

Captive Broodstock Management Plan
for Atlantic salmon at
Craig Brook National Fish Hatchery

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1. Introduction and objectives

The Atlantic salmon culture program at Craig Brook National Fish Hatchery (CBNFH) is a vital component of the overall restoration and recovery program for endangered and potentially listed salmon populations in Maine. The role of CBNFH in the restoration and recovery efforts of Atlantic salmon is to facilitate stock rebuilding until listed Atlantic salmon populations are self-sustaining above current conservation requirements.

The Captive Broodstock Management Working Group was established by the Maine Atlantic Salmon Technical Advisory Committee (TAC) and charged with “updating TAC Broodstock Collection and Spawning Protocols”. Since 1992, protocols used for broodstock collection, spawning and adult releases have been short term (Anon. 2000, Anon. 2001, Anon. 2002., Beland et al. 1995, Beland et al. 1997, Buckley 2002a, Burke and Tozier 2002, King et al. 1997).

There are two primary goals of the Broodstock Management Plan (BMP). The first goal is to describe and explain current broodstock management practices at CBNFH. Due to recent significant changes at CBNFH, culture techniques and production efficiencies have been improved and refined, although the overall program goals and guiding principles have not changed. The BMP details hatchery operations and describes the facilities and production practices, which aid in the evaluation of future management options at CBNFH. The second goal of the BMP is to provide a framework with quantifiable values to evaluate the hatchery program in relation to the management objectives. The BMP will provide both short and long term guidance for spawning, monitoring, and evaluation of the captive broodstock program to achieve its restoration and recovery goals. The BMP will serve as a working, evolving framework to guide management and propagation of the captive Atlantic salmon broodstock held at CBNFH. The BMP in part meets the genetic management plan requirement (requirement 6) of the Interagency Policy on Controlled Propagation of Species Listed under the ESA (65 FR 56916). Also, the BMP addresses parts 5 (Supplement wild populations with hatchery-reared DPS salmon) and 6 (Conserve the genetic integrity of the DPS) of the recovery program, and implementation of recovery plan task 5.1.1 (maintain river-specific hatchery and broodstock and continue to stock cultured fish in natal rivers) of the Draft Recovery Plan (NMFS and USFWS 2004).

1.1. Program goals

In November 1991, the U.S. Fish and Wildlife Service (FWS) designated five Atlantic salmon populations in Washington County, Maine as Category 2 under the Endangered Species Act (ESA). These populations were found in the Dennys, East Machias, Machias, Pleasant and Narraguagus rivers. Populations in the Ducktrap and Sheepscot rivers were subsequently added to this list. Following the Category 2 listing, the FWS and Maine Atlantic Sea-Run Salmon Commission (ASC) began a program to

preserve and recover the populations, as well as gain the information necessary to determine if listing under the ESA was warranted.

In 1991, the FWS and ASC began development of a river-specific broodstock program at the CBNFH. River specific broodstock collections were initiated in the fall of 1991 with the capture of 14 salmon from the Machias River, which resulted in the successful spawning of 3 female salmon (Buckley 1999). In 1992, the ASC and FWS developed a Prelisting Recovery Plan (Baum et al. 1992). The Prelisting Recovery Plan called for development of river-specific Atlantic salmon broodstocks to be used for supplementation, and genetic characterization of all Atlantic salmon stocks in Maine. The overall objectives of this plan were to begin recovery of the depleted populations of native Atlantic salmon in Maine, gather information regarding the population status, and to inform the public of the plight of the salmon and the need for restoration activities.

Due to low abundance of potential adult spawners in 1992, TAC recommended utilization of wild parr to establish a captive propagation program. Between 1992 and 2003, approximately 12,000 parr were collected from the Dennys, East Machias, Machias, Pleasant, Narraguagus, and Sheepscot rivers and reared at CBNFH (Buckley 1999, Buckley 2000, Buckley 2002a). The first parr collected in 1992 were spawned in 1994 and their progeny were released as fry in 1995. In 2000, populations in eight rivers (Sheepscot, Ducktrap, Narraguagus, Pleasant, Machias, East Machias, Dennys, and Cove Brook) were listed as an endangered Distinct Population Segment (DPS). Inclusion of propagation for the Penobscot River was to provide juveniles produced from wild adult salmon for stocking.

The overall goal for the broodstock program at CBNFH is to aid in the recovery and restoration efforts of Atlantic salmon in Maine, conserving the genetic legacy of Atlantic salmon in Maine until habitats can support natural, self-sustaining populations. Previously specified by Beland et al. (1997), the goals of the captive broodstock program at CBNFH include

- 1) Facilitate the recovery and restoration of the natural populations and minimize the risk of further decline or loss of individual populations
- 2) Conserve the genetic diversity within individual broodstocks, maintain the genetic characteristics and genetic variability for the populations maintained at CBNFH
- 3) Minimize the risk of artificial selection in the hatchery or through hatchery practices
- 4) Provide juveniles for stocking with genetic characteristics specific to the population of origin
- 5) Captively breed Atlantic salmon from the Penobscot River.

2. Craig Brook National Fish Hatchery

2.1. Facility description and operational requirements

CBNFH is the designated hatchery facility for the recovery and restoration efforts of the DPS Atlantic salmon rivers, and for the restoration efforts of the Penobscot River in Maine. The current river-specific program was implemented in 1992, and the guiding concepts remain the same today. The protocols, procedures and practices, however, have been reexamined and revised several times over the years, with the intent of using sound science for management.

CBNFH has adapted, and will continue to adapt to meet the needs of the Atlantic salmon restoration program. Some of these adaptations have included: construction and renovation of a receiving and isolation building in 1994 and 2002, respectively, construction of new broodstock and hatchery buildings in 1998, an addition to the Water Treatment Plant in 1999, and construction of a water pumping station in Alamoosook Lake in 2004. Future construction includes the Penobscot Broodstock Holding and Screening Buildings scheduled for 2005, and Wastewater Discharge Building in 2006.

Construction and renovation of CBNFH was completed to facilitate the management of the broodstocks with consideration of the requirements for maintaining separate populations following fish health and genetic protocols. Currently, CBNFH can rear up to seven separate populations, while adhering to biosecurity and genetic management criteria throughout the facility. All hatchery rearing rooms are completely enclosed with a single entrance. All incoming water lines are on a continuous loop. The feed lines have Swedish inlets to each individual tank or trough, and drop lines to each incubation stack. A common drain line is used for the discharge in each isolation room. All drain lines are joined together in a collection pit or collection line outside the isolation rooms. Table 1 describes the dimensions and equipment used in the fish rearing isolation units found in each building.

Table 1. Description of the type, size, number, life stage held, and capacity of the tanks listed by building used for Atlantic salmon culture at CBNFH.

Tank type	Tank size	Number of tanks per bay	Life stage held	Capacity (number)	Per unit type
<i>Receiving Building (50' x 200', 12 bays)</i>					
Oval	15' x 8'	15	Parr	250	tank
Oval	8' x 5'	5	Parr	150	tank
<i>Penobscot holding area</i>					
Swedish	36' x 36'	4	Sea-run Adults	200	tank
<i>Screening Building (36' x 127', 5 separate bays)¹</i>					

Oval	15' x 8'	2	Sea Run Adults	20	tank
<i>Broodstock Module Building (150' x 160', 6 separate rearing isolation units)</i>					
Swedish	18' x 18'	6	Adults	250	tank
			2 Year smolts	3,000	tank
<i>DPS Hatchery Building (53' x 220', 6 incubation and fry isolation units)</i>					
Vertical incubation stacks		8 ²	Green eggs	8,000	tray
			Eyed eggs	6,000	tray
			Alevin	6,000	tray
Troughs	1'2"x14'x10"	20 ³	Fry	25,000	trough
			Fingerling	5,000	trough
			0+Parr	2,500	trough
<i>Penobscot Hatchery Building (53' x 103', 1 incubation and fry isolation unit)</i>					
Vertical incubation stacks		28 ²	Green Eggs	8,000	tray
			Fingerling	5,000	tray
			0+Parr	2,500	tray
Troughs	1'2"x14'x10"	80 ³	Fry	25,000	trough
			Fingerling	5,000	trough

¹ The Screening Building for the Penobscot sea-run adults is scheduled for construction spring 2005.

² There are 16 trays per stack.

³ Each trough is in a series of two for reuse of water.

CBNFH has three water sources for hatchery operations. The primary water source is Craig Pond, a small 210 acre pond with a depth of 68'. The hatchery has two intakes in Craig Pond: one at 30' depth and one at 58' depth. The other major source of water is Alamoosook Lake, an 1100 acre lake with a depth of 28'. The hatchery has one intake in Alamoosook Lake at 10' depth. A third source for water is a 120 gallon per minute (gpm) well, which decreases as the well is in use.

The Water Treatment Plant receives and distributes all incoming water from the three water sources. There are six sand filters with the capacity of filtering 400gpm per filter, for a total of 2400 gpm processed. The filters remove debris and parasites from the water. After the water is filtered, it passes through UV chambers. There are five UV chambers used to kill bacteria and viruses in the water. Four of the UV chambers have the capacity to filter 600 gpm per chamber, and the fifth a capacity of 1200 gpm per chamber. Following passage through the UV chambers, the water passes through the vacuum degassers with oxygen injection. In the degassers, dissolved gases are stripped out of the water and oxygen is injected to the desired parts per million for salmon culture. The water is then distributed from the headbox to the hatchery via six individual distribution lines. Each line targets a specific part of the hatchery. The headbox was designed with the ability to mix water from two different sources for each of the distribution lines.

2.2. Hatchery facility limitations

The hatchery has three major limitations: wastewater discharge, water requirements, and space for operation and expansion. The most severe limitation is the wastewater discharge permit issued by the State of Maine for operations commencing in 2007. This permit allows Craig Brook NFH to discharge just over 100 lbs of phosphorous per year. All other discharge parameters specified in the permit are attainable. However, even with the best management practices for cleaning wastewater in the hatchery's new wastewater treatment building, scheduled for construction in 2006, the hatchery will not meet the phosphorous demands of the discharge permit.

The second facility limitation is the amount and temperature of the water required by the hatchery. To ensure that enough cold water from Craig Pond is available between the months of June and October, the hatchery pumps water from Alamoosook Lake between November and May. Alternating water sources allows Craig Pond to fill and be available for usage during the summer months, during which the hatchery requires 1400 gpm. Increasing the water usage for this time period to 1800 gpm (full production capacity) or greater would create severe shortages to the cold water supply and major problems with the camp owners on Craig Pond. A potential resolution of the cold water supply needs would be construction of a pipeline from Toddy Pond to the hatchery. Pipeline construction was evaluated in 1998 during facility reconstruction, however the cost was prohibitive and Champion Paper Company, who owns the water rights to Toddy Pond, placed a restriction on water usage during the months of August and September.

The third limitation to hatchery operations is the amount of land suitable for building. Although CBNFH is located on 135 acres, only 40 acres are suitable for construction because they are downstream of the future Water Treatment Plant. The amount of available land for expansion will be further reduced following the construction of the wastewater treatment facility in 2006.

An additional limitation of broodstock management at CBNFH is the containment of all broodstock at one site. In the event of a catastrophe (e.g. disease, dewatering, thermal excess, etc.), the survival of all broodstocks at CBNFH is threatened. With the exception of the Penobscot population which has a backup broodstock maintained at Green Lake National Fish Hatchery, there are no auxiliary broodstocks for the other CBNFH broodstocks. In the event of a catastrophe that resulted in the loss of any of the DPS broodstocks at CBNFH, each would need to be rebuilt with collections from the wild populations.

3. Current broodstock management

Protocols for broodstock collection, management and spawning were developed by the TAC and have been reviewed annually since 1995 (Beland et al. 1995, Beland et al. 1996, Beland et al. 1997, King et al. 1997, Anon. 2000, Anon. 2002). Due to differences in management practices at CBNFH between the captive DPS populations

(Sheepscot, Narraguagus, Pleasant, Machias, East Machias, and Dennys) and the Penobscot population, broodstock management for each group is described separately. Management differences between the DPS and Penobscot populations exist in collection protocols (juveniles are collected for DPS populations, adults for the Penobscot population), and the length of time each group spends in the hatchery. However, spawning protocols for both groups of fish are similar and therefore described jointly.

