2006 Annual Craig Brook National Fish Hatchery Atlantic Salmon Broodstock Report

Period covered: August 2005 – August 2006

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TAC Broodstock Committee
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This report is meant to fulfill the annual reporting requirements described in the Craig Brook National Fish Hatchery Broodstock Management Plan (BMP; Bartron et al. 2006). Identified within the BMP are specific components of the captive broodstock program to be monitored and assessed over both short and long time periods. The report follows the outline of the actions described in the "Program Goals and Assessment (Section 7)", and similarly Table 13 of the BMP which includes the data collection and assessment time frames. The report represents the first annual report; therefore all components identified within the evaluation framework are described.

The time period covered by this report is up to summer of 2006, including stocking information. Spawning activities of the fall of 2006 will be covered in the 2007 report.

In the course of the review of the BMP, and as part of the annual broodstock meeting, additional items relevant to Atlantic salmon broodstock management were identified for inclusion. Specifically, items suggested to be included into the BMP were a thorough description of the broodstock management practices at Green Lake National Fish Hatchery and a description of fry stocking processes. Also included are relevant memos presented by the broodstock committee to the TAC for the time period covered by this report: support for the implementation of an adult stocking program, and regarding the creation of a pedigree line for the Dennys River.

The annual broodstock meeting was held April 18, 2006 at Craig Brook National Fish Hatchery, and attended by members of the TAC Broodstock Working Group. Attendees included Denise Buckley, Tom King, Mike Kinnison, Greg Mackey, Paul Santavy, Tim Sheehan, and Meredith Bartron (members); also attending were Richard Dill and Carl Burger.

Table of contents

Attachments
Attachment 1. Trait monitoring summary 2006
Attachment 2. Trait monitoring 4.17.2006
Attachment 3. Penobscot Sea-Run Broodstock Information and Questions

1. List of each action and results of assessment or monitoring summary

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

Data collection time frame: Annual
Assessment time frame: 5 year

The broodstock database, MaineBroodstock.mdb, is maintained at Craig Brook National Fish Hatchery. The use of the database is currently used to record tagging, spawning, transfer within the hatchery and to other facilities, stocking, and other data for all Atlantic salmon maintained at the hatchery. The database is updated annually or more frequently as needed.

B. Use two-phased criteria to determine if implementation of spawning optimization program is needed to reduce potential for inbreeding.

Data collection time frame: Annual
Assessment time frame: Annual

The broodstock committee decided to implement the spawning optimization program for all DPS broodstocks held at CBNFH for the 2006 and future spawn years. Due to the integration of the program within the spawning database and availability of genetic data, there were no operational barriers to earlier hatchery-wide implementation and therefore no operational reason to implement according to the phased-in approach described in the Broodstock Management Plan (BMP).

C. Use two-phased criteria to determine if spawning between capture-years within populations is needed to minimize inbreeding.

Data collection time frame: Annual
Assessment time frame: Annual

One component of the captive broodstock management plan is to address spawning between versus within capture years. One way to monitor for the potential for spawning related individuals is to monitor the average pairwise relatedness value within each capture year (Queller and Goodnight 1989). Pairwise relatedness values were calculated for all available broodstock within each population by capture year (Appendix 1, Table 2). Calculating average pairwise relatedness within each capture year determines the average relatedness for individuals that may be spawned with each other. On average, the average pairwise relatedness value is low among all individuals within each capture year. To prevent spawning of related individuals, a spawning optimization program was used during the 2005 spawning of some of the broodstocks to calculate the relatedness of potential spawning pairs and ensure that value was below a relatedness threshold. Use of the spawning optimization program for all populations will occur in the 2006 spawn year.

Action Item: At this time, continue spawning within capture year and implement use of spawning optimization program in all populations for the 2006 spawning. Also, when spawning the pedigree lines for the Pleasant River, spawning should occur within a pedigree line, but making sure to cross between the captive and domestic portions of the pedigree lines.

D. Monitor levels of heterozygosity and estimates of inbreeding annually.

Data collection time frame: Annual
Assessment time frame: 5 year

Monitoring estimates of genetic diversity is an integral component to the broodstock management at Craig Brook National Fish Hatchery (CBNFH), and required by the CBNFH Broodstock Management Plan (Bartron et al 2006). Long and short-term maintenance of genetic diversity within the captive broodstocks is critical to preserving the endangered DPS populations. Monitoring of genetic diversity allows managers to minimize or change practices in the hatchery or throughout the Atlantic salmon program which may artificially reduce genetic diversity. See Appendix 1 for a summary of the genetic characterization and monitoring of the 2004 CBNFH parr broodstock collection.

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

Data collection time frame: Annual
Assessment time frame: 5 year

See attachments from Mike Kinnison (University of Maine) for a description of the trait variation monitoring program and preliminary results (Attachments 1 and 2). Dr. Kinnison and his students will continue photographing adults for three populations opportunistically for the foreseeable future. Monitoring of reproductive traits will continue, but not juvenile development due to lack of funding.

F. Implement stocking practices that equally distribute genetic groups (families) throughout stocking sites.

Data collection time frame: Annual
Assessment time frame: 5 year

Management efforts to achieve the goal of equal distribution of genetic groups can be divided into two components: a hatchery component and a stocking component.

Hatchery practices

Equal distribution of hatchery-produced families throughout stocking sites within the river-specific hatchery program was one of the management goals described in the BMP. To meet this goal, actions implemented in the hatchery include increasing the number of families contributing to each batch, and reducing the number of batches created per broodstock. Batches now reflect spawning date, so that families within each batch are of similar developmental stage. If not batched according to spawning date, mixing all families produced would result in stocking multiple developmental stages and therefore variable survival of stocked fry. Batching by spawn date standardizes the developmental stage within each batch, and results in the mixing of multiple families from each of the collection years within each river-specific broodstock. At time of stocking, fry are collected according to developmental stage from fry troughs and if possible, batches are stocked in multiple locations.

Complete mixing of family groups is not achieved through the implementation of these management changes. To completely mix all family groups, two different methods could be used. The first would be utilization of hormones during spawning to reduce the length of the spawning season. The use of hormones to facilitate spawning has not been incorporated for a number of reasons. The primary two reasons are to follow a natural maturation schedule, and that a condensed spawning season would present many staffing and operational difficulties due to the number of salmon that would be required to be spawned in a brief amount of time. A second method to mix family groups would be to postpone stocking until all fry are able to be stocked, and stock a river in a single event. By delaying spawning until all fry to be stocked have reached a suitable stocking size, the early-spawned fry would need to be started on a feeding regime. Additional considerations for water flows and temperature at time of stocking, and logistical and equipment requirements would need to be addressed. Stocking fry at multiple stages of early development may give some groups a selective advantage or disadvantage. Additionally, stocking later in the spring may result in unfavorable stocking temperatures, and may increase the threat to an entire cohort if a catastrophic event occurred during or following stocking.

Stocking practices

Please see Appendix 2 for a summary of the stocking practices used in the Maine Atlantic salmon program.

G. Implement collection practices that obtain representative genetic variation, including widespread field collection.

Data collection time frame: Annual
Assessment time frame: 5 year

2005 Parr Collection Summary (DPS)

Parr were collected from: Dennys, East Machias, Machias, Narraguagus, Pleasant, and Sheepscot river during the fall of 2005 (Table 1). Collection targets were achieved for the Machias, Narraguagus, Pleasant, and Sheepscot populations (Table 1). Parr collection targets were not achieved for Dennys and East Machias populations. Delivery dates ranged from mid August to late October (Table 1). Of the Pleasant parr collected, 98 were PIT tagged at the end of November for genetic analysis as part of the 2003 pedigree line.

Anecdotally, parr were generally smaller than normal for most populations. Based on hatchery observations, parr brought into the hatchery earlier in the season are more likely to transition to hatchery feed sooner, which leads to better condition the following spring.

