for the Ssa289 common allele (McConnell et al. 1997). The higher H₀ levels reported for Atlantic salmon strains in McConnell et al. (1997) resulted from the survey of several additional loci in that study. For the loci that were common to that study and the present one, the levels of H₀ are comparable in the wild and domestic strains.

The SJ strain was under domestication for longer than the GC strain, but also appears to have retained much of the genetic diversity present in the wild population. Atlantic salmon populations of the Bay of Fundy are genetically heterogeneous, but consistently distinct from other Nova Scotia populations (McConnell et al. 1997). The domestic SJ sample possessed two rare alleles at Ssa14, as well as the two common alleles observed in other Bay of Fundy populations (McConnell et al. 1997). The number of alleles observed at Ssa197 (12) and Ssa289 (3) in the SJ strain also were comparable to those observed in wild Bay of Fundy populations, although samples from the wild populations were smaller than those examined in the current study (and therefore may have underestimated allelic diversity relative to the present study).

Genetic differences observed between wild and domestic strains of salmon of Bay d'Espoir are sufficiently large to indicate that they form three very distinctive gene pools, consisting of the local Conne River populations, the introduced SJ strain and the transferred GC strain. The degree of allele frequency differentiation among them, at the microsatellite loci surveyed in this study, is sufficient to enable identification of relatively small (5%) contributions of escaped domestic salmon to samples taken from wild populations, but insufficient for reliable identification of individual escapees. However, given the large genetic distances among the three groups of fish, application of new Bayesian classification techniques such as described by Cornuet et al. (1999) and use of data from 10 or more microsatellite loci, accurate classification of individual fish to strain of origin (Conne, SJ or GC) would be technically feasible.

In the course of the present study, a preliminary DNA analysis found the Ssa289 allele 117 (allele 114 in this report) in adults but not smolts sampled from the Conne River, leading to speculation of possible SJ escapees among adults returning to the Conne River. However, Beacham and Dempson (1998) demonstrated that this allele was present consistently in adults sampled from the Conne River system in 1987 (prior to significant aquaculture-industry development), 1992 and 1993. In the 1992 and 1993 samples, the frequency of Ssa289 allele 114 was 0.04. If the only source of the allele was SJ fish, in which the allele frequency is 0.085, approximately half the adults sampled from the Conne River in 1992 and 1993 would have to have been aquaculture escapees. This is inconsistent with the absence of the SJ 'tag' alleles Ssa289 allele 116 and Ssa197 allele 202 in the same Conne River samples. Absence of the Ssa197 allele 192 and Ssa202 allele 249 in the 1992 and 1993 Conne River adult samples provides no support for any contention that Grand Codroy fish had contributed to the salmon-spawning population of the Conne River system. Thus, there is no indication that any hybrid or purebred SJ fish were included in the Conne River samples examined in this study, providing no evidence of introgression from the aquaculture strain into the wild populations.

As noted in the introduction, early Bay d'Espoir industry attempts to use wild stock from the LaHave River and from the Grey, Exploits and Conne rivers of Newfoundland revealed such gross aquaculture deficiencies as to undermine any rationale for pursuing quantitative performance evaluations. It is to the credit of the local industry that the performance evaluations of the present report were conducted on three full year classes, and to the credit of Canadian regulatory concerns that performance evaluation was implemented in the first place. Considering the regulatory framework that existed in 1989, its due-diligence requirements, and a growing concern about genetic impacts of aquaculture species on local wild stocks, there was little recourse at the time but to undertake development and evaluation of a wild salmon stock for Newfoundland aquaculture-industry use. The results of this study indicate that Newfoundland Atlantic salmon populations cannot be assumed to be a phylogenetically homogeneous group of closely related populations. A microsatellite survey of populations would provide an indication of the geographic organization of evolutionarily-distinct lineages in the province. In addition, a more comprehensive evaluation of the performance characteristics of wild strains under culture conditions should be undertaken prior to the considerable investment in selective breeding that would be required for such an endeavour. Finally, the fact that a native strain is used for development of an aquacuture broodstock does not eliminated concern about interactions between aquaculture and wild fish once the domestication process is underway.