3.1. DPS broodstock management and maintenance

3.1.1. Juvenile broodstock collection

Collection of juvenile Atlantic salmon for future broodstock purposes is guided by two primary objectives:

1. provide a river-specific egg source for stocking in DPS rivers, and
2. collect salmon parr from DPS rivers in a manner that represents the genetic variability in those rivers.

River-specific broodstock collection targets of parr, based on available habitat for fry, are set by ASC, NOAA Fisheries, and FWS through the TAC in advance of parr collections (Table 2). To increase the potential for incorporation of naturally spawned Atlantic salmon into the captive broodstock, data on the distribution of redds from two years previous are used to identify areas where natural reproduction has taken place. Collection locations and target numbers are selected for all accessible river sections where natural reproduction has occurred. Two or more collection locations are typically selected within 1000 meters of known spawning habitat to account for fry and parr dispersal. Target collection goals for parr are established by TAC, and are 250 parr for the Machias and Narraguagus, and 150 for the Dennys, Sheepscot, and East Machias.

Parr collection occurs annually from August to October. Parr are collected using backpack electrofishing equipment, and sampling efforts are either in conjunction with population assessment sampling, or during a dedicated broodstock collection trip, depending on the operational needs or preferences of the biologist responsible for sampling each river. Electrofishing sites for parr collection selected to recover hatchery-origin juveniles are based on the quantity and quality of riverine habitat. Hatchery-produced fry are stocked into river sections of suitable habitat, and collection efforts focus on these areas to recapture those stocked individuals as parr. Collection efforts are made in multiple sections throughout each river system. Parr broodstock are generally age-1, although age-2 parr are often captured. On rare occasions, sampling efforts may elect to collect age-0 parr, especially if there is reason to expect difficulties in capturing many parr in the following year. Younger parr (age-0) and/or smolts have occasionally been collected from the Pleasant River due to low recovery rates of older parr (Table 2).

Table 2. Broodstock collection summary for DPS captive fish held at CBNFH. All samples represent parr collections, except the Pleasant River, which also includes smolt collections. Data from USASAC reports.

River	Year					
	1999	2000	2001	2002	2003	2004
Dennys	150	131	159	315	276	151
East Machias	125	126	144	174	160	158
Machias	238	262	266	353	310	246
Narraguagus	255	259	259	260	264	245
Sheepscot	86	160	141	168	167	174
Pleasant-parr	0	67	0	0	119	102
Pleasant-smolts	-	60	15	4	3	-

Prospective broodstock from each river are either transported directly following collection to CBNFH, or held for several days in an oxygenated tank at the ASC Jonesboro headquarters (due to distance from hatchery). The short-term survival for both methods is approximately 100% for the captured parr. At CBNFH, the parr are brought to the hatchery’s receiving building, and within 24 hours receive a prophylactic 250 ppm formalin treatment for one hour. After the treatment, the parr from each river are placed in a fish rearing isolation unit for a quarantine period of 10-12 months. During this time period, the fish are observed for abnormal behavior (such as due to disease), and are non-lethally sampled for bacterial pathogens at the 10% assumed incidence level, using inoculation and culture from vents. After the quarantine period, each fish is implanted with a uniquely coded passive integrated transponder (PIT) tag and a fin clip is taken for genetic analysis (see 3.3.1). PIT tags facilitate the identification of an individual to river of origin, available genetic data, and spawning history. Once tagged, the fish are moved to the river-specific Broodstock Module. All tagging information including PIT tag codes, marking date, and capture date are entered into the CBNFH broodstock database.

3.1.2. Adult broodstock management

Adult Atlantic salmon are maintained in river-specific broodstock rooms, separately by capture year. Each capture year potentially represents multiple year classes due to the incorporation of age 1 and age 2 parr during juvenile sampling. For each population, typically three to four capture-year classes are held in each broodstock room. Currently, no adults are collected from the wild and introduced into the DPS broodstock populations.

3.2. Penobscot broodstock collection and management

Sea-run adults are used as broodstock for the Penobscot River. No captive broodstock for the Penobscot population are maintained at CBNFH, although domestic adults are maintained at GLNFH in case no sea-run adults return to the trap or for a fish loss at CBNFH. Adults to be used for broodstock for the Penobscot River are collected by ASC every year at the Veazie Trap, located at the upstream end of a fish ladder at the

Veazie Dam near Bangor, Maine. ASC tags incoming broodstock with PIT tags and collects biological data and tissue samples for genetic analysis. The survival to spawning for sea-run adults collected on the Penobscot is approximately 98-99 %.

A target number of 600 broodstock is needed for the Penobscot River restoration program. Target numbers are based on fry and smolt production and stocking requirements determined by ASC and TAC. The desired number of females and males is 400 and 200 (including 40 grilse) spawners respectively. Collection starts in May/June and continues until the target number is obtained, usually in mid July. During the later portion of the adult run, female broodfish are selectively targeted for collection to ensure the desired quota of females is collected.



Following fish health and biosecurity protocols, Penobscot broodstock are treated daily with 25 ppm of formalin for 6 hours to control fungus, *Ichthyophthirius*, and external parasites (Anonymous disease sampling protocol). Formalin treatment starts upon arrival of broodstock and continues until water temperatures drop below 10 C or until the end of September. All Penobscot broodstock are sampled for Infectious Salmonid Anemia (ISAV) prior to spawning by USFWS Northeast Regional Fish Health Lab (Lamar, PA).

3.3. Genetic evaluation of broodstock

Molecular genetic characterization of parr at presumed neutral loci is completed on an annual basis (prior to incorporation into broodstock). Responsibility for annual genetic characterization and interpretation of data resides with the USFWS, with genetic information standardized to the broodstock baseline to facilitate incorporation into an existing genetic database. Eleven microsatellite loci are currently used to characterize broodstock: Ssa197, Ssa171, Ssa202, Ssa85 (O'Reilly et al. 1996), Ssa14, Ssa289 (McConnell et al. 1995), SSOSL25, SSOSL85, SSOSL311, SSOSL438 (Slettan et al. 1995, 1996), and SSLEEN82 (GenBank accession number U86706). Genotypic data for DPS and Penobscot populations are maintained in a database by the USFWS Region 5 Conservation Genetics Lab at the Northeast Fishery Center (Lamar, PA). Parentage assignment uses genotypes from previously analyzed capture years, therefore incorporation of new loci or different markers must be completed in a method to allow for seamless utilization of the genetic broodstock database.

Evaluation of genetic diversity estimates for each broodstock population includes observed heterozygosity, number of alleles per locus, inbreeding coefficients, estimates of relatedness, and hierarchical comparisons of differences in allele frequencies. Comparisons of these estimates are made between capture years within river-specific populations, and between river-specific populations. Analyses are used to track maintenance of genetic diversity over time, and determine if populations are threatened by inbreeding or low effective population sizes (see section 3.4.4). Monitoring of heterozygosity will be used to detect if losses of greater than 5% over five years with a river-specific broodstock are occurring.

3.3.1. Genetic evaluation of broodstock-DPS

Parentage analysis of captured parr can be used to track efficiency of broodstock collections, determine the number of stocked family groups being recovered, and if parr of non-hatchery origin (wild reproduction or aquaculture escapees; see section 3.3.3. for broodstock screening procedures) may be present in broodstock collections. Evaluation of parentage analysis is used to monitor percentage of families recaptured for each broodstock in each capture year. Initial target recovery of percentage of families recaptured in parr collections is 75% of families stocked, however this threshold will be evaluated in coming years due to recent changes in stocking practices. As part of the genetic screening of parr, it is important to continue evaluation of within-capture year spawning to reduce the potential for spawning related individuals.

3.3.2. Genetic evaluation of broodstock-Penobscot

Fin clips for genetic analysis are obtained from all Atlantic salmon used for broodstock purposes trapped at Veazie Dam on the Penobscot River. Individuals that have not been sampled previously are PIT tagged to allow tracking of specific individuals through the spawning process and for future identification. Similar to uses for the DPS populations, genetic information is used to screen for potential aquaculture escapees, and to monitor estimates of genetic diversity within the population over time. Ultimately, genetic analyses provide the potential to track and identify the success of fry plants in specific riverine habitats.

3.3.3. Broodstock Screening

Maintenance of the genetic diversity of the Atlantic salmon (*Salmo salar*) broodstock held at Craig Brook National Fish Hatchery (CBNFH) is critical for population long-term preservation and viability. Captive broodstocks at CBNFH are maintained separately by river, and spawning occurs among individuals within a river to preserve river-specific genetic characteristics. To allow for some degree of natural selection to act on survival and fitness, fry are stocked into the river of origin and subsequently recaptured as parr. An additional benefit to this practice is the incorporation of progeny from wild-spawning adults into the captive broodstock system. Incorporation of offspring resulting from spawning adults from neighboring drainages would mimic natural low levels of straying among Atlantic salmon populations (Baum 1997). However, incorporation of wild parr increases the potential to incorporate progeny from adults not native to the DPS (i.e. aquaculture escapees). Incorporation of escapees or strays from aquaculture sources (e.g. net pens, other hatchery facilities) may confer negative fitness traits to the native wild populations, compete for resources, or reduce locally adapted diversity and reduce fitness. Annual screening also allows for monitoring of various estimates of genetic diversity within the broodstock (see section 3.3.1 and 3.3.2). Therefore, it is important to genetically screen captured parr to determine origin.

3.3.3.1. Genetic Screening Procedures

Individuals considered for future use as spawning adults in river-specific broodstocks at CBNFH are genetically analyzed to determine origin. Screening utilizes genotypes for 11 variable microsatellite loci (Ssa202, Ssa289, SsoSL311, SsoSL438, Ssa197, Ssa171, and Ssa85, Ssa82, Ssa14, Ssa25, and SsoSL85) provided to the USFWS by Dr. Tim King (USGS-Leetown Science Center). When parr from the Dennys, East Machias, Machias, Pleasant, Sheepscot, and Narraguagus are PIT tagged prior to transport to broodstock rooms, tissue samples are obtained for genetic analysis. Genetic screening and characterization occurs at this stage. Tissue samples are obtained from the Penobscot sea-run adults when individuals are transported for broodstock purposes to CBNFH. Genetic screening and characterization occurs prior to spawning, to allow removal prior to spawning.

Individuals screened are compared to river-specific broodstocks or other rivers in Maine. Baseline populations therefore include individuals from the Narraguagus, East Machias, Dennys, Ducktrap, Penobscot, Kennebec, Machias, Pleasant, and Sheepscot rivers, up to but not including the capture year analyzed, and minus individuals previously culled.

Assignment to population of origin is completed using GeneClass (Version 1.0.02; Cornuet et al. 1999). For each individual assignment to population of origin, simulations are done to determine the probability of assignment to each of the reference populations. The Bayesian assignment method (Rannala and Mountain 1997) within GeneClass is used when assigning unknown individuals to baseline populations.

3.3.3.2. Assignment criteria

Following continent of origin assignment testing required for the aquaculture industry to cull any non-North American individuals, a two-step hierarchical criterion based on probability of assignment to population of origin is used to classify individuals. First, an individual is identified for additional analysis if there is a less than 1% probability of assignment to the population an individual putatively originated. For individuals identified for potential removal with the first criteria, only individuals that have a less than 5% probability of assignment to any other Maine population (Narraguagus, East Machias, Dennys, Ducktrap, Penobscot, Kennebec, Machias, Pleasant, and Sheepscot) is be removed from the broodstock. This method was developed to increase the likelihood of correctly identifying individuals belonging to a specific population, while still allowing for the possibility of straying between neighboring drainages. Because other source populations not included in the baseline may have contributed to the unknown group of individuals, the use of the 2-step process and low probability of assignment thresholds aids in identification to any of the baseline populations used.