For the 2006 parr collections, it was recommended that the Dennys and East Machias be increased by 40-50 individuals to make up potential shortcomings in broodstock numbers and production from the 2005 collection year.

Overview of parr collection methods-DPS populations

Parr collection practices for some rivers target a large number of sites, and collect an equal number of parr per site. Other rivers have fewer collection sites and larger numbers of parr are collected per site. Some rivers have dedicated broodfish collection sites, for example due to field surveys occurring earlier than preferable for parr transfer to the hatchery. Greg Mackey (ASC) provided parr collections by collection site for the Dennys, East Machias, and Machias (Table 2 a-c). Included were numbers of fry stocked per site, the percentage of total fry stocked per site, the number of broodstock collected the following year as parr, and the percent of broodstock collected from each site.

Penobscot broodstock collection

Richard Dill (ASC) presented information regarding average fecundity per females over time (Attachment 3). Tom King (FWS) discussed changes to operations (building construction, water supply, O_2 saturation, and change from shipping green to eyed eggs) that may have impacted fecundity and general operations. General information regarding fecundity calculations:

-Fecundity is back calculated from eyed to green eggs.
-15-20% error in identification of sex at trap
-75-80% of the Penobscot run is brought in for broodstock
-85-95% of the Penobscot run is of hatchery origin
-Grilse are used for spawning, and this component of the spawning males has remained constant over time. However, the proportion of grilse observed in the run has increased over time.

Management options to reduce artificial selection of run-timing

1. Cycling of adults collected to allow for representation of individuals from throughout the run, while ensuring that sufficient numbers of adults are collected for production purposes. Individuals would be stocked out prior to spawn from early part of run if not needed for production.
2. Bring in later run females
3. Increase the number of males collected

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

Data collection time frame:	Annual
Assessment time frame:	Annual

Effective population size

Estimates of effective population size and genetic analysis of parentage are used to monitor contributions of broodstock to future generations. Estimates of effective population size (N_e) were calculated based on the number of individuals spawned at

CBNFH per broodstock. Spawning records (contained within the database MaineBroodstock.mdb) were used to calculate both the number of females and males spawned at CBNFH within each broodstock. Effective population size was calculated based on

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

for number of males (N_m) and number of females (N_f) spawned, both based on initial spawning, and according to the number of parents contributing to the parr collections. Additional estimates of effective population size (such as variance and temporal effective population size) will be calculated in the future as pedigree and a sufficient time-series of genetic data becomes available.

Effective population size estimates based on the number of individuals spawned varied between and within rivers based on management practices and egg production requests (Table 3). Production requests from TAC determine the number of females spawned (egg production) for each specific river, and therefore in conjunction with broodstock spawning guidelines, production requests guide N_e. Similarly, as production requests vary by river (a function of habitat available to be stocked), the number of parr collected (future broodstock) is also determined by TAC and production requests. Larger rivers with more available of habitat require greater production and more individuals for spawning (e.g. Machias, Narraguagus, and Penobscot), than smaller rivers (e.g. the Pleasant and East Machias). Therefore, the average N_e for the Machias and Narraguagus (average N_e =211.3 and 234.2 respectively, Table 3) is greater than that for the Dennys, East Machias, and Sheepscot (average N_e= 136.8, 139.5, and 132.3 respectively, Table 3). The N_e for the Pleasant River is very low due to limited number of individuals available to establish the broodstock.

To increase the number of broodstock available, pedigree lines have been established within the hatchery (see section I). The first spawn of the first pedigree line established was in 2005, and resulted in an increased N_e. The N_e for the Penobscot broodstock is the largest among all broodstocks spawned at CBNFH (average N_e=448.0, Table 3), a function of the different management practices used for the Penobscot population relative to the DPS populations maintained at CBNFH. Broodstocks aren't maintained captively for the sea-run adults from the Penobscot River. Following collection at Veazie Dam, adults are transported to CBNFH for holding until spawning. The number of adults spawned and N_e within the Penobscot broodstock is dependant on river returns, but can be supplemented through the use of the domestic broodstock (first generation progeny from sea-run Penobscot spawning at CBNFH) if adult returns are low.

Genetic parentage analysis

Genetic analysis of parentage was used to determine hatchery parental contributions to parr collections for the DPS broodstocks. The assumption was made that collected parr were either age 0+, 1+, or 2+ (i.e. for the 2003 collection year, 0+ parr were from spawn year 2002, 1+ parr were from spawn year 2001 and 2+ parr were from 2000). Genetic parentage analysis was completed using Cervus (Marshall et al. 1998).

Results from the genetic parentage assignments were compared to spawning records to determine accuracy of assignments. Only assignments to known spawning pairs were considered for additional analyses. Information is not presented for the Pleasant due to the use of the pedigree line (see Section I).

The overall percentage of parr collected for broodstock assigned to known spawning pairs is low for all broodstocks (Table 4). The low percentage of assignment to known spawning pairs can be attributed to a number of factors: marker inheritance issues, laboratory or human error in the processing of samples, missing genotypes for adults (potential parents) or parr, or the low percentage could be due to the capture of offspring resulting from natural reproduction. To address potential marker issues, beginning with the 2005 capture year parr, a new suite of microsatellite markers tested for parentage assignment capabilities in known families will be integrated into the marker suite analyzed. Laboratory practices continue to be subjected to a variety of quality checks for consistency in results. Screening of adults in the hatchery prior to spawning to make sure complete genotypes are available for all adults (instituted in the 2005 spawn year) may also increase assignment capabilities as complete genotypes would be available for all potential parents. Alternately, some of the parr could be the result of natural spawning by adults or precocious parr.

I. 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.

Data collection time frame:	Annual
Assessment time frame:	5 year

Pleasant River pedigree line

Two pedigree lines have been created for the Pleasant River. Pedigree lines are referenced by the year they were (or would be) collected as parr in the wild, as collection years are maintained separately in the hatchery, within each broodstock room by tank. The first Pleasant pedigre line, the 2003 line was comprised of 2001 (spawn year) domestics and 2003 (capture year) captive parr, have spawned once. The second Pleasant pedigre line, the 2005 line was comprised of the 2003 (spawn year) domestics and 2005 (capture year). Excess 2003 (spawn year) domestics, the domestic component of the second pedigreed line, were released in spring 2006 as 2 year old smolts with the other juveniles produced from that spawn year for smolt stocking (Table 5). A third pedigreed line for the Pleasant River will be established following the 2006 spawn year.

Genetic results from the 2005 Pleasant pedigree line

A pedigree line for the Pleasant River broodstock was established following in the 2003 spawning season. During the 2003 spawning year, a total of 11 females and 12 males were spawned to create 30 families. Families were mixed following swim-up stage. To create the domestic line, 60 individuals per family were retained in the hatchery (Table 5). At the time of genetic tissue sampling and PIT tagging, a total of 1579 individuals from the domestic line were sampled (Table 5). During parr collection, a total of 98 were collected in the Pleasant River in 2005 to represent the captive line (Table 5).

Genetic determination of parentage was completed using genotypes at 11 microsatellite loci, and using Cervus (Marshall et al. 1998). Genotypes were available for all putative parents, however could be liked directly to only four female parents and 10 male parents. Using unmatched genotypes, links between lab sample id and PIT id could be deduced for 6 females based on patterns of parentage analysis using spawning records. Therefore, genotypes for 10 females and 10 males could be determined, and only four of the 30 families identified could not be conclusively determined based on only one parent (or neither) genotype being available.