It is to be expected that aquaculture broodstocks will perform differently in different environments (i.e. display genotype x environment interactions) with respect to characteristics important to aquaculturists (i.e. incidence of early maturation, susceptibility to endemic pathogens, growth, FCR). Beyond the conservation objective underpinning the rationale for use of local stocks, there still remains economic incentive for developing a strain that performs well in a cold-water "frontier" environment (Tlusty et al. 2000) like Bay d'Espoir and the few areas of the south coast of Newfoundland that may be suitable for salmon farming. Given the Norwegian experience with development of Atlantic salmon brood stock, it is apparent that there can be long-term benefits to a structured-breeding program. However, the costs inherent in such a program, both for hatchery infrastructure and for the ongoing annual operating expenses of a pedigreebreeding program, are well beyond present industry financial capacity. Any such initiative in the future, if it is to succeed, would have to be based on a clear understanding of the logistics and costs of such a program and a priori commitment of all interested parties that the costs and expertise required for such a program can and will be maintained for the required time frames.

ACKNOWLEDGMENTS

In response to requests from the Bay d'Espoir Development Association in the late 1980s, and in consideration of the precautionary regulatory framework that existed at the time, DFO directed that the association be given access to a limited number of Grand Codroy salmon spawners. Science Branch personnel were directed to assist development association employees to procure broodstock from the Grand Codroy River, after consulting with salmon-angler associations. The wild-stock compensation program to mitigate the effects of spawner removal on wild-stock production for the Grand Codroy River was implemented by DFO and funded under the Canada-Newfoundland Inshore Fisheries Development Agreement. The cooperation of the North Shore Bay of Islands Development Association, in providing access to their Hughes Brook incubation facility, was an essential component to the Grand Codroy compensation strategy. The Salmon Preservation Association for the Waters of Newfoundland was instrumental in its contribution to planning both the wild-stock procurement logistics and to developing the fry-stocking compensation strategy for the Grand Codroy River.

The level of cooperation and interactions among industry, resource user groups and government that took place over the decade of this experiment was exemplary. All of the individuals prominent in undertaking and pursuing the various components of the strain-performance initiative are commended for their foresight, diligence and responsiveness to both common and divergent needs amid diverse constraints.

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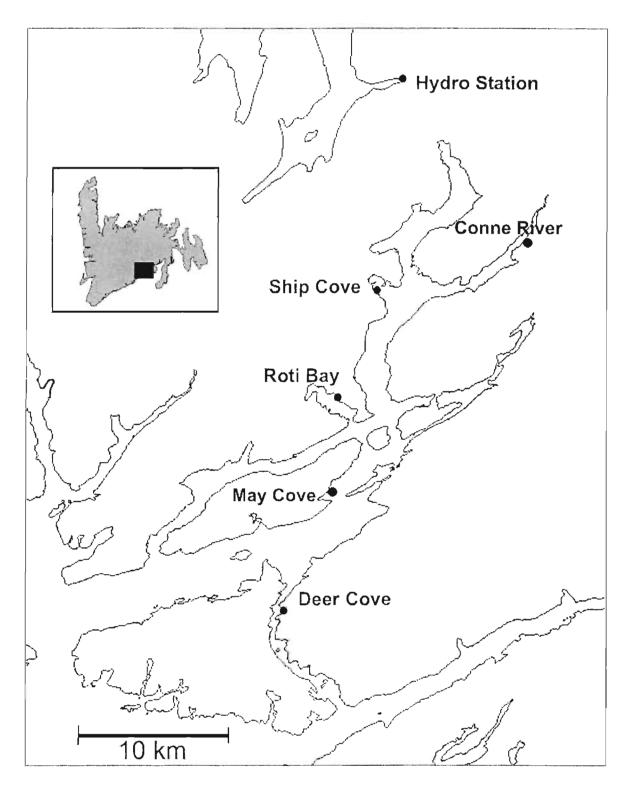


Figure 1. Bay d'Espoir site locations referenced for Grand Cordoy salmon strain evaluations.

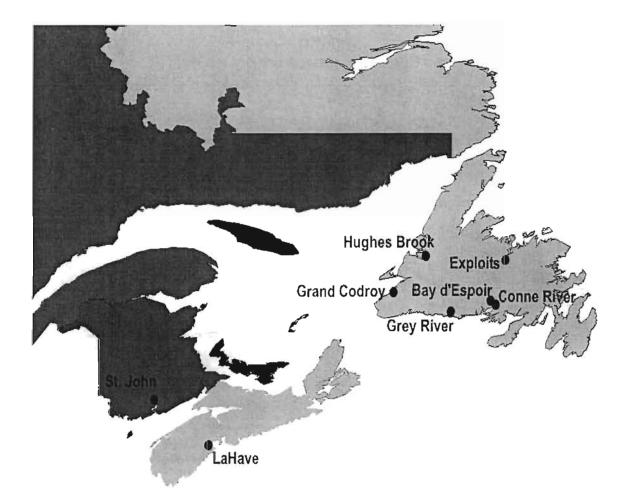


Figure 2. Geographic locations of Atlantic salmon stocks and sites involved in broodstock evaluations in Newfoundland.