3.4. Spawning

Due to low numbers of returning adults to rivers, the captive broodstock at CBNFH has been used as the primary source to produce juveniles for restoration stocking efforts. Due to the reliance on the captive population to sustain Atlantic salmon in Maine, it is necessary to maintain genetic variation inherent in the captive broodstock. Therefore, the primary goals for spawning strategies implemented at CBNFH are:

1. preserve genetic variation inherent to each of the genetically unique river populations maintained at CBNFH,
2. ensure the long-term maintenance of genetic variation,
3. minimize the potential for inbreeding or domestication selection and associated reductions in fitness in the wild.

Because all broodstock are PIT tagged, rearing and spawning histories can be tracked for each individual. PIT tag numbers of males and females spawned and pertinent information (i.e. egg quality, blood in milt) about spawning pairs is recorded into a Microsoft Access database (see section 5.1). Individual spawning histories are then able to be tracked by PIT tag to assess number of times spawned, mate, and disposition of offspring.

3.4.1. Spawning strategy

At present the risk of domestication selection is reduced, although probably not eliminated, by attempts to rear and spawn fish using methods and practices chosen to avoid overtly biasing the survival or reproductive success of individuals with different trait values. Assessment of possible selection risks and methods to mitigate against artificial selection will likely represent a continuous effort. However, more directed strategies are in place for preserving genetic diversity and limiting inbreeding through spawning approaches.

Spawning occurs in November, following the natural cycle of maturation for Atlantic salmon. Current spawning protocols require single paired matings, occurring within the same broodstock year class. Individuals that have not previously spawned (“first-time spawners”) are preferentially selected, and individuals are only spawned once within the spawning season (Table 3). The spawning hierarchy is applied to both DPS and Penobscot populations. The basis for the current protocol of using first-time spawners is to maximize the number of fish contributing to the broodstock program and more approximately equalize the contribution of individuals to future generations. The value of these attributes is that they better facilitate the contribution of rare or unique alleles to future generations (Kinnison et al. 2003). For example, through equalizing contributions, the genetic material from a limited number of individuals is prevented from becoming overrepresented in a population due to artificial mating practices, reducing the potential of losing rare or unique alleles through genetic drift or “bottlenecking”. However, due to the shortage of first time spawners in some spawning years, it is necessary at times to reuse a small portion of broodfish spawned in previous years to meet fry production targets (Table 3). Individuals used for spawning are randomly chosen based on the ripeness of the available fish to spawn on a particular day

(with the exception of the use of the spawning optimization program, see Section 3.4.2). Spawning sex ratios for the DPS populations are one female to one male, with no (or limited re-use of males). Spawning sex ratios for the Penobscot population are also one female to one male, but males are often re-used to reflect the female bias in broodstock collection. Future broodstock management evaluations will examine this practice and evaluate alternative broodstock collection and spawning practices for the Penobscot River.

Broodstock from the DPS populations are held at CBNFH for multiple years. Although spawning protocols focus on the utilization of individuals that have previously not spawned, retaining adults for multiple years acts as a genetic reservoir if needed, or if the first spawning event for an individual was not successful. In years when production goals are met with first time spawners and a surplus of first time spawners exist, the eggs of individuals not spawned are stripped and discarded to improve their egg production in future years.

Table 3. Decision-making hierarchy for spawning.

Step	Decision
1	All first-time spawners will be spawned in a population. For populations with a pedigree broodstock line, domestic individuals are used in conjunction with captive individuals as the two groups represent a single genetic group.
2	Repeat spawners will be used when first-time spawners cannot meet egg take requirements. Repeat spawners will also be used if inadequate genetic diversity occurs within the first-time spawners. This may occur in situation where broodstock collection is low and or there is substantial mortality in the hatchery in the first-time spawner groups.

3.4.2. Spawning optimization program

To ensure the long-term genetic viability of the endangered stocks, it is important to minimize potential deleterious genetic impacts of captive propagation. Through the use of PIT tags, genetic monitoring, and tracking of individuals at CBNFH, a program was designed that structures matings to avoid breeding close relatives and limit the accrual of inbreeding within the captive populations (Coombs et al. 2004). The PIT tags of five females and five males ready to be spawned are scanned into computer program prior to mating. Using existing genotype information for the identified individuals, the program identifies optimal mate pairs that will maximize the genetic distance between individuals for the five resulting matings, using each individual only once. The program outputs the PIT tag numbers for the male and females to be paired during spawning. Gametes are not pooled; milt is added to the eggs of only one female. The use of five individuals of each sex to choose from during the spawning process was determined based on a combination of factors, including: the spawning setup in the hatchery, space constraints, and computer simulations which demonstrated no significant decrease in the

proportion of shared alleles when more than five individuals of each sex were available to spawn. The resulting pairing information is saved by the program and transferred to the broodstock database for tracking purposes.

The spawning optimization program was tested at CBNFH during the fall 2004 spawning. Implementation of the program will follow a two-tiered evaluation (see section 3.4.3.). Because the Penobscot adults are drawn from a larger population relative to the DPS populations, the threat of spawning related individuals is much lower in the Penobscot population. Although spawning pairs are documented in the Penobscot broodstock program, implementation of the spawning optimization program is not currently planned; however this will be evaluated annually.

3.4.3. Implementation of software and changes to current spawning protocols

To improve the efficiency and effectiveness of spawning at CBNFH to achieve the primary goal of long-term maintenance genetic diversity, a two-tiered assessment plan will be implemented to establish:

1. if capture year classes will be crossed within a particular population, and
2. if the spawning optimization program will be used in a particular population.

Standard hatchery practice is to sort broodstock prior to spawning to estimate the number of sexually mature individuals. Based on the individuals estimated to spawn within each population, the proportion of shared alleles can be estimated for random mating within capture year, and random mating between capture years. If the averages of these proportions are higher than averages for the same population in recent years (e.g. show evidence of increased risk of inbreeding through time), then the spawning optimization program must be used. Current plans at CBNFH are to phase in the spawning optimization software approach and eventually use this approach for all DPS populations by 2006.



3.4.4. Effective population size

The effective population size (N_e) represents the number of individuals successfully contributing to subsequent generations. This number may be substantially lower than the census number of individuals in a population, due to varying ages at maturity, opportunities to reproduce, or poor reproductive output. The effective population size for each of the broodstocks at CBNFH can be calculated based on the data contained in the spawning database, using the formula

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

where N_m is the number of males spawned, and N_f is the number of females spawned.

Table 4 describes the N_e for the captive broodstocks at CBNFH. Also described in Table 4 is the number of adults available to spawn in a given year, which is typically much larger than the N_e . Due to changes in spawning practices (to first-time spawners,

see Table 3), increased parr collections in some years for specific research requests, numbers of adults per population vary both within populations by collection years, between populations, and relative to N_e . Differences between N and N_e include: not all adults are sexually mature that year (may have spawned previous year), not spawning all mature individuals to limit egg or fry production, retention of surplus adults in the hatchery for use as a repeat spawner, or for surplus stocking (see Section 3.6.1).

Implementation of the surplus spawner stocking program (Section 3.6.1; Table 10) will work to reduce the number of spawners surplus to production, demographic, or genetic needs maintained at CBNFH.

In some populations, there is a large difference between the N_e and N (Table 4). Differences are the result of increased parr collection efforts in for the Machias and Narraguagus (Table 2). Within the Dennys River, the high N available versus the N_e is due to the retention of an additional spawner year class. Most broodstocks at CBNFH consist of three spawning year classes (ages 3, 4, and 5), however the Dennys broodstock consists of four spawner year classes (ages 3, 4, 5, and 6) to meet the additional smolt and fry production requests. Therefore, although the census population size for some broodstocks is above 500, the effective number of spawners for most broodstocks is less.

Table 4. Estimates of effective population size (N_e) for broodstocks at CBNFH based on spawning records of the number of males and females spawned each year, by population. Also included is the total number of adults (N) available per population for each spawn year (not including parr broodstock collections).

Population	Spawn Year	Number Females	Number Males	N_e	N
Dennys	1999	56	45	99.8	124
	2000	64	47	108.4	183
	2001	82	72	153.4	291
	2002	68	68	136.0	379
	2003	78	77	155.0	451
	2004	83	85	168.0	449
East Machias	1999	56	58	114.0	276
	2000	69	55	122.4	231
	2001	67	56	122.0	312
	2002	92	94	186.0	377
	2003	92	80	171.2	380
	2004	57	65	121.5	423
Machias	1999	120	119	239.0	503
	2000	108	81	185.1	208
	2001	108	91	197.5	555
	2002	111	111	222	665
	2003	94	95	189.0	676
	2004	115	120	234.9	690
Narraguagus	1999	139	117	254.1	585
	2000	137	106	239.0	474
	2001	93	81	173.2	450

	2002	159	148	306.6	671
	2003	115	113	228.0	669
	2004	96	109	204.2	690
Pleasant	2001	13	13	26.0	n/a
	2002	19	19	38.0	126
	2003	11	12	23.0	732*
	2004	23	23	46.0	699*
Sheepscot	1999	57	56	113.0	224
	2000	60	42	98.8	225
	2001	56	41	94.7	254
	2002	101	72	168.1	332
	2003	91	80	170.3	346
	2004	75	74	149.0	417

* Reflects large number of individuals held for smolts and as part of domestic pedigree line.

Various general guidelines for threshold effective population sizes for captive populations have been proposed in the conservation literature. For example, an N_e of greater than 50 is proposed to minimize inbreeding depression and reduce the potential for reduced viability or reproductive fitness of individuals within a population (Franklin 1980). Alternately, an N_e of greater than 500 is proposed to maintain constant genetic variance in a population (Franklin 1980, Soule 1980, Lande 1988). In addition, the effective population size can be calculated to be a relationship between the effective numbers of natural spawners in addition to the captive effective population size (Waples 2002). Because the majority of the endangered Atlantic salmon are maintained in the hatchery, the effective number of natural spawners can be assumed to be low to a non-significant amount. The primary component of effective spawners is the hatchery population. Evaluation of the data in Table 3 demonstrates that for most of the populations, the effective population size was below the recommended $N_e = 500$ threshold. Therefore, maximizing the effective population sizes (eg. increasing the number of parr collected and the number of individuals reproducing in a given year), monitoring spawning practices to reduce the potential for inbreeding, and monitoring of estimates of genetic variation over time in the hatchery broodstocks is critical to detect any decreases or losses in the amount of genetic variation over time within the broodstocks.

There are numerous options to increase the effective size of each of the broodstocks at CBNFH. The two most practical options are to 1) implement pedigree lines for each of populations at CBNFH and increase the number of individuals retained following integration of the captive and domestic portions, or 2) increase the number of parr collected from each river. Both concepts result in increase operation loads to the hatchery, potentially reducing the number of capture year classes able to be maintained concurrently, result in surplus egg production, and would require increased staffing and operational costs at the hatchery. Increased collection of parr from the wild may result in incorporation of wild-spawned offspring (resulting from wild or stocked hatchery adults),

but does not ensure equal genetic representation from the hatchery populations (and thereby may result in a loss of genetic diversity and increased inbreeding potential).

3.4.5. Distribution (stocking) adult broodstock

After being held in the hatchery for approximately four years and having fulfilled their genetic and demographic contribution as part of the broodstock, adult broodstock are released to their river of origin. Individuals are released into the lower reaches of each river, typically in December after spawning. Further research is needed into the survival of these individuals, as well as alternate release sites and times (see section 3.6).

3.5. Alternate broodstock management options

To meet the genetic criteria for broodstock management, meet production goals, and operate the hatchery within operational parameters, additional permutations of spawning, culling, and stocking exist. For example, if the number of parr collected for broodstock is increased, but production goals remain constant, a proportion of eggs sufficient for production goals could be retained, and excess eggs (at green or eyed stage) could be used for fry stocking programs, culled, or for other purposes. Alternately, retaining representatives from all families (the use of pedigreed broodstock, see section 3.5.1) can reduce the risk of not collecting representatives from all families during parr collection. However, the goal of the BMP is not to list all possible options, but to provide a framework for evaluation and a basis for adaptive management. New and innovative management options can and should be developed as needed to meet the goals of the broodstock program.