Parentage was assigned to 24 of the captive group (released as fry and recaptured as parr) and 679 of the domestic group (retained in the hatchery). Of the 30 families created, 24 were detected in the offspring. All juveniles were screened for continent-of-origin and river/region probability of assignment according to broodstock screening protocols. Forty-seven juveniles were identified to be culled (5 captive and 42 domestic) based on river/region screening, none based on continent-of-origin screening. Of the parents that can be linked to lab id and PIT id, none were identified to cull, however, five individuals from the parent capture year were identified to cull based on river/region screening (implemented following spawning) and were potentially spawned, resulting in the identification of the offspring to cull.

Family group contribution was equalized among all offspring according to retention protocols: all captive offspring were retained (24 with parentage assigned, 69 without), and 183 offspring from the domestic line (all with parentage assigned) were retained. Of the remaining juveniles, 1354 will be stocked as pre-smolts into the Pleasant River.

Dennys River Pedigree Line

A large number of putative aquaculture-origin Atlantic salmon escaped from net pens in Canada in the summer of 2006. To mitigate for potential incorporation of putative aquaculture offspring into the 2007 parr collection from the Dennys River, a pedigree line was established. The domestic portion of the pedigree line will be retained at the hatchery and serve as the primary source for the 2007 parr broodstock collection. The captive portion of the pedigree line will be stocked into the Dennys River in the spring of 2006 into areas where spawning by putative aquaculture-origin individuals was not observed. Collection of parr for broodstock will occur in the fall of 2007, targeting the fry stocking areas. All parr collected will be screened prior to incorporation into the broodstock. See Appendix 4 for the letter to TAC regarding the establishment of the 2007 Dennys Pedigree Line.

J. Continually monitor trait variation and avoid undesirable selection resulting from stocking and collection practices.

Data collection time frame:	Annual
Assessment time frame:	5 year

See Section E. (Continually monitor critical trait variation and avoid inadvertent selection resulting from spawning practices) for related information. Additionally, incorporation of the spawning optimization program acts to reduce artificial selection and identifies mating based on minimizing relatedness among spawners.

K. Annual genetic characterization of parr (DPS) and sea-run adults (Penobscot). Track changes in estimates of genetic diversity such as heterozygosity, alleleic variation, partitioning of variation within and among populations.

Data collection time frame:	Annual
Assessment time frame:	5 year

Summary of estimates of genetic diversity for the broodstock collected between 1994 and 2004 are provided in Appendix 1, Table 2. The 2005 capture year parr are currently being genotyped and culling results will be provided to the hatchery prior to spawning.

L. Seek funding for genetic characterization of parr for DPS populations, Penobscot adults, and the assessment of additional ATS populations of interest.

Data collection time frame:	Annual
Assessment time frame:	Annual

Funding for annual genetic characterization of CBNFH broodstock, the sea-run adult Penobscot broodstock, and domestic Penobscot broodstock maintained at GLNFH was provided by USFWS.

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

Data collection time frame:	Annual
Assessment time frame:	5 year

Annual spawning reports for both DPS and Penobscot can be found in Table 6 and Table 7. Production resulting from the 2005 spawning can be found in Table 8. In addition to fry production, eyed eggs and parr were produced for the Sheepscot River and smolts were produced (including pedigree line production) for the Pleasant River.

Adult stocking

Adults are typically stocked post-spawn into the estuaries or lower river regions of their natal river. At times, adults which are no longer needed to meet production requests, and which had already been spawned at CBNFH in previous years, are available for pre-spawn stocking into their natal river. Stocking of river-specific adults prior to spawning occurred in the East Machias, Machias, and Sheepscot populations in the fall of 2005. Approximately 50 males and females were stocked into each river (2006 USASAC report).

Action Item: A letter was sent to TAC in support of adult pre-spawn stocking into suitable spawning habitat. See Appendix 5.

N. 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.

Data collection time frame:	Annual
Assessment time frame:	5 year

Based on adult returns provide (from 2006 USASAC report), adult returns were not sufficient to result in re-assessment of stocking and production goals by Maine ASC. Stocking and production goals are presented to the TAC through a formal request from Maine ASC.

O. Maintain genetically viable, river-specific broodstocks for fry or other life stages to be used for supplementation.

Data collection time frame:	Annual
Assessment time frame:	5 year

One of the goals of broodstock management at CBNFH is the maintenance of genetically viable, river-specific broodstocks. To achieve these goals, genetic characteristic are monitored, incoming broodstock are screened, attention is paid to spawning practices, and hatchery practices are tracked. A summary of genetic monitoring and spawning practices are provided in other sections of this report.

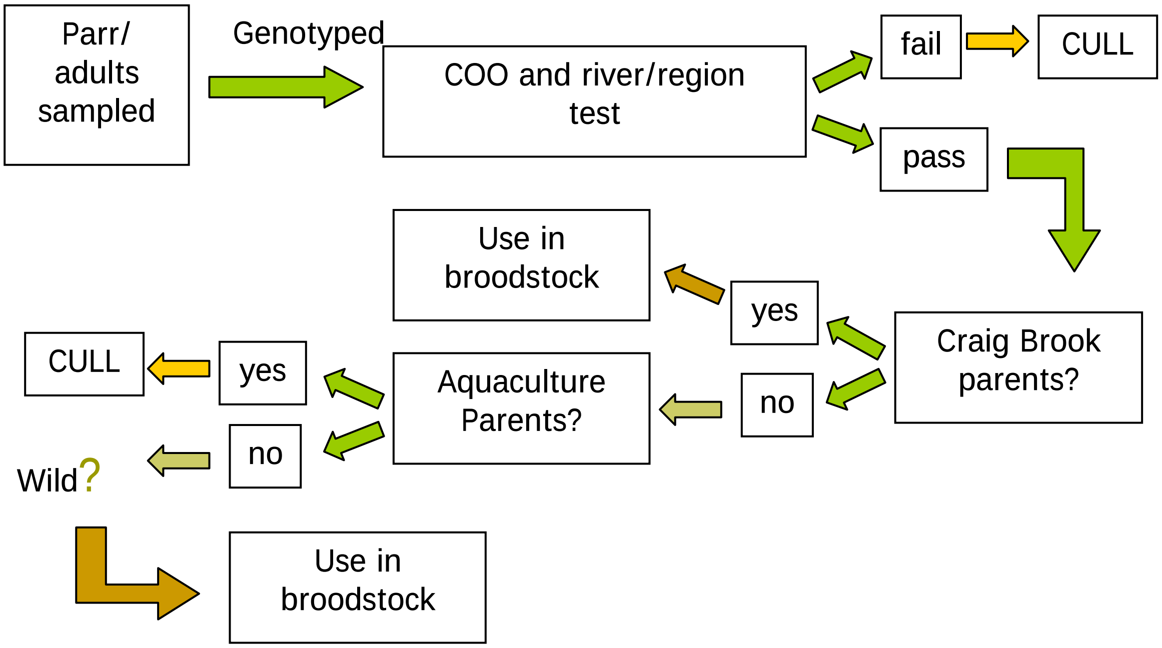
Broodstock screening

Culling summaries for Atlantic salmon broodstock at Craig Brook National Fish Hatchery can be found in Tables 9 and 10. Broodstocks analyzed represent parr with the exception of Penobscot, which are screened as adults. Culling methods screen according to continent of origin determination, and probability of assignment to putative river of origin and Maine populations.

Future changes to broodstock culling practices

Permit conditions for Atlantic salmon aquaculture companies in Maine require a mark that can identify sub-lot within a hatchery/stocking site, to be implemented this year. Cooke Aquaculture has recently become the sole Atlantic salmon aquaculture company operating in Maine. Cooke has decided to use parentage analysis (using microsatellite loci) as the mark. NOAA-Fisheries and FWS has been working with Maine DEP, ACOE, and Cooke to develop appropriate marking procedures, including identification of appropriate marker suites, database to manage stocking, genetic, and tracking data, and quality control and quality assurance procedures to allow analysis and identification of potential aquaculture escapees.