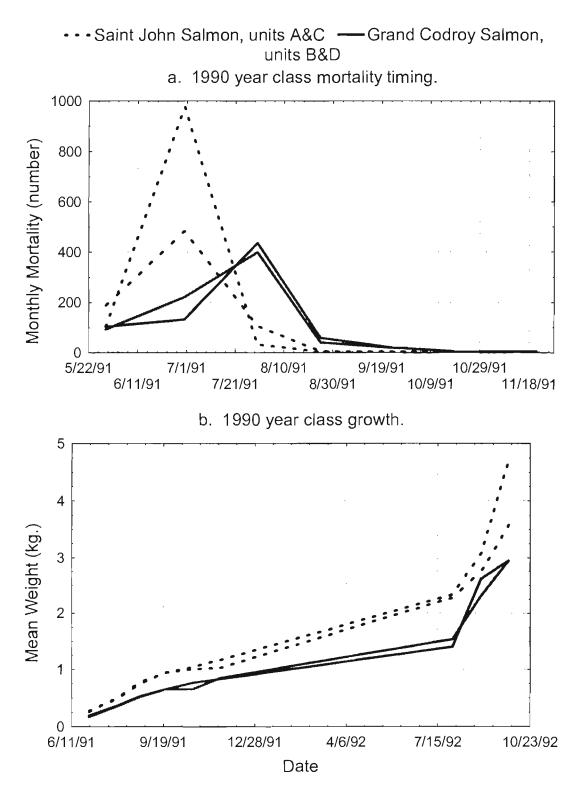


Figure 3. 1990 year-class growth and mortality (industry inventory data available only for the 1st year of marine rearing).

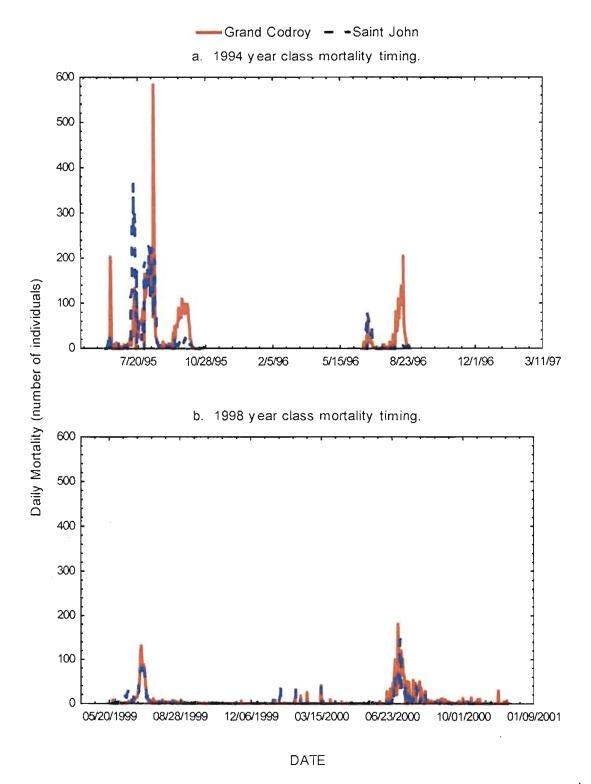


Figure 4. Incidence of mortality for 1994 and 1998 year classes of Grand Codroy and Saint John River Atlantic salmon in Bay d'Espoir aquaculture cages.