3.5.1. Pedigree broodstock

Currently the restoration efforts of the Atlantic salmon program focus on stocking fry and recapturing parr to incorporate some amount of natural selection into the life cycle of Atlantic salmon. However, when the recovery of stocked individuals is low or distributed unevenly among family groups, the potential for reducing genetic diversity within the broodstock and increasing the potential of inbreeding is increased. The current proposed method minimize with these risks is to implement a “pedigree broodstock”. Pedigree broodstocks are established through the use of an equal number of offspring per family set aside from normal production schedule. This subgroup is divided in half at time of fry stocking, and half of the group is stocked following normal fry stocking procedures, and the other half is retained at the hatchery. Pedigree broodstocks therefore consist of two components: a “captive” group consisting of parr collections from the river following fry stockings, and a “domestic” group, which was retained in the hatchery. Genetic determination of parentage for the captive and domestic groups allow for retention of representatives from all family groups, should there be some families that are not collected in the field.

The domestic, supplemental broodstock would provide a genetic resource from which representatives of families might be drawn to supplement field collected genetic

variation. Where possible, wild capture fry that have been exposed to natural selection in the wild would be used preferentially, but captive fry would be used to supplement the wild collection to equalize familial representation in the broodstock. In addition, progeny of wild spawning adults may be identified and these parr will be incorporated into the spawning population. In combination, this approach would maximize effective population sizes and inclusion of genetic diversity, while at the same time allowing for some natural selection in the wild. Due to the family equalizing nature of this approach, selection on among family variation in the wild may be obscured; however, this loss would likely be offset by avoidance of acute loss of diversity and reduce domestication selection in very small-sized populations. In the long run, the program may benefit from utilizing information on the relative performance of family lines in the wild (or captivity) to further optimize the breeding program.

The use of a pedigree broodstock program was implemented for the Pleasant River in 2001, due to low recoveries of stocked parr in 2000. Most of the fry resulting from spawning in the fall of 2001 were stocked into the Pleasant River, while approximately 600 (representing a portion of all families created during the fall 2001 spawn) were retained at CBNFH. Subsequent genetic analysis of individuals recovered during parr broodstock collections and individuals held at CBNFH allowed evaluation of family representation in each group. Parentage determination using genotypic data for parents and offspring allowed for identification of familial contribution to each group of offspring. All individuals that spent a year in the river were held and family representation and contribution was equalized using captive individuals. Excess individuals (due to hatchery space limitations, and holding limitations) were stocked back into the Pleasant River.

Specific operational aspects of pedigree lines are being refined through the implementation of pedigree lines for the Pleasant River population. For example, refinement of the number of eggs to retain per family is needed to reduce both the number of individuals maintained at the hatchery, and therefore the number of individuals required to be tagged and analyzed for familial representation, while not compromising the survival of each family in the broodstock. Refinement of these and other operational parameters prior to hatchery implementation is required to meet stocking deadlines (primarily smolt-stage stocking of surplus individuals), and to continue to provide adequate space in the hatchery for captive parr and maintenance of multiple capture-year classes of adult broodstock.

Implementation of pedigree lines

Pedigree lines may be established at CBNFH when:

- The number of broodstock for a particular population is low (less than collection target),
- When there is a threat of few or no hatchery or wild spawned parr being recovered, or

- If loss of family variation through general parr collection practices is projected to cause appreciable losses in local population diversity in the near future.

For all populations captively held at CBNFH, recent changes in stocking practices have equalized the distribution of family groups throughout each river system, and parr recovery efforts will more evenly target both stocked and naturally produced parr. These management changes will be monitored for the next two years to determine if the recovery of stocked families has increased. A proposed target for recovery of 75% of the families stocked will be initially used for evaluation of the recapture years following the changes in stocking practices. However, this recovery target may be altered (increased or decreased) on a river-specific basis when considering overall parr recovery success, number of adult spawners, and if stocking of pre-spawn surplus hatchery adults has been implemented. Results will be annually reviewed and evaluated by the Broodstock Management Committee (ad-hoc TAC committee), and any recommendations to management would be discussed. Also, if for any reason parr recaptures for any system decrease significantly, the implementation of pedigree lines will be discussed by the committee.

3.5.2. Artificial induction of spawning

Current broodstock spawning follows the natural annual maturation schedule of adult Atlantic salmon to minimize artificial manipulation or selection. Alternative management strategies could be incorporated to increase the percentage of ripe individuals available for spawning. Strategies could include manipulation of water temperature and lighting regimes, the use of hormones to induce uniform ripening within a broodstock, or the use of cryopreservation to create “sperm banks” to increase the number of males available to fertilize eggs throughout the spawning season. Induction of spawning to condense or alter the timing of the spawning season is not currently being considered.

3.5.3. Adult broodstock collection-DPS

Collection of sea-run adults could be another source of broodstock for some of the DPS populations. The ASC has operated adult Atlantic salmon trapping facilities on the Narraguagus, Pleasant, and Dennys rivers, however presently an adult trap is only present on the Narraguagus River. Collections of offspring from returning adults are the primary pathway to incorporate genetic material from sea-run adults. If recovery and restoration plans determine that collection of sea-run adult salmon to the DPS broodstock is necessary, adults could be collected from the trap on the Narraguagus River. A quarantine period for fish health and genetic screening would be required; afterwards individuals could be integrated into broodstock operations for each specific river.

Data collection at the adult trapping facility on the Narraguagus River is limited to the collection of biological data and tissue samples from adults prior to upstream migration. Collection of adults from the Narraguagus River for broodstock purposes would require

significant changes in project staffing, equipment, and procedures from current practices. Changes necessary to facilitate adult broodstock collections include:

- increase staffing and equipment to collect adult salmon for broodstock from existing traps on the Narraguagus River;
- modify equipment to enable movement of adult Atlantic salmon from weir to transport vehicles;
- repair or replace the Pleasant and Dennys river weirs due to poor existing condition of structures; and
- construct new weirs on DPS rivers currently without weir structures.

Future integration of wild adults into each river-specific captive broodstock should be considered as a viable management option. However, recent returns to the DPS rivers with broodstock held at CBNFH are very low so the benefits from trapping and incorporation of sea-run adults into the broodstock program should be considered in relation to the benefits of natural reproduction and the cost and time requirements necessary to make the efforts feasible (Table 5).

Table 5. Adult returns to the DPS rivers. Returns are provided only for the rivers that have counting structures. Data from USASAC reports.

River	Year					
	1999	2000	2001	2002	2003	2004
Dennys	3	3	21	2	10	1
Narraguagus	32	23	32	8	21	11
Pleasant	0	3	11	0	2	1

3.5.4. Smolt Broodstock Collection-DPS

Smolts may be collected as broodstock through the current established smolt trapping and sampling programs for the DPS populations maintained at CBNFH. For example, smolt collections were used in part to found the current Pleasant River broodstock. Smolts have undergone further natural selection than parr, and so may theoretically offer benefits to the population from this perspective.

Smolts offer a viable option for broodstock collection. However, smolts present some additional challenges: 1) a smolt trapping program must be in place to capture the smolts, 2) smolt trapping is staff intensive, 3) smolts are at a delicate life history stage and present greater handling challenges than parr, 4) successful smolt trapping is not guaranteed due to weather, flows, equipment failure and smolt abundance, 5) smolt collections may require additional biosecurity and rearing resources at the hatchery.

Smolts should be considered as a backup strategy to parr broodstock collections at this point. However, the use of smolts as a source for broodstock should not be ruled out as a potential standard method of broodstock collection because they have survived to an additional critical life history stage compared to parr, and they have imprinted on their

natal waters. Therefore, if released as spawning adults, their reproductive success may be greater compared to other captive adults.

3.6. Management of surplus broodstock

Management of DPS salmon populations reared at CBNFH and integration into management strategies for salmon in the wild is complex. This relationship is perhaps the key component to successful use of CBNFH as a restoration tool for the endangered populations of salmon in Maine. The complexity arises from the challenges of genetic and demographic thresholds that CBNFH must meet, coupled with evolving broodstock management approaches and management of fish in the wild.

3.6.1. Surplus stocking of adult Atlantic salmon

Annually, there are sexually mature adult salmon that are surplus to the genetic and demographic egg production needs at CBNFH. Surplus adults are defined as individuals that have previously spawned, and due to hatchery space constraints and previous genetic contributions of these individuals, they are considered surplus to hatchery spawning needs. As part of the restoration strategy for the DPS Atlantic salmon populations, use of these adults is of interest to increase the amount of natural selection exposed to the populations during their life cycle, increase the reproductive potential of these individuals, and restore a historically occurring component to the natural ecosystem. To be able to use these fish for targeted restoration actions as spawners in the wild, they must be identified at the hatchery in a timely manner that is also relevant biologically (evidence of sexual maturation is observed just prior to spawning). These fish are identified as sexually mature surplus typically by mid-October, and only at that point are numbers available to managers to determine stocking potential through evaluation of previous genetic contribution, estimates of the number of first-time spawners, and comparison of spawning potential to egg and fry production requests. To appropriately manage these surplus adults, agencies must develop stocking plans for each population so that when surplus adults do arise, a plan is already in place to utilize them. The decision-making pathway for adult salmon is complex. The hierarchical decision-making process to decide which fish to spawn is given in Table 3. Stocking or other distribution of surplus adult Atlantic salmon is outlined in Table 6. See also section 4.7 for a description of adult stocking.

Table 6. Decision-making hierarchy for surplus adult salmon

Step	Decision
1	First time or repeat spawners, required for spawning will be retained in the hatchery for the duration of the spawning season.
2	First-time spawners will be retained as repeat spawners for 1-2 years.
3	Repeat spawners should be held as backup to the first-time and pedigree spawners beyond the 1-2 year time period only if there is a genetic or demographic need.
4	Repeat spawners that have fulfilled their role in the program should be

	stocked as sexually mature adults into or near viable spawning habitat prior to spawning according to an adult stocking plan.
5	Surplus repeat spawners should be stocked in the estuary or extreme lower river in December, according to TAC recommendations.
6	Any other surplus adult fish that was not stocked with the intention of natural spawning should be stocked in the estuary or extreme lower river in December, according to TAC recommendations.

3.6.2. Surplus stocking of juvenile Atlantic salmon

Juvenile life history stages of salmon (egg-smolt) may become surplus to the program for various reasons. However, these surpluses should be used in a beneficial manner for restoration. Each life history stage has target times of year for stocking that are related to their development and natural environmental conditions that are conducive to their survival and integration into the natural population. Table 8 provides life history stages and desirable times of year when they should be stocked, when specific management actions must be taken, when management decisions must be made, and when certain analyses need to be conducted by in order to identify surplus and make stocking decisions.

For example, the use of pedigree broodstock lines while important to management, create large temporary surpluses of fish in the hatchery. To appropriately utilize the pedigree lines and stock excess fish during biologically relevant life stages, genetic analysis to obtain genotypes and determine parentage and familial representation in both the domestic component (those maintained at the hatchery) and captive components (from the same parental source as the captive group, only stocked as fry to expose to natural environments) must be completed and excess individuals identified prior to smoltification in May. To accomplish this task, the domestic component is PIT tagged and fin clipped in June or July, as age 1+ parr. Genotyping of these individuals begins immediately. Captive parr are captured from the river in September or October; fin clips are taken immediately and sent to the FWS Region 5 Conservation Genetics Lab for genotyping and parentage analysis. Results of familial contribution, and determination of individuals to retain and those to stock are provided to the hatchery in March or April so stocking can occur prior to the smolt stage with the goal that stocked juveniles will undergo the smoltification process and emigrate to the marine environment (see section 3.5.1.).

3.7. Hatchery management implementation timeline

Timing of management activities critical to hatchery operations at CBNFH is complex but critical to adhere to biologically important life stages of Atlantic salmon. Table 7 provides temporal guidelines for key annual information dissemination and decision-making. The goal of Table 7 is to provide a framework that allows the entire hatchery system to operate with as few unexpected events as possible and to be as integrated into the management of the natural populations as possible.

Table 7. Management cycle and operational framework for CBNFH.