Figure 1. Broodstock screening of parr and adults at CBNFH will be modified to reflect these changes.



P. Follow biosecurity protocols, continue consultation with USFWS Northeast Region Fish Health Center.

Data collection time frame: Annual
Assessment time frame: Annual

Biosecurity procedures continue to be followed at CBNFH. Revisions to existing protocols for the Penobscot Spawning Pools will be made as needed to reflect changes to the facility following construction, and protocols will be completed for the new Penobscot Receiving Building and Wastewater Treatment Building.

Q. Continue development of Penobscot Receiving Building to aid in the isolation of potential fish health issues and maintain Downeast Receiving Building.

Data collection time frame: Annual
Assessment time frame: Annual

The Penobscot building renovations will be completed for receiving Penobscot adults this summer. The ISA building will be completed for screening and receiving next year. The wastewater treatment plant has not yet begun construction, however, it is planned to be completed and functional January of 2007.

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

Data collection time frame:	Annual
Assessment time frame:	Annual

The ISA building as part of the screening for wild-caught adult salmon from the Penobscot will be completed and allow for isolation by day of capture for ISA screening to occur prior to movement into the Penobscot broodstock tanks.

No information was presented at the meeting regarding fish health issues.

3. Tables

Table 1. Parr collections received at Craig Brook National Fish Hatchery during the fall of 2005.

Strain	# collected (target)	Delivery Dates	Comments
Dennys	120 (150)	10/21-10/22/2005	-
East Machias	105 (150)	10/17-10/18/2005	-
Machias	265 (250)	10/19-10/20/2005	-
Narraguagus	256 (250)	8/15-9/14/2005	-
Pleasant	101 (100)	10/5/2005	98 parr PIT tagged on 11/30/2005
Sheepscot	165 (150)	9/25-9/29/2005	-

Table 2. 2005 broodstock collection summary based on parr recapture and numbers stocked, calculated by ASC for the Dennys (a), East Machias (b), and Machias (c). Numbers indicate the number of fry stocked per group, and the number of broodstock from each group collected as parr (groups previously were stocked into specific sites, now groups are mixed). Percentage stocked represents the percentage of fry stocked of the total number of fry stocked, and the percentage contribution of that group to the number of broodstock collected.

a. Dennys

Group	Number stocked	Broodstock collected	% stocked (as fry)	% broodstock collected
C3	57,689	28	27	27
C4	79,000	0	37	0
C5	48,620	24	23	23
C6	28,600	53	13	50
C7	0	0	0	0
total	213,909	105	100	100

b. East Machias

Group	Number stocked	Broodstock collected	% stocked (as fry)	% broodstock collected
C1	45,900	0	12	0
C2	109,202	0	27	0
C3	24,150	0	6	0
C4	38.640	48	10	46
C5	35,429	39	9	37
C6	70,023	0	18	0
C7	75,086	18	19	17
total	398,430	105	100	100

c. Machias

Group	Number stocked	Broodstock collected	% stocked (as fry)	% broodstock collected
C1, C2, C8	203,893	0	45	0
C3	66,893	0	15	0
C4	62,509	24	14	9
C5, C7	29,610	0	7	0
C5	23,800	0	5	0
C6	43,405	97	10	37
C7	24,025	144	5	54
total	454,135	265	100	100

Table 3. Effective population sizes based on the number of males and females spawned for the DPS and Penobscot broodstocks for the spawn years 1999 to 2004.

Spawn	Broodstock						
Year	DE	EM	MA	NA	PE	PL	SH
1999	99.8	114.0	239.0	254.1	572.0	x	113.0
2000	108.4	122.4	185.1	239.0	303.8	x	98.8
2001	153.4	122.0	197.5	173.2	414.8	26.0	94.7
2002	136.0	186.0	222.0	306.6	344.0	38.0	168.1
2003	155.0	171.2	189.0	228.0	549.9	23.0	170.3
2004	168.0	121.5	234.9	204.2	503.5	46.0	149.0
Average	136.8	139.5	211.3	234.2	448.0	33.2	132.3

Table 4. Results of genetic parentage analysis for parr broodstock collections. The number of parr assigned to known spawned hatchery parental pairs, by collection year and spawn year according to parental assignments.

a) Percentage of parr with parentage assigned to known spawning pairs from each collection year for each broodstock.

Broodstock	Capture year	Number screened	Number correctly assigned parentage	Percentage correctly assigned
Dennys	2001	101	25	24.8
	2002	270	42	15.6
	2003	142	32	22.5
	2004	148	40	27.0
East Machias	2001	135	41	30.4
	2002	168	78	46.4
	2003	156	95	60.9
	2004	154	71	46.1
Machias	2001	244	23	9.42
	2002	336	133	39.6
	2003	248	152	61.3
	2004	247	100	40.5
Narraguagus	2001	248	52	21.0
	2002	243	80	32.9
	2003	252	145	57.5
	2004	234	114	48.7
Sheepscot	2001	300	44	14.7
	2002	157	98	62.4
	2003	160	82	51.3
	2004	149	82	55.0

b) Number of correctly assigned parr from each broodstock by collection year to year spawned.

Collection	Spawn	Broodstock				
Year	Year	DE	EM	MA	NA	SH
2001	1998	-	-	-	-	-
	1999	25	41	23	51	44
	2000	0	0	0	1	0
2002	1999	2	7	17	8	2
	2000	39	71	116	72	96
	2001	1	0	0	0	0
2003	1999	1	1	4	0	0
	2000	0	11	17	19	1
	2001	29	83	131	126	81
	2002	2	0	0	0	0
	2003	0	0	0	0	1
2004	1999	0	1	1	0	0
	2000	0	3	1	0	0
	2001	1	17	18	12	4
	2002	39	46	80	101	78
	2003	0	4	0	1	0

Table 5. Summary of Pleasant pedigree lines created at CBNFH.

Name	Spawn year	Captive coll. year	Number families	Number eggs retained (family)	# retained for broodstock	
					Domestic	Captive
2003	2001	2003	37	n/a	136	113
2005	2003	2005	30	60	183	93

Table 6. Spawning summary for the broodstocks spawned at CBNFH, and average fecundity over time.

Population	Capture Year	# Females Spawned	# Males Spawned	2005 Average Fecundity	5-Year Average Fecundity	Change in Fecundity
Dennys	2000	19	16	4,860	7,916	(3,056)
	2001	19	22	5,778	7,888	(2,110)
	2002	4	4	4,164	4,260	(96)
	2003	43	43	2,177	2,539	(362)
East Machias	2001	2	1	8,995	6,976	2,018
	2002	16	17	3,769	5,585	(1,816)
	2003	70	47	2,748	2,878	(130)
Machias	2001	56	51	3,895	7,452	(557)
	2002	15	26	4,891	5,046	(155)
	2003	89	55	3,114	2,631	483
Narraguagus	2001	49	49	7,224	7,028	196
	2002	14	14	3,645	4,688	(1,044)
	2003	83	76	2,378	2,757	(379)
Penobscot	2005	296	167	8,403	8,583	(180)
Pleasant	2001	56	41	-	-	-
	2003	43	49	2,591	-	
Sheepscot	2001	11	9	9,310	7,972	1,338
	2002	3	3	4,093	4,667	(574)
	2003	56	43	2,536	2,876	(341)

Table 7. Total green eggs, % eye up, and % survival to fry (from green eggs to swim-up fry) for the 2005 spawn year populations held at CBNFH.

Population	Total Green Eggs	% eye up	# remaining (4/17/06)	% survival to fry
Dennys	412,445	81	329,461	80
East Machias	280,882	80	212,019	75
Machias	670,394	87	578,831	86
Narraguagus	446,528	88	376,590	84
Penobscot	2,449,988	91	2,210,477	90
Pleasant	297,785	84	264,280	88
Sheepscot	238,846	77	186,571	78

Table 8. Annual assessment of production at CBNFH. Data from 2005 U.S. Assessment Committee database (draft).