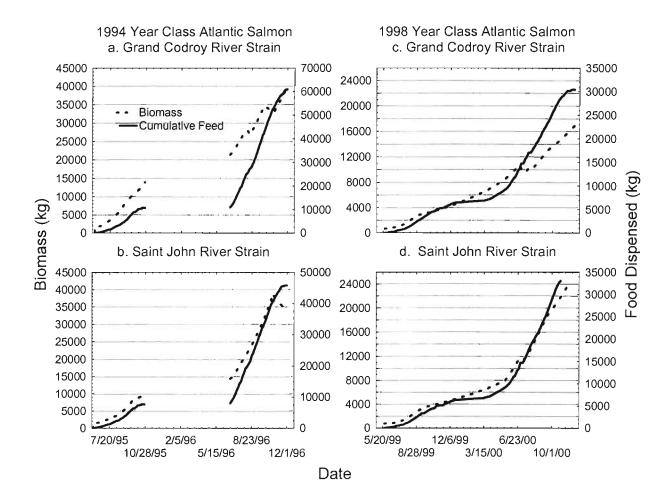
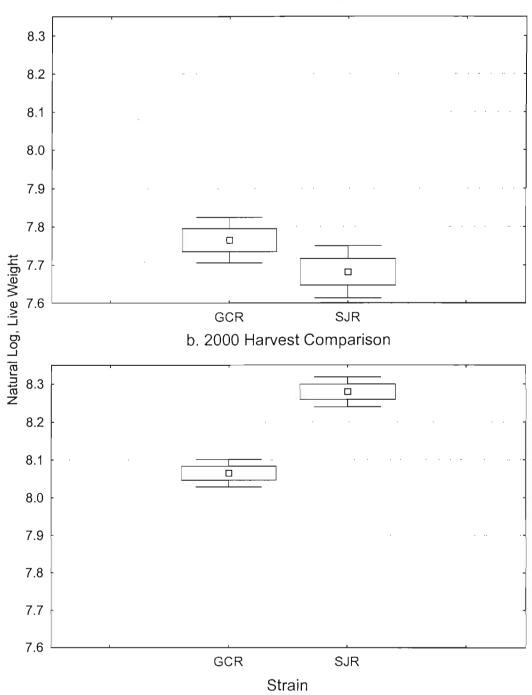


Figure 5. Feed dispensed and accumulated biomass for the 1994 and 1998 year classes of Saint John and Grand Codroy salmon in Bay d'Espoir.



Mean <u>Mean±SE</u> Mean±95% Confidence
a. 1996 Harvest Comparison

Figure 6. Comparison of harvest weight (log10 transformed) statistics for the 1994 and 1998 year classes of Grand Codroy and Saint John River Atlantic salmon from Bay d'Espoir aquaculture.

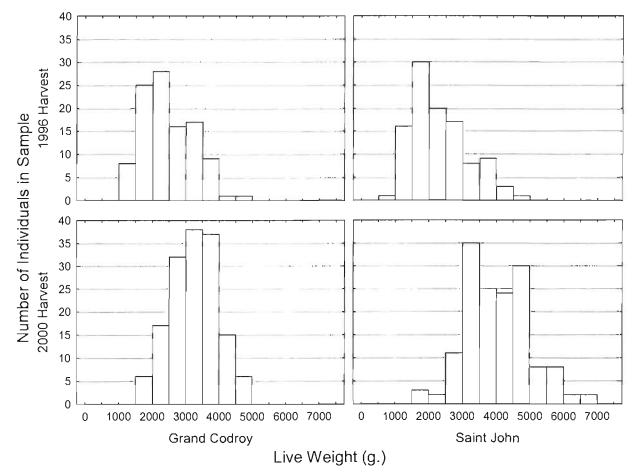


Figure 7. Distribution of harvest-sample weights for the 1994 and 1998 year classes of Grand Codroy and Saint John River Atlantic salmon from Bay d'Espoir aquaculture.

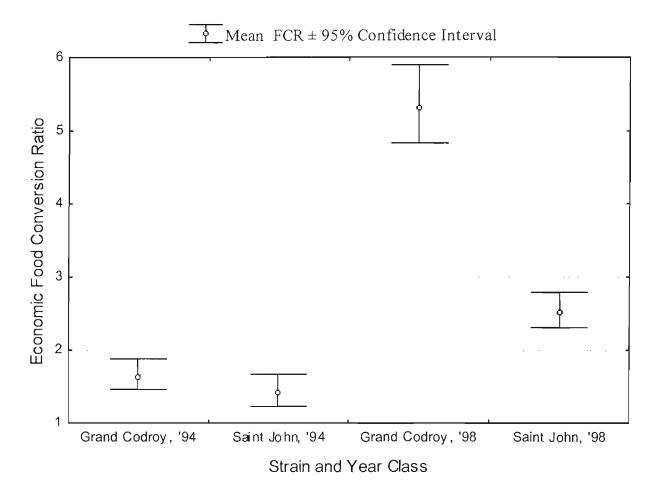
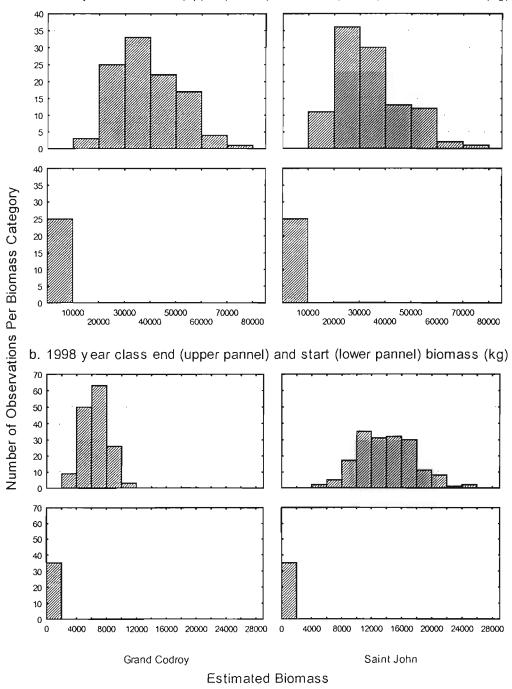