Step	Date	Life Stage	Action	Description
1	August	Post-smolt	Transfer	Captive parr collected the previous year are transferred into the CBNFH Broodstock Building following a year of quarantine for fish health evaluation in the Receiving Building; individuals are PIT tagged, and tissue samples are taken for genetic analysis (and sent to the FWS Region 5 Conservation Genetics Lab).
2	August	Parr: Domestic portion of pedigree line	Assessment	Domestic parr that are part of pedigree lines are PIT tagged and genetic samples are taken and sent to the FWS Region 5 Conservation Genetics Lab for genotyping and parentage analysis.
3	August-October	Parr: Captive portion of pedigree line	Receive	Parr are captured from rivers according to ASC established collection targets, and brought into the CBNFH receiving building.
4	September	Eggs	Request: Egg production	ASC staff makes egg requests to TAC for management and research purposes.
5	October	Parr: Captive portion of pedigree line	Assessment: Genetic parentage analysis for captive component of pedigree line	Fin clips are taken for genotyping and parentage analysis at the FWS Region 5 Conservation Genetics Lab from river origin parr collected that same year (tissue samples taken a year ahead of normal schedule so results can be provided from parentage analysis and stocking of surplus pre-smolts can occur prior to smolt). See Appendix 3.
6	October - Mid	Adult	Assessment: Sexual maturation of adults	CBNFH staff sort adult broodstock to determine maturation level of each individual adult.
7	October - Mid	Adult	Assessment: Spawning history of mature adults and estimates of production	CBNFH uses results from determination of mature spawners and sorts individuals based on first-time, repeat, and surplus spawners, and immature adults. Demographic, genetic, and spawning history is incorporated and hierarchy for spawning criteria (Table 3) is followed.
8	October -	Adult: Surplus	Stocking:	Surplus repeat spawners are stocked

	Mid	sexually mature	Surplus sexually mature adults pre-spawn	into previously specified waters as early as possible in order to allow them to reproduce naturally, following previously identified stocking and evaluation plans.
9	October-Mid	Adult: Broodstock	Assessment: Pre-spawning genetic review	Review of individuals to be spawned, following drainage-specific goals. Ensure culled individuals have been removed from broodstocks, review genetic characteristics of spawning broodstocks within populations and capture-year classes, determination of spawning optimization software implementation (see Section 3.4.3.).
10	October - November	Adult: Broodstock	Spawning	Recording of spawning pairs, adherence to spawning protocols, fish health procedures, and any additional guidance.
11	November	Eggs	Incubation	For the domestic portion of a pedigree line a small aliquot of eggs from each family are separated. Eggs surplus to stocking or pedigree needs are moved into 1) surplus fry production, 2) non-fry stock uses such as egg planting or smolt production, 3) research, 4) stocking in non-natal drainages (if approved by appropriate agencies), 5) destroyed.
12	December	Adult	Stocking: Surplus adults post-spawn	Surplus adults still on station will be released in December in the lower reaches of their natal river, according to TAC recommendations. Spawned out first-time spawners are held on station following the protocol in Table 3.
13	January-February	Eyed eggs	Assessment: Shocking	Eggs are shocked, total mortalities recorded, and the number of fry projected is reported to agencies.
14	January-February	Eyed eggs	Transfer	Eggs may be diverted to GLNFH for smolt rearing (e.g. Penobscot and Dennys rivers smolt programs). Some may be retained at CBNFH for 0+ parr or 1+ smolt rearing. In all cases, these groups should be genetically representative of the entire population unless management or research

				considerations warrant otherwise.
15	March-April	Parr: Pedigree Broodstock	Assessment: Genetic characterization of captive parr for pedigree line	Captive and domestic juveniles have been genetically analyzed and familial representation has been determined. Individuals surplus to the pedigree line (fish that are already genetically represented by sufficient siblings) are identified and results provided to CBNFH to sort and stock surplus as smolts in April-May.
16	March-April	Juveniles	Supplementation: Alternative founding methods for pedigree line	In some cases, parr or smolts may be retained to form or augment a pedigree line. In these cases, the fish retained shall be genetically representative of the entire population.
17	April- May	Smolt	Stocking: Surplus domestic pedigree line	Stocking of portion of domestic pedigree line that were identified as surplus due to equalization of familial representation between captive and domestic parr. Surpluses are stocked into river of origin prior to smolting.
18	April-June	Fry and smolts	Stocking: Fry and smolts	Fry and smolts (if held) are stocked into rivers through cooperative efforts between ASC and USFWS according to stocking protocols outlined in this plan.
19	June	All	Assessment: Status on station	All fry (that are not part of a domestic pedigree line) have been stocked. All surplus adults have been released. The remaining adults include those who have not yet spawned, and those who are being retained for future spawning. Parr rearing to fall parr (part of pedigree lines) or other life stages are still on station.
20	July	Parr	Genetic characterization: Parr for broodstock	Results from genetic characterization of parr (not including pedigreed populations) are provided to CBNFH and the Broodstock Management Committee. Results include list of individuals to be culled (see Section 3.3.3).
21	Year-round	Parr through Adult	Rearing	The parr (for broodsock) are reared until first-time spawners and the cycle starts again.

4. Production targets

Production targets for CBNFH and the life stage of Atlantic salmon produced by CBNFH are determined by ASC and TAC through evaluation of available river habitat. Production targets for CBNFH are established by the TAC (Table 8). ASC makes river-specific requests to TAC at the annual fall meeting, and those requests are passed on to CBNFH. Atlantic salmon fry are most commonly stocked in Maine, however other life stages stocked into Maine DPS rivers are smolts, 0+ parr (fall parr) and 1+ parr, and eggs (Table 9). In addition, adult salmon are stocked into rivers and estuaries following use in the broodstock at CBNFH (see 3.4.5. for proposed alternate methods). However, due to stocking requests CBNFH primarily produces fry, with some limited smolt production for the Pleasant River. If adult returns increase, natural reproduction could be incorporated into calculations which would reduce the number of hatchery-produced salmon requested. Presently, ASC stocking plans are being revisited.

Table 8. Production targets for CBNFH as requested by TAC from 2002 to 2004 for the current DPS rivers and the Penobscot for restoration purposes only. Numbers include total requests for fry, parr, and smolts combined. Production requests for other stocking purposes, such as school programs, research, or non-DPS stocking are not included.

River	2002	2003	2004
Dennys	232,100	358,800	358,800
Machias	424,650	424,650	546,700
East Machias	208,197	208,198	208,197
Pleasant	-	127,800	142,800
Narraguagus	300,000	315,000	370,000
Penobscot	1,515,000	2,676,000	2,686,000
Sheepscot	194,380	194,380	206,380

Multiple life history stages are currently or have been previously produced for stocking at CBNFH. Each life history stage has an optimal time to be stocked due to certain growth, diet, physiological, or other need (Table 9). Production and hatchery operations are then guided accordingly to best meet these optimal stocking times for the life stage being produced.

Table 9. Key life history stages and optimal stocking times

Life history stage	Optimal stocking time
Eggs-green	November (immediately following spawning)
Eggs-eyed	January-February (following eye up)
Fry	April-May
0+ Parr (fall parr)	September
1+ Parr (Fall parr)	September
Smolt	April-May (just prior to smolting)
Post-Smolt	Rear to adult and stock as sexually mature adult



4.1. Production capacity at CBNFH

The current operation and setup of CBNFH allows for production of up to 500,000 fry per DPS fry room, and 2,000,000 for the Penobscot production room. Current fry production levels for some of the rivers (with the exception of production for the Machias River) are lower than maximum hatchery capacity (Table 8). However, full capacity production is not required in some of the rivers where habitat-associated fry requests from ASC and TAC are more limited (Table 8).

4.2. Production capacity at GLNFH

Similar to production at CBNFH, production of Atlantic salmon at GLNFH is determined by requests made by ASC or other parties through TAC. GLNFH produces smolts for stocking in the Penobscot from sea-run adults, eyed eggs (transferred to another hatchery for stocking as fry) from domestic Penobscot adult broodstock, and parr and smolts for the Dennys river from spawning of the Dennys broodstock at CBNFH. Production capacity is currently at maximum at GLNFH based on available rearing space for the smolt program. Egg production for the domestic Penobscot strain could increase, but currently is limited due to cost and staffing constraints.

4.3. Fry stocking

The number of fry to stock in a particular area is determined by ASC based on the amount of unused rearing habitat in a stream or stream section. Habitat data (in GIS form) is used to describe the amount of habitat available in a stream under low water conditions (summer flows). Available riverine habitat is expressed in 100 m² units (units). Stocking targets are calculated based on Atlantic salmon densities ranging between 50 and 100 fry per unit, determined to be the optimal density for Atlantic salmon fry (Elson 1957, Elson 1975). However, target densities can be evaluated and adjusted accordingly based on past electrofishing data of juvenile abundance and distribution, and environmental data such as water temperature and pH. To determine the number of fish to stock in a river, the number of habitat units are multiplied by the target density of fish desired:

$$\text{number of fry} = (\text{fry/unit}) * \text{number of units}$$

The number of fry produced can be adjusted by varying the number of adults spawned to meet production requests (see Table 8 for production requests). In some cases, fewer adults than possible are spawned to intentionally reduce production. If fry production is below requested targets, fry distribution is adjusted accordingly primarily through reducing the density of fry stocked per unit.

The timing of fry stocking into rivers is primarily based on fry development and river flows. Spawning occurs over the course of a month, and therefore fry development can represent a variety of developmental stages at time of stocking. To standardize

stocking size and developmental stage, fry are stocked into rivers according to spawning date: individuals with earlier spawning dates are more developed and ready to be stocked before those with a later spawning date. Other stocking strategies have been and are currently used in the Narraguagus River and the Sheepscot River (2005 fry stocking) where specific families are grouped together to form separate genetic batches, and are stocked separately into specific sections of the river. This process allows the use of genetic determination of parentage to be used to track and correlate hatchery releases and survival with specific stocking locations. However, to more evenly distribute families throughout river systems (allowing natural selection to act on the greatest number of families and increase recapture of diversity), families are currently batched by spawn date, mixed, and stocked into various sections of the rivers. Further research on optimal stocking size, time, and developmental stage is needed and currently underway. This work could increase fry productivity or reduce mortality.

4.4. Smolt stocking at CBNFH

Smolt production is based on the number of adults returning to a river, and is established by ASC through TAC. To determine the number of smolts stocked, the desired number of adults is divided by the ocean survival rate, providing the number of smolts required to return the optimal (based on available habitat) number of adults

$$\text{number of smolts} = \text{number adult returns} / \text{ocean survival rate}$$



Multiple methods are used to stock smolts. Similar to fry protocols, batching of specific families is used for some sections of the Penobscot River. Batching allows for identification of specific stocking locations and to correlate hatchery releases and survival with specific stocking locations.

Smolt production currently is limited to the Pleasant, Dennys, and Penobscot rivers. At CBNFH, smolts are raised on an experimental basis for the Pleasant River. Fertilized eggs from the Dennys and Penobscot populations are transferred from CBNFH to Green Lake NFH (GLNFH) to be raised to smolt stage and then stocked.

4.5. Production at GLNFH

GLNFH produces approximately 600,000 smolts and 300,000 parr (stocked in the fall) for stocking into the Penobscot River. From spawning of the Dennys River captive broodstock at CBNFH, approximately 56,000 smolts are produced for stocking into the Dennys River. In addition, 35,000-40,000 parr are produced for the Dennys River. Parr stocking in correlation with smolt stocking is the result of reducing the number of fish per tank at the hatchery to accommodate the increased size of the fish due to growth. Stocking of parr typically occurs in the fall, prior to smolt stocking in the subsequent spring. Smolts produced at GLNFH are considered age 1+, resulting from spawning a year and a half prior. Approximately 750,000 eyed eggs from the domestic Penobscot broodstock produced at GLNFH are transferred to the Saco River hatchery for fry stocking in the Saco River.