Strain	Eyed Eggs	Fry	Parr	Smolts
Dennys	-	215,172	-	-
East Machias	-	215,748	-	-
Machias	-	476,211	150	-
Narraguagus	-	352,263	-	-
Penobscot	-	1,898,411	-	-
Pleasant	-	76,187	-	5,933
Sheepscot	8,812	201,100	15,882	-

Table 9. Total number of individuals identified to be removed from each broodstock based on genetic screening results for continent-of-origin and river/region testing methods.

	Capture year: total number culled						
Drainage	1994-8*	1999*	2000	2001	2002	2003	2004
Dennys	14	0	2	1	7	2	2
East Machias	1	0	1	6	0	1	1
Machias	1	0	8	5	15	1	11
Narraguagus	0	0	6	1	8	7	6
Penobscot	0	0	13	13	2	1	15
Pleasant	0	4	18	0	0	1	0
Sheepscot	4	0	1	5	3	4	4

*results for culls not separated by reason

Table 10. Number of individuals to be removed from the broodstock from genetic screening results due only to continent-of-origin testing.

	Capture year: number culled due to COO				
Drainage	2000	2001	2002	2003	2004
Dennys	1	0	2	1	0
East Machias	0	0	0	0	0
Machias	2	5	1	0	1
Narraguagus	0	0	3	3	0
Penobscot	5	9	0	0	0
Pleasant	0	0	0	1	0
Sheepscot	0	0	0	2	0

Appendicies

APPENDIX 1. Genetic characterization of the 2004 Craig Brook NFH parr broodstock collection

Summary of estimates of genetic diversity for the broodstock collected between 1994 and 2004 for the DPS broodstocks, and between 2002 and 2006 for the Penobscot broodstock are provided in Tables 1 and 2. Parr collected for broodstock purposes are genetically characterized as part of the broodstock screening process prior to incorporation into the broodstock.

Methods

Tissue samples from DPS broodstock parr were provided by Craig Brook NFH. Parr are PIT tagged and tissue sampled concurrently so that individuals can be tracked throughout their life cycle within the hatchery. Tissue samples from sea-run adults captured at the Veazie trap on the Penobscot River were provided by Maine Atlantic Salmon Commission, and samples analyzed from the Penobscot reflect only those spawned for broodstock at CBNFH. Data for the CBNFH DPS and Penobscot broodstocks up to and including the 2001 collection year were provided by Dr. Tim King (USGS Leetown Science Center, Leetown, West Virginia). Data for the 2002 collection and subsequent years were generated by the FWS Northeast Conservation Genetics Lab at the Northeast Fishery Center in Lamar, Pennsylvania. DNA was extracted using the PurGene method (Gentra Technologies). Eleven microsatellite loci analyzed: Ssa14, Ssa289 (McConnell et al. 1995), SSOSL25, SSOSL85, SSOSL311, SSOSL438 (Slettan et al. 1995 and 1996), SSa85, Ssa171, Ssa197, Ssa202 (O'Reilly et al. 1996), and SSLEEN82 (GenBank accession number U86706). PCR conditions followed those described in King et al. (2001). PCR products were electrophoresed on an ABI 3100 (Applied Biosystems, Foster City, California), and analyzed using GeneScan and GenoTyper (Applied Biosystems, Foster City, California). Genotypes were standardized between labs to allow for comparison of data.

Estimates of alleleic diversity, allele frequencies, observed and expected heterozygosity, and inbreeding coefficients were obtained using GDA (Lewis and Zaykin 2001). Exact tests for Hardy-Weinberg disequilibrium were also obtained using GDA, and Bonferroni correction to significance levels were made for multiple comparisons (Rice 1989). Mean pairwise estimates of relatedness based on Queller and Goodnight (1989) were calculated using Spagedi (Hardy and Vekemans 2002). Allelic richness estimates in each broodstock were calculated using FSTAT (Goudet 2001). To determine partitioning of genetic variation within and among populations, F-statistics were estimated both with and without population hierarchies based on Weir (1996) and Weir and Cockerham (1994), using GDA (Lewis and Zaykin 2001). Bootstraps for the f-statistics were also calculated to determine the significance of estimates at the 95% confidence interval.

Results

Results presented will focus on the average estimates calculated by broodstock between 1994 and 2004 for the DPS broodstocks held at CBNFH, except for the Pleasant River (1995-2004), and the Penobscot broodstock (2002-2006). The timeframe for the Penobscot broodstock was chosen to reflect known broodstock, rather than all adults returning to and sampled at Veazie Dam.

Average sample size for all broodstocks (DPS and Penobscot) ranged from 135 in the East Machias to 460.9 in the Penobscot (Table 1). This large range is due to fewer broodstock sampled on an annual basis for the DPS broodstocks compared to the Penobscot River. Within the DPS broodstocks, multiple year classes are spawned in a given year, so therefore annual collections of (typically a single) year-class range from 100-250 per broodstock river depending on river and collection target. Within the Penobscot, multiple year classes are returning to spawn in a given year, and production goals for the Penobscot are higher compared to the DPS populations. Therefore, the differentiation in sample size between rivers is a function of collection targets, production goals, and life-stage sampled.

Deviations from Hardy-Weinberg equilibrium were observed in all populations and most collection years, most likely due to sampling juvenile life stages for the DPS populations consistent with the Allendorf-Phelps effect (Allendorf and Phelps 1981). Due to the range in sample size, the average number of alleles per locus (Table 1) could be potentially biased due to sampling effects. Allelic richness is a preferred measure for comparison of diversity due to the standardization of allelic diversity according to sample size (Petit et al. 1998). Allelic richness ranges from 5.52 in the Dennys River to 8.88 in the Penobscot (Table 1). Both the Dennys and East Machias parr collections had the lowest alleleic richness within the DPS populations, compared to 8.40 and 8.71 in the Machias and Narraguagus respectively (Table 1). The Pleasant and Sheepscot populations were intermediate (Table 1). Examination of allelic richness estimates within each broodstock annually revealed consistent estimates over the time period examined (results not presented). Observed heterozygosity was similar among all populations, again lowest in the Dennys River (H_o=0.555; Table 1), but highest in the Sheepscot (H_o=0.577; Table 1). Inbreeding estimates were lowest in the East Machias (f=-0.018; Table 2), and highest in the Narraguagus (f=0.002; Table 1). The average mean pairwise relatedness (r_{xy}) within each broodstock collection ranged from -0.007 in the Pleasant River (Table 2), to 0.003 in the Penobscot River (Table 2).

F-statistics were calculated to estimate population differences (Weir and Cockerham 1994). Calculations were made among all populations without any hierarchies, and again with individual parr or adult collections grouped across years by drainage. When all populations were examined separately, f (F_{IS})=-0.0036, F (F_{IT})=0.0098, and Θ_p (F_{ST})=0.0133. The estimates of F and Θ_p were both significant according to 95% confidence intervals. When hierarchies were used to group individual collections by river drainage (e.g. all Sheepscot collection years considered as subpopulations to a single Sheepscot population), the results were: f =-0.0036, F =0.0108, Θ_s=0.0144 and Θ_p=0.0076. The estimates of F, Θ_s, and Θ_p were considered significant according to 95% confidence intervals. The results indicate that differences between individual broodstock year collections are significant, and that when considered by drainage, differences between populations are also significant.

Discussion

A few caveats are important to address prior to interpretation of the genetic results. First, samples from the DPS populations represent collections of parr. Therefore, there is a high likelihood that related juveniles are part of the collections, which may bias some of the genetic population analyses (i.e. deviations from Hardy-Weinberg equilibrium; Allendorf and Phelps 1981). The practice of collection of parr was determined to be necessary to collect broodstock as all of the DPS populations have very low numbers of returning adults. Collection of parr was thought to be a method to incorporate offspring from any returning adult spawner, and also to allow some degree of natural selection to hatchery produced stocked fry if and when the hatchery fry are reincorporated into the broodstock during parr collections.