Figure 8. 1994 and 1998 year class FCR comparisons for Grand Codroy and Saint John River Atlantic salmon strains in Bay d'Espoir (.



a. 1994 year class end (upper pannel) and start (lower pannel) biomass (kg)

Figure 9. Distribution of biomass estimates (smolt and harvest) for the (a) 1994 and (b) 1998 year classes of Grand Codroy and Saint John Atlantic salmon in Bay d'Espoir.

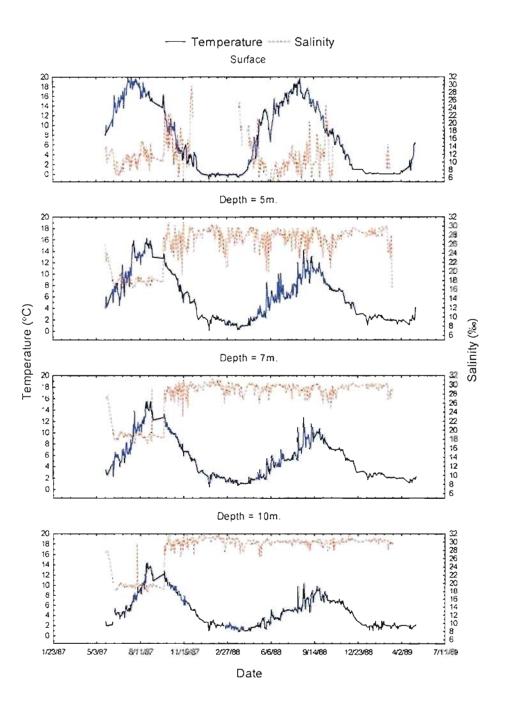


Figure 10. Temperature and salinity fluctuations in the upper-water column for the industry's main overwintering site in Roti Bay for the two-year interval of May, 1987 through April, 1989.

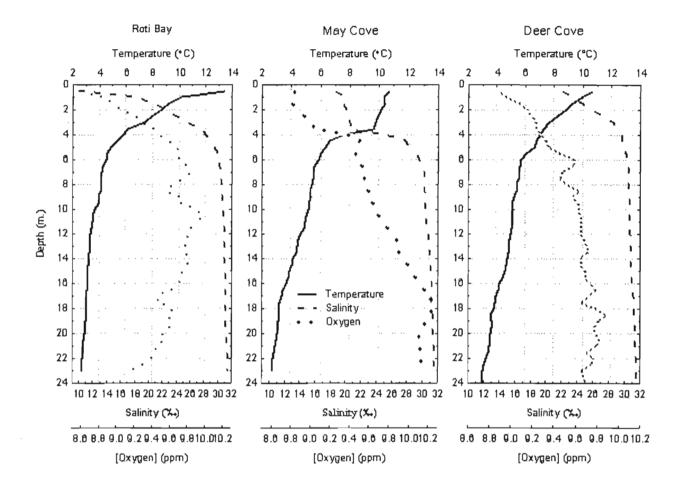
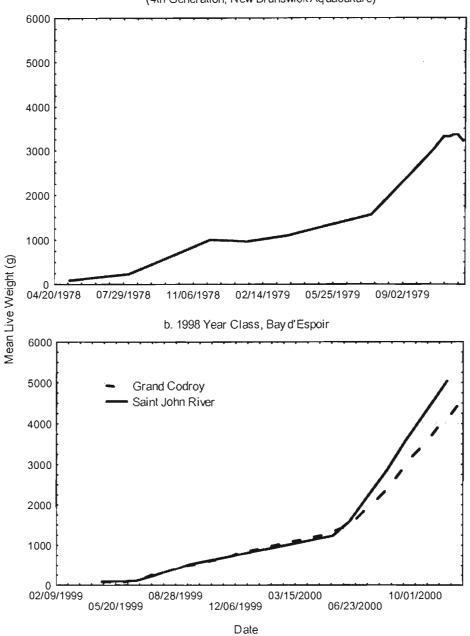