4.6. Egg and 0+ parr stocking

Eggs are used for experimental stocking purposes. The number of eggs stocked (i.e. planted in the stream substrate or in a streamside incubating device), is determined using the same method as for fry, with the incorporation of the survival rate from embryo to the emergent fry stage. Survival rates are affected by the type of incubation method used, and can vary among incubation sites. Therefore, the number of eggs to be stocked is divided by the expected survival rate to emergence.

$$\text{number of eggs} = (\text{fry/unit} * \text{number of units}) / \text{survival to emergence}$$

Age 0+ parr (fall parr) may also be stocked where there is a need to reduce biomass in the hatchery. Therefore, hatchery operational requirements determine how many fall parr must be stocked, rather than the demographic needs of the population. Optimal fall parr stocking densities have not been calculated, however, fall parr stocking numbers could be estimated using methods similar to estimation of fry densities. A range of target densities would be required, and these densities have not been established in the Maine stocking program.

4.7. Adult stocking

Current hatchery stocking programs focus on stocking juvenile (fry, parr, and smolt) life stages into river or estuarine environments. Stocking reproductively viable adults has not been extensively used as a management strategy due to a variety of concerns. Spawning activity by hatchery adults could result in redd superposition of the wild adult redds, over-representation of hatchery broodstock genes in the population, and competition of resulting offspring with those from wild reproduction. Due to the low number of wild adults returning to spawn, changes in hatchery practices to standardize individual reproductive contribution, and assessment of juvenile densities and rearing habitat, concerns about hatchery and wild competition have been reduced. Although stocking hatchery adult salmon may result in some competition, the potential to increase natural reproduction, the exposure of different life stages to natural selection, and to return a historically important component to the ecosystem can contribute to restoration and recovery efforts. Therefore, the following guidelines were developed for implementation and use of sexually mature adult hatchery Atlantic salmon.

- Adults should be reared to size and sexual maturity that is consistent with natural size and timing of spawning.
- Adults should be stocked in areas where reproductive habitat exists
 - Areas to stock adult fish should be identified *a priori* in an adult stocking plan. These areas should be approved for adult stocking before any adult stocking is intended. When adults become available, these areas shall be used.

- Experimental areas should be established to allow for more rigorous evaluation, and for stockings that are LOW RISK to DPS populations should biological conflicts arise.
- Adults should be stocked at reasonable times of year (we do not know when it is best to stock, but whatever time is chosen should be done so with assessment in mind). October is the most likely time, but other times of year may be explored.
- Adults should be stocked in areas where they will have minimal impact on wild spawners (redd superimposition or high levels of competition on the spawning grounds).
- Adults should be stocked in areas that will not confound ongoing research.
- When possible, adult movement out of the stocking area should be limited or monitored.
 - Monitoring of adult movement can be done through the use of weirs, natural barriers (beaver dam or water fall, etc.), PIT tag array, telemetry, and/or systematic visual observation
- A monitoring and or assessment plan should be developed.
 - Monitoring can include redd counts, spawning observations, sampling redds, emergent fry trapping, juvenile surveys for young-of-year and/or parr through electrofishing or snorkeling, and genetic parentage analyses
- Adaptation of management activities
 - Stocked adult spawners may require modification of planned management activities such as stocking other life histories in the same area.
- Stocking adult fish should be operated under an adaptive management paradigm. Future stocking efforts should build upon the success and shortcomings of past stockings.

4.8. Selection of individuals for alternative supplementation or culling

Given potentially large differences in survival and reproductive success for individuals allocated to alternative supplementation, or even culling, it is important that selection does not inadvertently introduce variation in family performance or undesired selection. Selection of different families for alternative fates could contribute to substantial variation in relative family performance with concomitant effects on effective population size and genetic variation. It is also important that individuals or families selected for alternative uses not be selected with respect to ecologically important and heritable traits. For example, “grading” of fry, parr, or smolts based on size may result in significant and undesirable selection on growth and correlated traits if the resulting lots have different reproductive success (survival or offspring production). To avoid these undesired effects of alternative supplementation approaches or culling, it is recommended that lots be sampled proportionately from all families or batches and that grading be avoided when possible in favor of less biased subsampling.

4.9. Summary of distribution of Atlantic salmon at CBNFH

Broodstock management and hatchery production of Atlantic salmon at CBNFH is a complex process. Table 10 presents the primary life history stages, rearing pathways, and dispositions that result from broodstock management.

Table 10. Summary of life stages held and distribution of salmon at CBNFH.

Life History Stage	Primary Disposition	Secondary Disposition	Tertiary Disposition
Egg	Reared to fry.	Research, egg planting, etc. if specific APPROVED study plan in place.	Destroyed if surplus and no other uses.
Fry	Stocked into rivers according to ASC management needs.	Research, fry stocking, etc. if specific APPROVED study plan in place.	Destroyed if surplus and no other uses.
Pedigree line-domestic fry	Reared in hatchery until pre-smolt.	Reared to first-time spawner.	Stocked as smolt if genetically redundant.
0+ Parr	Stocked into rivers according to ASC management needs.	Research, parr stocking, etc. if specific APPROVED study plan in place.	Destroyed if surplus and no other uses.
Captive 1+ and 2+ parr	Reared in hatchery until first time spawn.		
Smolt	Stocked into rivers according to ASC management needs.	Research, smolt stocking, etc. if specific APPROVED study plan in place.	Destroyed if surplus and no other uses.
Pedigree line-domestic smolt	Stocked as pre-smolt if genetically redundant.	Held in hatchery until first spawn.	
First-Time Spawner	Spawned in hatchery.	Held for additional years to spawn again if needed.	
Repeat Spawner	Spawned if needed for demographics or genetics.	Stocked prior to spawning as sexually mature spawner into viable spawning habitat.	Stocked in estuary as stripped fish in December.

5. Data collection and management

The collection and management of data is an integral component to broodstock management, as it is used to track biological, hatchery-related, and environmental information that relate to the captive broodstock program. Evaluation of the data collected can be used to assess and improve the management of captive populations. The overall goals of the data system are to:

1. allow evaluation of the spawning program,
2. monitor production, offspring quality (e.g. egg size, fry size), and survival rates at critical life history stages,

3. allow evaluation of the progress toward captive broodstock management goals, and
4. allow evaluation of post-stocking performance of the stocked fish.

Due to the complexity of the captive broodstock program, there are numerous independent databases and data collection activities. These range from hatchery practices and production, to assessments of survival and population dynamics in the field, to stocking and quantitative population genetics issues.

5.1. Database System Structure

Broodstock program data are managed through the Atlantic Salmon Information System (AS-IS), an array of linked inter-agency databases. The system is based around MaineSalmon.mdb, a central reference library database that houses standardized codes for most aspects of salmon biology, and a hierarchical geographic naming system. Many specialized databases connect to MaineSalmon.mdb, allowing operation and collection of standard codes. Table 11 represents an overview of the AS-IS databases relevant to broodstock management. Additional databases not presented here address other components of Atlantic salmon biology and management. New databases are added, or existing databases are revised, as needed.

Table 11. Databases in the Atlantic Salmon Information System

Database	Main Focus	Description	Lead Agency	Time Period
MaineSalmon.mdb	central storage for standard codes	Contains standard codes and hierarchal naming structures used by ASC, FWS and NOAA.	ASC	
MaineBroodstock.mdb	CBNFH captive broodstock management, egg and fry production	Broodstock inventory, traits, and demographics, spawning data; egg, fry, parr and smolt production at CBNFH	USFWS	1999 -present
MaineStocking.mdb (MaineStocking-v1.mdb)	Releases of Atlantic salmon	Numbers, ages, population, family groups, stocking locations, dates	USFWS	2004 -present (1957-2003)
Flow2.0.mdb	USGS stream gauge data	Updatable data from USGS for stream gauges on streams within the DPS	USFWS	1992 -present
Climate2.0.mdb	Climate data	Updatable data from [NEED SOURCE] for Maine	USFWS	1992 -present
CBNFHTemp.mdb	CBNFH rearing conditions	Water temperatures at CBNFH, collected at discrete locations or streaming from the water treatment plant into broodstock and incubation modules. Particular attention to rearing schedule.	USFWS	1992 -present
Efish.mdb	Juvenile population assessment	Population estimates, broodstock collection numbers, length and weights, biological samples, etc.	ASC	
SmoltsArchive.mdb	Smolt assessment	VIE, clip, punch, physiology, scale samples observed and applied. Includes all smolts sampled, observed and applied marks, tissue, gill samples taken, and injuries observed.	NOAA	

AdultTrap.mdb	Adult returns	Origin, capture dates, biological data, and disposition of adult returns	ASC	
WaterTemperature.mdb	Water temperatures	Storage and analysis of data collected by various stream monitoring devices	ASC	
TBA	Water quality	Water quality data (under construction).	NOAA, ASC	
Habitat	GIS data	Spatial habitat data in GIS	ASC	
Genetics	Genetic data for Maine Atlantic salmon stocks	Genetic characterization data for CBNFH broodstock as well as other representative samples from stocks within the DPS	USFWS	1993-present
CBNFH DI Tracking	Fry developmental indices	Used to track developmental indices of fry	USFWS	Annual

5.2. CBNFH broodstock database (*MaineBroodstock.mdb*)

MaineBroodstock.mdb is the central database for the management of the captive broodstock populations at CBNFH (Figure 1). The database addresses two major components of hatchery management: broodstock management and juvenile salmon production. Broodstock management data encompasses incoming wild broodstock and associated health screening, biological sampling, tagging and genetic characterization. Broodstock are tracked, via individual PIT tag numbers, within the database to maintain an accurate inventory for each population and collection (year) class. The matings are tracked on an individual level and as paired matings; therefore, the parents of each offspring are known. The database is also used to track individual families of eggs (product of a single paired mating) and genetic batches (groups of families) through their rearing until time of release. In the course of tracking families, new programmatic changes such as diversion of eggs for river-specific smolt production, research projects, and development of pedigreed broodstock can also be managed through the database. The transition between the Broodstock Database and the Stocking Database occurs when fish transition from rearing in the hatchery to preparing for release.

5.3. Broodstock data flow

The flow of data related to broodstock management is shown in Figure 2. Juvenile parr are collected annually for the broodstock program. The inventory is refined as genetic and health information is analyzed, fish are culled due to health issues or genetic origin (e.g. non-DPS ancestry). During spawning, mating data are recorded and resulting families are tracked through family groupings and subsequent release locations. Data regarding release (stocking) are managed in MaineStocking.mdb. Information related to survival and condition of the stocked fish, as well as their wild counterparts, is collected and stored in the appropriate databases (AdultTrap, Efish, SmoltsArchive, etc.).

Figure 1. Generalized structure of MaineBroodstock.mdb broodstock database system outlining two major components, broodstock rearing and production.