The second caveat is that sample sizes are a reflection of management goals for future production in the hatchery. Production goals are based on available habitat for juvenile salmon, and therefore the populations from larger river drainages have increased production goals compared to smaller river drainage basins. Target parr collection numbers for the Machias and Narraguagus are 250 parr, for the Dennys, East Machias, and Sheepscot the target is 150 parr, and for the Pleasant, this target is 150 parr (Table 1). These collection targets result in a bias in sample size for each broodstock, and potentially may result in a bias in the number of alleles observed per locus. However, as broodstock collections are generally large (>100), the sample size may adequately reflect the genetic diversity of each broodstock (Table 1). Estimates of effective population sizes also follow this pattern (2006 Annual Report) because calculations are based on the number of males and females spawned per year. Differences in N_e within and between broodstocks are a function of production targets and the required number of individuals to be spawned.

Genetic sampling of a non-random group of potentially related juveniles may also have implications to estimates of population structure. Analyses of the population structure within and among the broodstocks indicate that significant genetic differences exist between collection years within each broodstock (Θ_s=0.0144), potentially due to the sampling of related juveniles (cohorts). When the individual collection years are grouped by broodstock population and all populations are compared (the DPS broodstocks and the Penobscot population) by drainage, significant genetic differences among drainages were observed (Θ_p=0.0076). Grouping populations across collection years may reduce the bias effect from the individual parr collections, and would be more similar to the population structure (diverse age structure) to a random sample of a distributed population.

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Table 1. Summary of average estimates of genetic diversity for broodstock at CBNFH. DPS population averages between 1994 and 2004, except Pleasant River (1995-2004).

Estimates for Penobscot populations are averaged from 2002 to 2006.

Broodstock	Sample size	Number alleles /locus	Allelic richness	Heterozygosity (exp.)	Heterozygosity (obs.)	Inbreeding (f)
Dennys	138.7	8.90	5.52	0.552	0.555	-0.004
East Machias	135.0	8.44	5.75	0.559	0.569	-0.018
Machias	229.9	8.68	8.40	0.564	0.563	0.001
Narraguagus	261.3	9.23	8.71	0.568	0.567	0.002
Penobscot	460.9	9.24	8.88	0.571	0.571	-0.001
Pleasant	155.7	8.06	6.27	0.571	0.571	-0.001
Sheepscot	138.6	8.08	7.54	0.569	0.577	-0.014

Table 2. Mean pairwise relatedness values within capture years (Queller and Goodnight 1989). Abbreviations are as follows: Dennys (DE), East Machias (EM), Machias (MA), Narraguagus (NA), Penobscot (PE), Pleasant (PL), and Sheepscot (SH).

Capture	Drainage						
Year	DE	EM	MA	NA	PE	PL	SH
2000	-0.086	-0.004	0.001	-0.002	x	x	-0.005
2001	-0.006	-0.007	-0.004	-0.004	x	x	-0.002
2002	-0.001	-0.006	0.002	0.004	0.000	x	-0.006
2003	-0.007	-0.007	-0.004	0.000	0.021	-0.004	-0.006
2004	-0.007	0.000	-0.004	-0.004	-0.003	-0.011	-0.007
2005	x	x	x	x	-0.002	x	x
2006	x	x	x	x	-0.002	x	x
Average	-0.021	-0.005	-0.002	-0.001	0.003	-0.007	-0.005

APPENDIX 2. Summary of stocking practices for the Maine Atlantic salmon program.

Greg Mackey, Maine Atlantic Salmon Commission

A variety of stocking methods are used in the DPS rivers. Generally, fry are stocked into each river using either clump or point stocking methods. Clump stocking is where a small group of fry are stocked into ideal habitat in multiple locations throughout the stocking site. Point stocking is where all fry designated for a specific stocking site are stocked into a single location within the site. Point stocking typically occurs at the most upstream point of a site to allow for natural downstream dispersion throughout the site. The determination which stocking method used (point or clump) is made by ASC, and varies by river according to site and river conditions (flow, temperatures, or other factors). Greg Mackey (ASC) provided the following information about river-specific stocking methods.

For both methods, fry are first distributed to the stocking locations from Craig Brook NFH in quart coolers with aerators and supplemental oxygen. Two primary transport methods are used distribute fry to the stocking site: canoe and by foot-stocking. For most of the sites to be clump stocked, fry are transported in oxygenated coolers in canoes. For some clump and point stocking sites, fry may be stocked directly from the hatchery truck to the river via buckets. Foot stocking is accomplished by carrying small amounts of fry in five gallon buckets and releasing them in intervals throughout a small section of habitat. Rarely, though used in a few sites or dependant on water conditions, fry are transported to the stocking sites in an oxygenated backpack cooler and distributed either by clump or point stocking methods.

Clump stocking

Before the stocking trip, biologists draft an estimate of the number of fry to be stocked in each major section of habitat within each site. Biologists use habitat databses, local knowledge of the streams, maps, and GPS coordinates to locate each habitat section.

Field staff travel to each section of habitat (usually by canoe) and release fry using a small aquarium dip net. The fry are placed in shallow water, usually near the margins of the streams. Ideal habitat is small cobble, although substrate type varies considerably. Quite a bit of on-site judgment is required because of the high variability in the habitat. The fry are stocked in “clumps” of between roughly 1000 and 5000 individuals, depending on the size and characteristics of the habitat, however this number can vary. The goal is to place fry in habitat where they can quickly find refuge and then volitionally spread out.

The following streams are clump stocked*:*

Dennys
East Machias (except Northern Stream, see below)
Machias
Pleasant

Narraguagus (except Baker Brook and Narraguagus mainstem near Bog Brook, see below)
Sheepscot
Ossippee (Also see Saco River, below)

Point Stocking

Northern Stream, East Machias

ASC biologists performed an experimental point stocking on Northern Stream, East Machias drainage. All fry for the entire section (approximately 1.5 km long) were stocked at the top of a major habitat section (top of study site) at a rate of 100 per unit for the entire section. The focus of this study was initial and summer distribution of stocked fry.

Narraguagus Mainstem near Bog Brook

A large point stocking at Bog Brook confluence covers about 550 units of habitat over a 3 km stretch of river. The habitat is more or less continuous, in that there are no large deadwater sections. ASC biologists stock at about 125 per unit and all the fry are stocked at the same location and disperse throughout the reach over time. The dispersal of fry is monitored by standard electrofishing effort as part of the BGEST sampling.

Baker Brook, Narraguagus

ASC also has a smaller scale point stocking site at Baker Brook, a small coldwater tributary in the upper Narraguagus. ASC biologists stock about 166 units of habitat at about 100 per unit. The fry are again point stocked at one location above the electrofishing index sites.

Sandy River, Kennebec

Point stocking is also performed on the Sandy River primarily due to the small number of fry released. In general, one or two release locations are chosen with fry releases at densities between 25 and 100 fry per unit. Electrofishing surveys are conducted to monitor dispersal, growth and to gain insight into habitat quality.

Saco River

The majority of fry releases in the Saco River drainage are point stockings. The only exception is the Ossipee River which is usually stocked via canoes similar to other large rivers. The point stocking is generally done in and around road crossings primarily because the streams are small and access is difficult.

Other Stocking Strategies

Penobscot and Aroostook Rivers

Point stocking is typically used for the Penobscot for smolt stocking, and Aroostook Rivers. ASC is beginning to investigate habitat quality in these systems, and ASC deliberately stocks ample fry in and around the electrofishing sites to ensure that the observed electrofishing fish densities are associated with habitat suitability rather than lack of recruitment (i.e. stocking). Fry stocking allocations are based on quantities of fry habitat recorded in the ASC habitat database.