Figure 11. Comparison of "typical" water-column profiles (June 15, 2000) among study sites.



a. Sutterlin et al (1981) Saint John River Strain (4th Generation, New Brunswick Aquaculture)

Figure 12. Comparative illustration of weight increases of (a) 4th generation Saint John River strain salmon in the Bay of Fundy relative to (b) Bay d'Espoir records of the 1998 year class of Grand Codroy and Saint John River strain salmon.

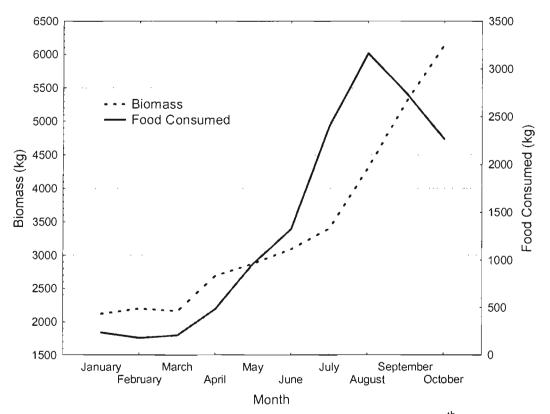


Figure 13. Food consumption and biomass progression for 4th generation Saint John River strain Atlantic salmon from the Bay of Fundy (data from Sutterlin et al 1981).

Thermograph		Thermal	Water Temperature (C at 9m)		
Unit	Month	Units ^a	Mean	Min	Max
1446	October	287	9.3	7.2	11.9
(Cascade, 3n)	November	200	6.7	4.7	8.3
	December	151	4.9	0.8	5.8
	January	123	4.0	0.6	4.6
	February	89	3.2	0.8	4.1
	March	77	2.5	1.4	3.8
	April	57	1.9	1.6	3.9
	May	70	2.3	1.9	4.6
	Total Units	1054			
6248 (SJR mixed)	October	296	9.5	7.5	11.7
	November	. 210	7.0	5.3	8.6
	December	160	5.2	3.8	6.1
	January	134	4.3	2.8	4.9
F	February	99	3.5	2.3	4.4
	March	86	2.8	1.6	4.0
	April	66	2.2	1.9	3.2
	May	79	2.5	2.2	3.5
	Total Units	1129			
6435	October	297	9.6	7.5	11.7
(GCRxSJR)	November	211	7.0	5.6	8.6
	December	162	5.2	3.8	6.1
	January	135	4.4	3.1	4.9
	February	100	3.6	2.3	4.4
	March	88	2.8	1.7	4.1
	April	67	2.2	1.9	3.1
	Мау	80	2.6	2.2	3.5
	Total Units	1141			
6439	October	300	9.7	7.7	11.7
(GCR mixed)	November	214	7.1	5.6	8.7
	December	164	5.3	4.0	6.1
	January	138	4.5	2.9	5.0
	February	102	3.7	2.5	4.6
	March	90	2.9	1.7	4.3
	April	70	2.3	2.0	4.0
	May	83	2.7	2.3	3.5
	Total Units	1161			

Appendix 1. Thermograph summary, Roti Bay: winter 1999/2000.

Appendix 1 (Cont'd.)

Thermograph		Thermal	Water Temperature (C at 9m)		
Unit	Month	Units ^a	Mean	Min	Max
8549	October	288	9.3	7.3	12.0
(SJR female)	November	200	6.7	5.3	8.3
	December	149	4.8	3.6	5.7
	January	121	3.9	2.6	4.5
	February	87	3.1	2.2	4.0
	March	76	2.4	1.4	3.7
	April	57	1.9	1.5	2.6
	May	71	2.3	1.9	3.1
	Total Units	1049			

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^a The average measure of thermal units from the five units is 1107 for the October/99 through.