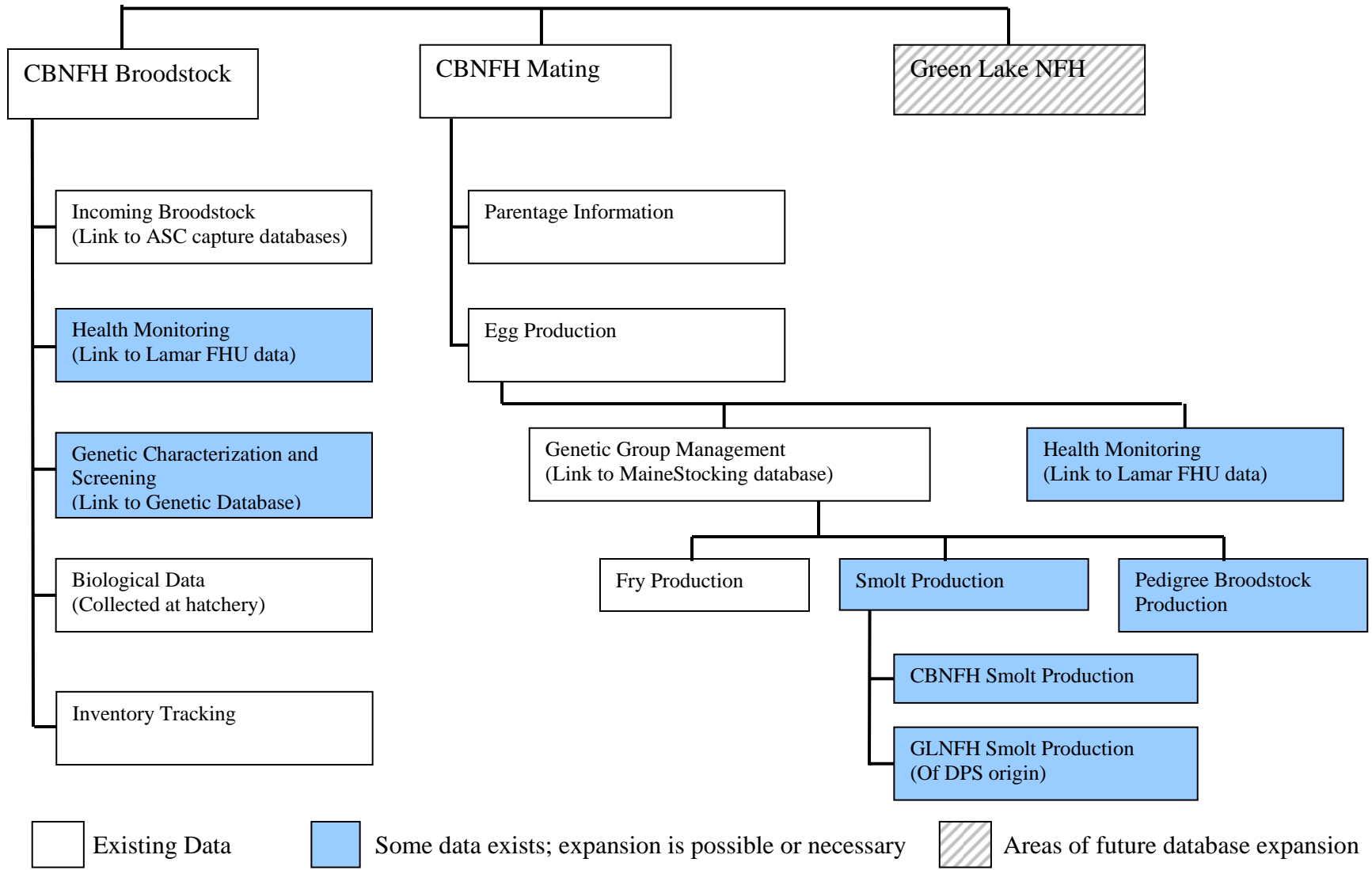
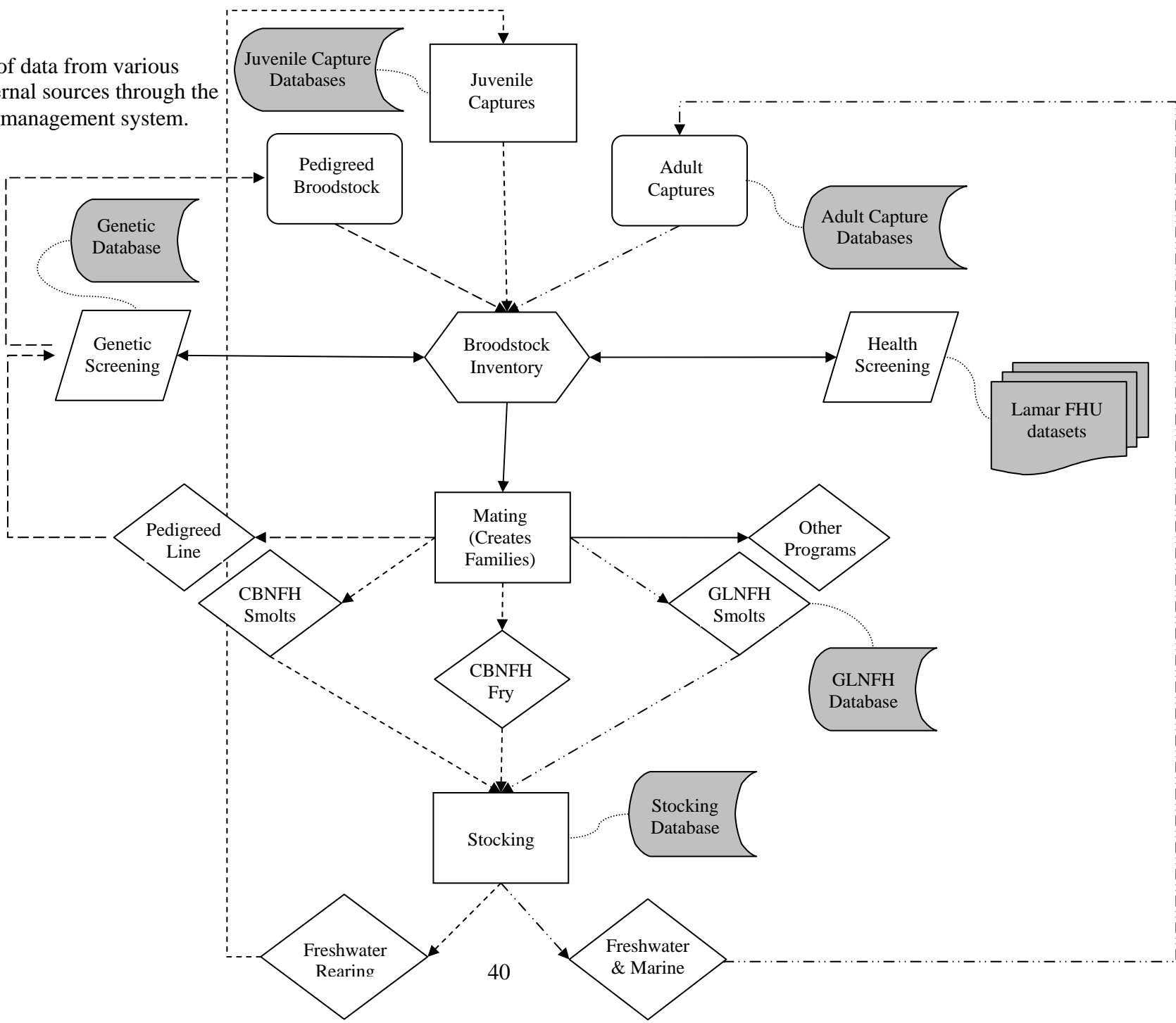


Figure 2. Flow of data from various external and internal sources through the broodstock data management system.



5.4. Conceptual areas of importance to broodstock management: data considerations

The data management process addresses a complex system of interrelated hatchery practices, health and genetic issues, and field activities, all of which generate a substantial amount of data. The data management system has been designed to allow biologists and hatchery managers to address hatchery operations, management, and research issues to more effectively manage and evaluate Atlantic salmon in Maine. Managing captive broodstock populations requires balancing the operational limitations of the hatchery, optimal genetic management of the populations, and the demographic needs of the populations. Table 12 lists conceptual areas of importance to the captive broodstock program with a list of the types of data that may be required for assessment, as well as the databases where those data exist. Also noted in Table 6 are databases and data collection components that do not currently exist. In addition, a list of documents that have been used to manage broodstock and as operational plans at CBNFH is presented in Appendix 1.

Table 12. Conceptual areas of importance to the captive broodstock program with a list of the types of data that may be required for assessment, as well as the databases where those data exist. Also noted are areas where databases and data collection do not currently exist.

<i>Area of Interest</i>	<i>Database / Data Source</i>	<i>Possible Data Set Expansion</i>
<i>1. Evaluation of Spawning Program</i>		
Spawning methodology	In operational plans	
Mating schemes	MaineBroodstock.mdb	
Cross-population matings		Not currently investigated
Spawning dates, seasonal influences	MaineBroodstock.mdb	
Spawning histories of individuals	MaineBroodstock.mdb	
Production of discrete families	MaineBroodstock.mdb	
Cohort(s) spawned	MaineBroodstock.mdb	
Captive generation (F1, F2 etc.)	MaineBroodstock.mdb	
<i>2. Production: Fecundity, Milt Quality, and Survival Rates</i>		
Fecundity	MaineBroodstock.mdb	Expand to parameters beyond current volumetric measurements
Milt Quality		Not currently investigated
Sexual maturity rates	MaineBroodstock.mdb	

Survival to developmental milestones	MaineBroodstock.mdb	Relate survival rates to condition of offspring at time of release
Tracking families / population	MaineBroodstock.mdb	
Tracking genetic groups	MaineBroodstock.mdb MaineStocking.mdb	
<hr/>		
<i>3. Demographic Management of Captive Broodstock</i>		
Demographics of broodstock	MaineBroodstock.mdb Capture databases	
Broodstock sources	MaineBroodstock.mdb Capture databases	
Genetic characterizations	Genetics Database	Link broodstock to capture sites
Phenotypic traits	Maintained by Univ. of Maine Orono	Incorporate into MaineBroodstock.mdb
<hr/>		
<i>4. Post-Release Performance</i>		
Rearing conditions at hatchery	Environmental Suite	
Developmental index at stocking	MaineStocking.mdb	relate to parr and smolt survival in capture databases
Lifestage stocked	MaineStocking.mdb	
Density of Stocking		Derived from stocking data and habitat data.
Stocking method	MaineStocking.mdb	
Stocking location	MaineStocking.mdb	
Environmental conditions at time and location of release	MaineStocking.mdb, Environmental Suite	relate to parr and smolt survival in capture databases
In-river rearing conditions	Environmental Suite	
Juvenile survival	Electrofishing, Smolts	
In-stream condition of juveniles	Electrofishing, Smolts	
Origin of juveniles	Electrofishing, Smolts, Genetic Database	Link to MaineBroodstock.mdb through parentage analysis

Origin of returning adults	Adult Trap, Genetic Database	Link to MaineBroodstock.mdb through parentage analysis
Numbers of returning adults	Adult Trap	
Demographics of adults	Adult Trap	

6. Fish health and biosecurity protocols

Fish health monitoring and management are critical to the longevity of captive broodstock maintenance at CBNFH. Two primary documents have been implemented to guide fish husbandry procedures and integration of wild individuals into the captive broodstock: 1) the Craig Brook NFH Biosecurity Plan (2001b), and 2) a Disease Sampling Protocol for Wild Parr from Maine's Downeast rivers held for broodstock (anon.). Fish health and disease sampling is monitored by CBNFH staff and the USFWS Region 5 Fish Health Lab, in Lamar, Pennsylvania.

Necropsies are performed on all mortalities for both DPS and Penobscot broodfish. Necropsies are performed by the hatchery staff, following training and protocols established by FWS Fish Health Biologists to collect the appropriate samples to conduct necropsies. Tissue samples and related information required to determine cause of mortality are sent to the USFWS Region 5 Fish Health laboratory for analysis.

In accordance with the biosecurity procedures guidance outlined in the Craig Brook NFH Biosecurity Plan (2001), the broodstock for each river are kept isolated, and no transfer of broodstock or equipment between river-specific bays is allowed for any purpose.

7. Program goals and assessment

Management of the captive broodstocks held at CBNFH has been focused on the primary goal of the program, which is aiding in the recovery and restoration efforts of Atlantic salmon in Maine. As such, many of the management practices implemented at the hatchery have been developed to achieve those goals. By stepping down this primary goal into two parts to identify specific objectives and specific management actions, this plan can provide a template for assessment and review of the broodstock program. This document serves as a "living" document; as such, modifications can be made to current protocols as new science and techniques are developed, and as a result of creative, innovative strategies for restoration and recovery. Therefore, this document provides an adaptive framework to meet the needs of the Maine Atlantic salmon hatchery-based recovery program partnership.

Overall program goal: Aid in the recovery and restoration efforts of Atlantic salmon in Maine

1. Goal: Maintain the genetic characteristics and variability for the populations maintained at CBNFH in a manner that preserves and favors the potential for adaptation to wild habitats.

A. Objective: Conduct spawning operations in ways that promote retention and incorporation of potentially critical genetic variation in future generations and that minimize the risks of artificial or domestication selection. Maintain average pairwise relatedness below 0.02 within each river-specific capture year.

Action: Utilize broodstock database to track spawning history for all salmon held for broodstock purposes.

Action: Use two-phased criteria to determine if implementation of spawning optimization program is needed to reduce potential for inbreeding (section 3.4.3).

Action: Use two-phased criteria to determine if spawning between capture years within populations is needed to minimize inbreeding (section 3.4.3).