Sandy River, Kennebec

Other release strategies are currently being investigated on the Sandy River. Small numbers of eggs, green and eyed, have been buried in portion of the drainage to gain insight into the use of eggs as a restoration/enhancement tool. Alevin are allowed to develop naturally and disperse when conditions are appropriate. Electrofishing surveys are conducted to monitor dispersal, survival and densities.

APPENDIX 3. Description of hatchery practices at Green Lake National Fish Hatchery

Paul Santavy, Hatchery Manager, Green Lake National Fish Hatchery

Green Lake NFH (GLNFH) serves as a smolt production facility, and is primarily used for production for the Penobscot River. There are two primary sources of Penobscot juveniles for production at GLNFH: wild sea-run adults obtained from Veazie Dam on the Penobscot River that are spawned at CBNFH, and production at GLNFH by a domestic Penobscot broodstock. The domestic Penobscot broodstock also results from the wild adults from Veazie Dam spawned at CBNFH, but is retained to maturity for spawning at GLNFH (versus stocked as smolts). Offspring from the domestic broodstock is used primarily to offset production shortfalls from the wild sea-run adults due to low number of adults available for broodstock, or for production to be stocked in rivers other than the Penobscot. However production by the domestic broodstock has been limited to fry production in the past. If the numbers of sea-run returns for Penobscot broodstock decrease, the use of the domestic line for smolt production should be considered to increase the effective population size for that spawn year. Use of the domestic Penobscot broodstock for smolt production, for which smolts have higher rates of return than fry, would be consistent with the goals of the BMP to conserve the genetic diversity of the individual broodstocks. Although the primary goal of production at GLNFH is the smolt stage, other earlier life stages are also available to be stocked as a by-product of fish growth and increased space demands as part of the smolt-rearing process.

Egg stage

Wild Penobscot strain eggs are taken at Craig Brook National Fish Hatchery (CBNFH) and placed by discrete family into individual incubators. At the eyed egg stage, an equal number of eggs from each family are separated, mixed according to spawn date, picked, and then transported to Green Lake National Fish Hatchery (GLNFH). Here they are placed in incubators, where all the families within a particular egg-take date (lot) are mixed, but the eggs from different lots are kept separate by spawn date. This is how they are kept until they hatch and are moved to the fry tanks. Dennys strain eggs are handled and moved in the same manner

Domestic F2 Penobscot eggs are taken at GLNFH and kept on-station. The families are mixed as they are placed in incubators, but the eggs from different lots are kept separate. At the eyed egg stage, they are picked and placed back into the incubators, still keeping different lots separate. These eggs are distributed to various partners shortly after pick, and at this time none are hatched on-station.

Fry stage and movement

As alevin develop into fry, they are moved into the fry tanks at the appropriate developmental index. They are grouped within lots for placement into the tanks (usually 3 incubators / tank). Fry from different lots are normally not mixed into a fry tank unless they were taken as eggs in very close proximity to one another (i.e. up to 5 days). The various lots are tracked from incubators into the fry tanks, so as each lot can be identified in the fry tank room. By the time the fry have developed enough to be moved to the outside pools, they are at an equal level of development and size.

Transfer among pools

When fry are moved from the fry tanks into the pools, a random group of fry are removed from each and every fry tank in equal numbers to fill one pool. There are typically 4 fry moves to the pools, each spaced apart by 1-2 weeks. When all the fry are out to the pools, 1 complete row (out of 3 total rows) of pools is filled, and every pool has a representative population from every fry tank.

In mid-summer, a split occurs in which the parr from the 1st filled row are split to fill the 2nd row. Each pool is split to the adjacent pool in the next row, so that the 2nd row also has a representative population from every fry tank.

In fall, grading of all parr occurs. During grading, graded-out parr are moved to load-out raceways in preparation for stocking. The remaining smolt (currently still parr) are split again to fill the 3rd row. Every pool still has a representative population from each fry tank, minus any graded-out parr.

Penobscot Domestic Broodstock

The Penobscot domestic broodstock maintained at GLNFH is created annually from a representative portion of each family created at CBNFH. Typically the domestic broodstock is used for fry production, however the use as production for smolts should be considered if sea-run returns for broodstock are low. Use of the domestic line for smolt production if needed would increase the effective population size for that given spawn year, and may act reduce the likelihood of long-term genetic bottlenecks resulting in a reduction of genetic diversity in the Penobscot River population.

Eyed eggs are shipped to GLNFH from families created at CBNFH. Prior to the fall parr grading, an equal number of fish from each of the pools filled during the 1st fry tank move (typically 8 pools) are collected. These fish represent a population from all of the fry tanks, all of the lots, and as representative of all of the families as possible. They are moved into the hatchery building to be held until they are ready to spawn. Each fish is only utilized once for egg and milt production during its captivity at the hatchery. Only 4 year-old females (except the occasional rare 5 year-old that hasn't produced yet) are used for egg production when spawning. If a 3 year-old female becomes gravid, it is stripped and the eggs are discarded. The female is then held an additional year and used for production if she again becomes gravid. Each female is mated with 1 male, and the male may be either 3 or 4 year-olds. All fish that are used for production are released back into the Penobscot River below Veazie Dam after spawning activities have concluded.

Due to the lower than expected return of Penobscot adults to the Veazie trap, a portion of the domestic broodstock maintained at GLNFH was used to supplement fry stocking in the Penobscot River basin in 2005. GLNFH transferred domestic origin eyed eggs to CBNFH; the resulting fry were raised at CBNFH and were stocked into the East Branch of the Penobscot in 2006.

The domestic broodstock from the Penobscot River, held at GLNFH is typically not genotyped for broodstock tracking purposes. The parents of the domestic line, the sea-run Penobscot adults spawned at CBNFH, are genetically screened prior to spawning, so the screening of the domestics is not required for broodstock culling purposes. When production from the domestic line is needed for stocking into the Penobscot River, tissue samples are obtained from each parental spawning pair for future parentage analysis and tracking purposes for future returns.

APPENDIX 4. Dennys pedigree line letter to TAC

Memo

To: TAC Members
From: TAC Broodstock Planning Work Group
Date: June 5, 2006
Re: Establishment of a pedigree line for the Dennys River (2007 Parr Collection Year)

A large escapee event from Canadian Atlantic salmon aquaculture facilities in Passamaquoddy Bay was documented in 2005. Monitoring of the Dennys River identified eight potential aquaculture escapee adults, however the weir on the Dennys River was not in place and it was assumed that additional aquaculture escapees had migrated upstream to spawn. Redd surveys in the fall of 2005 were difficult due to high water, so surveys were conducted in the spring of 2006 to document potential spawning activity (Greg Mackey, ASC, pers. comm.). These surveys identified over 100 redds in Cathance Stream (a tributary to the Dennys River) and in the Dennys River mainstem. Genetic analyses are being currently conducted to determine the origin of the eight Atlantic salmon captured from the river. Attempts to sample pre-emergent fry from seven redds this spring did not result in the capture of any fry. Plans to sample young-of-the-year in the area near the redds to determine the origin of the spawning adults are underway, although these efforts may be confounded by the spring 2006 stocking of Craig Brook National Fish Hatchery (CBNFH) fry in the same stream reaches.

Visual evaluation of the captured adults indicated aquaculture origin, based on scale growth pattern analysis, fin condition, and body morphology (Greg Mackey, ASC, pers. comm.). Therefore, there is a high probability that the large number of observed redds were from additional escaped aquaculture adults.