Action: Monitor levels of heterozygosity and estimates of inbreeding annually.

Action: Continually monitor critical trait variation and avoid inadvertent selection resulting from spawning practices.

B. Objective: Develop stocking and recovery strategies to limit loss of genetic diversity and minimize risks of artificial selection. Initial target recovery of parr from 75% of families stocked per drainage.

Action: Implement stocking practices that equally distribute genetic groups (families) throughout the stocking sites.

Action: Implement collection practices that obtain representative genetic variation (i.e. majority of artificial and wild spawned families), including widespread field collection.

Action: Use genetic determination of parentage to identify percentage of families recovered from stocking events, and monitor yearly to evaluate stocking practices to maximize recovery.

Action: Implement pedigree lines if recovery of families is significantly lower than target recovery goal and management actions undertaken to increase percentage recovered have not been realized.

Action: Continually monitor critical trait variation in broodstock and avoid artificial selection resulting from stocking and collection practices.

C. Objective: Monitor estimates of genetic diversity in annual river-specific parr collections. Target loss of heterozygosity over five-year increments no greater than 5% in each captive broodstock.

Action: Annual genetic characterization of parr (DPS) and sea-run adults (Penobscot). Track changes in estimates of genetic diversity such as

heterozygosity, allelic variation, partitioning of variation within and among populations.

Action: Seek funding for genetic characterization of parr for DPS populations, Penobscot adults, and the assessment of additional Atlantic salmon population(s) of interest.

2. Goal: Provide juveniles for stocking purposes with genetic characteristics specific to population of origin and associated local habitats.

A. Objective: Conduct spawning and hatchery operations to achieve target stocking goals. Meet TAC egg/fry river-specific production requests.

Action: Annually assess hatchery production to evaluate changes in fecundity, percentage of the broodstock reproducing, and reproductive success to stocking size. Produce annual spawning report.

Action: Evaluate production goals as a function of adult returns (natural reproduction) and optimal juvenile stocking densities. Adjustments to production requests would incorporate this into fry target goals.

Action: Maintain genetically viable, river-specific broodstocks for fry or other life stages used for supplementation.

B. Objective: Implement Fish Health and Biosecurity protocols to reduce the risks of disease outbreak or the introduction of disease into CBNFH.

Action: Follow biosecurity protocols, and continue consultation with USFWS Northeast Region Fish Health Center.

Action: Continue development of Penobscot Receiving Building to aid in isolation of potential fish health risks and maintain Downeast Receiving Building.

Action: Monitor broodstocks for evidence of disease; continue work with USFWS Northeast Fishery Center Fish Health Unit to identify causes of mortalities and to review hatchery procedures to minimize introduction or spread of diseases within the hatchery.

7.1. Timeline for evaluation

Annual evaluation of the management actions for the program goals and objectives can be used to determine if revision to broodstock management is warranted. Annual and five-year evaluations are the initial responsibility of the BMC; however additional comment, review, and participation by additional parties can be incorporated as needed. Reassessment of these program goals and objectives every five years can

determine if new management actions are warranted, if program goals need revision, or if specific management targets need modifications. Evaluation timelines and the responsible agencies are specified in Table 13. Changes to hatchery management described in this plan as determined by the BMC and participating agencies will be incorporated in the following manner:

1. Amendments are approved by the BMC for minor or moderate changes. Changes will be documented and attached as appendices to the BMP.
2. Major changes to broodstock management approved by the BMC will result in a plan revision of the BMP, incorporating previous appendices as needed and applicable.

Table 13. Specific evaluation timeframes for each action item listed by objective and goal, including the agencies responsible for initial evaluation. The TAC Broodstock Management Committee is the party responsible for initial annual and five-year reviews.

Goal	Objective	Action	Data collection time frame	Assessment time frame	Responsible agencies	
Maintain the genetic characteristics and variability for the populations maintained at CBNFH in a manner that preserves and favors the potential for adaptation to wild habitats	Conduct spawning operations in ways that promote retention and incorporation of potentially critical genetic variation in future generations and that minimize the risks of artificial or domestication selection. Maintain average pairwise relatedness below 0.02 within each river-specific capture year.	Utilize broodstock database to track spawning history for all salmon held for broodstock purposes	Annual	5-year	USFWS	
		Use two-phased criteria to determine if implementation of spawning optimization program is needed to reduce potential for inbreeding (section 3.4.3).	Annual	Annual	USFWS	
		Use two-phased criteria to determine if spawning between capture years within populations is needed to minimize inbreeding (section 3.4.3).	Annual	Annual	USFWS	
		Monitor levels of heterozygosity and estimates of inbreeding annually.	Annual	5-year	USFWS	
		Continually monitor critical trait variation and avoid inadvertent selection resulting from spawning practices	Annual	5-year	USFWS	
		Develop stocking and recovery strategies to limit loss of genetic diversity and minimize risks of artificial selection. Target recovery of parr from 75% of families stocked per	Implement stocking practices that equally distribute genetic groups (families) throughout stocking sites.	Annual	5-year	USFWS, ASC, TAC

	drainage.				
		Implement collection practices that obtain representative genetic variation (i.e. majority of artificial and wild spawned families), including widespread field collection.	Annual	5-year	USFWS, ASC
		Use genetic determination of parentage to identify percentage of families recovered from stocking events, and monitor yearly to evaluate stocking practices to maximize recovery.	Annual	Annual	USFWS
		Implement pedigree lines if recovery of families is significantly lower than target recovery goal and management actions undertaken to increase percentage recovered have not been realized.	Annual	5-year	USFWS, TAC
		Continually monitor critical trait variation and avoid undesirable selection resulting from stocking and collection practices.			
	Monitor estimates of genetic diversity in annual river-specific parr collections. Target annual loss of heterozygosity no greater than 5% in each captive broodstock.	Annual genetic characterization of parr (DPS) and sea-run adults (Penobscot). Track changes in estimates of genetic diversity such as heterozygosity, allelic variation, partitioning of variation within and among populations	Annual	5-year	USFWS
		Seek funding for genetic characterization of parr for DPS populations, Penobscot adults, and the assessment of additional ATS populations of interest.	Annual	Annual	USFWS
Provide juveniles for stocking purposes with genetic characteristics specific to population of origin and associated local habitats.	Conduct spawning and hatchery operations to achieve target stocking goals. Meet TAC egg/fry river-specific production requests.	Annually assess hatchery production to evaluate changes in fecundity, percentage of the broodstock reproducing, and reproductive success to stocking size. Produce annual spawning report.	Annual	5-year	USFWS
		Evaluate stocking goals as a function of adult returns (natural reproduction) and optimal juvenile stocking densities. Adjustments to production requests would incorporate these into fry target goals.	Annual	5-year	USFWS, ASC, NOAA
		Maintain genetically viable, river-specific broodstocks for	Annual	5-year	USFWS

		fry or other life stages used for supplementation.			
	Implement Fish Health and Biosecurity protocols to reduce chance of disease outbreak or the introduction of disease into CBNFH.	Follow biosecurity protocols, continue consultation with USFWS Northeast Region Fish Health Center.			
		Continue development of Penobscot Receiving Building to aid in the isolation of potential fish health issues and maintain Downeast Receiving Building	Annual	Annual	USFWS
		Monitor broodstocks for evidence of disease; continue work with USFWS Northeast Fishery Center Fish Health Unit to identify causes of mortalities and to review hatchery procedures to minimize introduction or spread of diseases within the hatchery.	Annual	Annual	USFWS

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Appendix 1. Data and technical information sources related to broodstock management.

Data Type	Title or File Name	Author or Data Steward	Time Period	Major Topics / Keywords
Report	Prelisting Recovery Plan for Maine Wild Atlantic Salmon Populations	E.T. Baum, J. Marancik, P.R. Nickerson	1992	Outlines steps required to recover stocks of Atlantic salmon in Maine in response to the Category 2 listing of seven Downeast rivers
Report	Summary of Maine Atlantic Salmon Collections for Broodstock and Genetic Analyses 1990 – 1998	D.B. Buckley	1990-1998	Summarizes genetic sampling and broodstock collections from 1990 through 1998 including numbers collected, locations disposition of samples and fish
Report	1999 Summary of Maine Atlantic Salmon Broodstock and Genetic Collections.	D.B. Buckley	1999	Summarizes genetic sampling and broodstock collections including numbers collected, locations, disposition of samples and fish; includes revised sample and broodstock collection numbers for 1991 through 1998
Report	Summary of Activities in 2000 and 2001 Related to Maine Atlantic Salmon Broodstock Management and Genetic Sample Collections	D.B. Buckley	2000-2001	Summarizes genetic sampling and broodstock collections in 2000 and 2001 including numbers collected, locations and disposition of samples/fish
Report	Spawning Protocols to Maintain Genetic Integrity in River-Specific Atlantic Salmon Stocks at the Craig Brook National Fish Hatchery	T. King	1995-1997	Outlined a spawning matrix involving crosses males and females from separate year classes
Report	Atlantic Salmon Spawning Activities at Craig Brook National Fish Hatchery 1990 – 1997	T.A. Copeland, T. King, D. Bean.	1990-1997	Summarizes spawning procedures, number of fish spawned per cohort by river, and green eggs produced from 1990 through 1997
Report	Summary of Spawning Procedures at Craig Brook National Fish	D.B. Buckley	1999-2001	Summarizes revised spawning procedures, number of fish spawned by cohort and river,

	Hatchery 1999 – 2001			take dates, introduces broodstock database, maturation rates, for 1999 through 2001
Report	Summary of Spawning Procedures at Craig Brook National Fish Hatchery 2002	P.M. Burke, D.L. Tozier	2002	Summarizes spawning procedures, numbers spawned by cohort and river, take dates, introduces broodstock database, maturation rates for 2002
Report	Stocking Report	T.A. Copeland	1998	Descriptions of stocking equipment, methodologies and numbers of fish stocked in 1998
Report	Spawning 2001	Anonymous	2001	Summarizes spawning procedures, numbers spawned by cohort by river, take date summary for 2002
Working Paper	Broodstock collection recommendations to the Technical Advisory Committee	Beland et al	1995	Recommended target broodstock collection numbers to meet fry stocking goals for each river based on specific management goals
Working Paper	Broodstock collection recommendations to the Technical Advisory Committee	Beland et al	1996	Revised broodstock target numbers; began building foundation of broodstock management and spawning protocols for future goals of the program
Working Paper	Broodstock collection recommendations to the Technical Advisory Committee	Beland et al	1997	Developed principles to guide breeding program based on 4 management goals
Working Paper	Amendments to Spawning Protocols at CBNFH – 2000	Anonymous	2000	Revises spawning protocols in 1997 document based to increased production and tracking capabilities at CBNFH; initiates single-pair matings
Working Paper	Amendments to Spawning Protocols at Craig Brook National Fish Hatchery – 2002	Anonymous	2002	Recommends steps to minimize coancestry of parents and equalize broodstock contribution; initiates spawning broodstock only at age 4

Working Paper	Amendments to Spawning Protocols at Craig Brook National Fish Hatchery – 2001	Anonymous	2001	Revises steps to equalize broodstock contributions by keeping eggs from paired matings in proportion to each parents previous spawning history
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Appendix 2. Request for specific production change for Atlantic salmon at Craig Brook National Fish Hatchery.

Proposed requests are due to Craig Brook NFH one month prior to the TAC meeting which this request will be formally made to determine operational feasibility.

Applicant Information:

1. Date:
2. Applicant name:
3. Applicant agency:

Request Information:

4. Proposed request:

5. Number of Atlantic salmon requested:
6. Life stage of Atlantic salmon requested:
7. Purpose of request:

8. Spawn year for request to begin:
9. Stocking season and year:

10. Proposed method to distribute excess individuals (due to tank capacity limits, as fish grow the numbers need to be reduced periodically, either through splitting into additional tanks, or for stocking into river):

11. Time (season/year) of excess distribution:
12. Life stage of excess distribution:
13. Proposed location for distribution of excess:

14. Method for evaluation and monitoring of study:

15. Will genetic assessment be required (i.e. for family group determination)?

Appendix 3. Flow chart of current broodstock management at CBNFH.