Broodstock collections for the Dennys and other DPS populations maintained at CBNFH incorporate parr, which potentially include two age classes (age 0+ and age 1+). Resulting juveniles from these putative aquaculture redds could be incorporated into broodstock collections occurring in 2007 and 2008, with potential greatest impact to the 2007 collection. To reduce the potential of incorporation of aquaculture offspring into future broodstocks, collection methods could be adjusted: avoidance of areas where putative aquaculture redds were identified, increased numbers of parr collected, and increased stringency of genetic screening of the parr.

Although the above methods may be utilized, establishment of a pedigree line to ensure recovery of sufficient broodstock is necessary to ensure long-term maintenance of genetic diversity in the Dennys River broodstock. Similar pedigree lines have been established in the Pleasant River broodstock, due to low numbers of parr recovered. Currently, the CBNFH Broodstock Management Plan (USFWS, 2006) considers three criteria for the establishment of a pedigree line at CBNFH:

1. The number of broodstock for a particular population is low (less than collection target),
2. When there is a threat of few or no hatchery or wild spawned parr being recovered, or
3. If loss of family variation through general parr collection practices is projected to cause appreciable losses in local population diversity in the near future.

Due to the aquaculture escape event, we propose the establishment of a pedigree line for the 2007 collection year for the Dennys River broodstock (the Dennys 2007 pedigree line). Establishment of a pedigree line for the Dennys River broodstock would meet the above criteria under 1) the threat of few wild parr being recovered, or 3) due to loss of family variation in broodstock (due to overrepresentation of putative aquaculture offspring in parr collection). The implementation of the 2007 Dennys River is due to the aquaculture escape event, and implementation of additional pedigree lines in the Dennys River is not currently planned for future years.

The proposed pedigree line for the Dennys River would be established from fry resulting from the 2005 spawn year, which would have been represented the primary year class present in the 2007 parr collection. A subset of fry from the 2005 spawn year was retained at Green Lake National Fish Hatchery (GLNFH) for smolt production. Currently, fry are grouped at GLNFH into five tanks according to spawn date, and within each tank are representatives of each family created on that specific spawn date. Due to excess individuals being retained to account for any mortality during grow-out to smolt size, retention of individuals from each spawn date would not represent a significant decrease in smolt production.

Current parr broodstock collection target for the Dennys is 150 parr, however a greater number would be retained to establish the pedigree line. Retention of 60 individuals per tank would result in 300 parr retained, to be considered the 2007 parr broodstock collection. Increased total broodstock number for the pedigree line is important for multiple reasons: to allow for additional mortality to maturity, to account for potential decreases in future broodstock (the 2008 parr broodstock collection) due to the abundance of aquaculture parent origin juveniles, and to maximize the probability of retaining representatives of each family created during the 2005 spawn.

The 300 Dennys River fry will be transferred from GLNFH to CBNFH this spring (2006). PIT tags would be inserted at appropriate size, and tissue samples would be obtained following the Broodstock Management Plan. Genetic characterization of the 2007 Dennys pedigree line would follow standard protocol. If necessary, reduction in broodstock number can be achieved following genetic determination of parentage and equalization of familial contribution (Broodstock Management Plan), and stocking of excess at pre-smolt stage. Fry production and stocking would continue as standard practice. Determination if the 2007 parr collection from the Dennys River will occur would be made following sampling in fall 2006 to assess location and abundance of putative aquaculture fry from redds observed in the Dennys River mainstem and

Cathance Stream. Parr broodstock collection for 2006 will occur as scheduled, with increased collection targets due to low collection numbers in 2005, and to account for screening of putative aquaculture-origin individuals.

APPENDIX 5. Adult stocking letter to TAC

Memo

To: TAC Members

From: TAC Broodstock Planning Work Group

Date: June 5, 2006

Re: Stocking adult broodstock from Craig Brook National Fish Hatchery

Current broodstock management of Atlantic salmon at Craig Brook National Fish Hatchery relies on the maintenance of salmon at various life stages. Fry are the primary life stage stocked; parr and smolts are also stocked in some instances as the by-product of grading for stocking larger life stages (i.e. as part of a pedigree line). However, stocking adult hatchery Atlantic salmon has generally not been used as a restoration/recovery strategy.

Past disposition of adult broodstock resulted in the stocking of spent adults into non-spawning habitats (estuaries), after spawning in early winter. Justification for stocking non-reproductively viable adults into non-spawning habitat included concerns about unequal contribution of individual hatchery adults to the population (with additional spawning opportunities), competition caused by stocking multiple lifestages in the same areas, the avoidance of super-imposition of redds by hatchery adults onto wild redds, confounding issues when interpreting redd count data, and competition between hatchery-spawned and wild-spawned juveniles in rearing habitat.

Many concerns about stocking of adults have been addressed through previous monitoring and evaluation efforts, or could be addressed by implementation of a structured adult stocking program design. Genetic evaluation of the hatchery family contribution in parr broodstock collection, records of individual spawning histories, and clear spawning protocols reduced the concern for unequal reproductive contribution to production. Consistently low spawning escapement has decreased the probability of disturbance of natural redds and reduced the potential for competition with naturally-produced juveniles.

Following current broodstock management protocols, both reproductively viable and post-spawn adults are often available for stocking. Due to changes in spawning protocols, increased numbers of adults maintained, and improvements to the hatchery (resulting in increased fecundity and decreased early mortality), retention of adults for more than two spawning cycles is not always required to meet production goals. As additional broodstock years become reproductively mature, the older year classes are available for stocking pre-spawn if not needed for genetic or production goals.

Therefore, as a by-product of current hatchery management, pre-spawn adults are available for stocking purposes.

Stocking reproductively-viable adults into spawning habitat prior to spawning is consistent with current recovery/restoration management goals for Atlantic salmon in Maine. In-river spawning by hatchery adults would incorporate an additional component of natural selection into the hatchery program, and may prove to be an effective management strategy for restoration and recovery. Stocking of reproductively viable Atlantic salmon adults in or near spawning habitat could be accomplished in such a manner to allow for monitoring and evaluation of stocking efforts, while potentially minimizing negative impacts where wild adults may be spawning.

The TAC Broodstock Management Committee strongly supports stocking of pre-spawn adult hatchery broodstock as an important component and management strategy in the recovery and restoration of Atlantic salmon in Maine. Consistent with current early life-stage stocking into river of broodstock origin, adult broodstock would be stocked back into river of broodstock origin. However, as with stocking hatchery Atlantic salmon (at any life stage) into a river system(s) other than that of broodstock origin under experimental conditions, careful evaluation on a case by case basis would be needed.

The Broodstock Management Committee encourages TAC to support the stocking of pre-spawn adults. To facilitate stocking of adult Atlantic salmon, we encourage:

1) identification of stocking sites for pre-spawn adults
2) development of appropriate monitoring and evaluation programs, and
3) approval of adult stocking permits

well in advance of stocking reproductively viable adult to facilitate the implementation of this program.

Determination of the number of reproductively viable adults is typically not known more than one month prior to ahead of spawning due to manual sorting and maturity assessment in the hatchery. Therefore, there is a short time-frame prior to hatchery spawning for stocking of pre-spawn adults to occur, and pre-identification of appropriate stocking locations is critical to management. Ideally, pre-spawn adult stocking sites should contain adequate spawning habitat, be isolated from areas known to have frequent wild spawning, and should not be stocked with other life history stages. Monitoring for pre-spawn adult stocking should consist of at least one electrofishing site to assess reproductive success, and ideally may entail monitoring of stage-specific abundance (i.e. fry emergence to large parr or smolt stages). Inclusion of a monitoring program is considered critical to the evaluation of an adult stocking program and its contribution to recovery and restoration efforts.

Attachment 1. Trait monitoring summary 2006

Maine Atlantic Salmon Trait Monitoring

Michael T. Kinnison
and
Nathan F. Wilke

1865 THE UNIVERSITY OF MAINE

Objectives of Initial Trait Monitoring Project

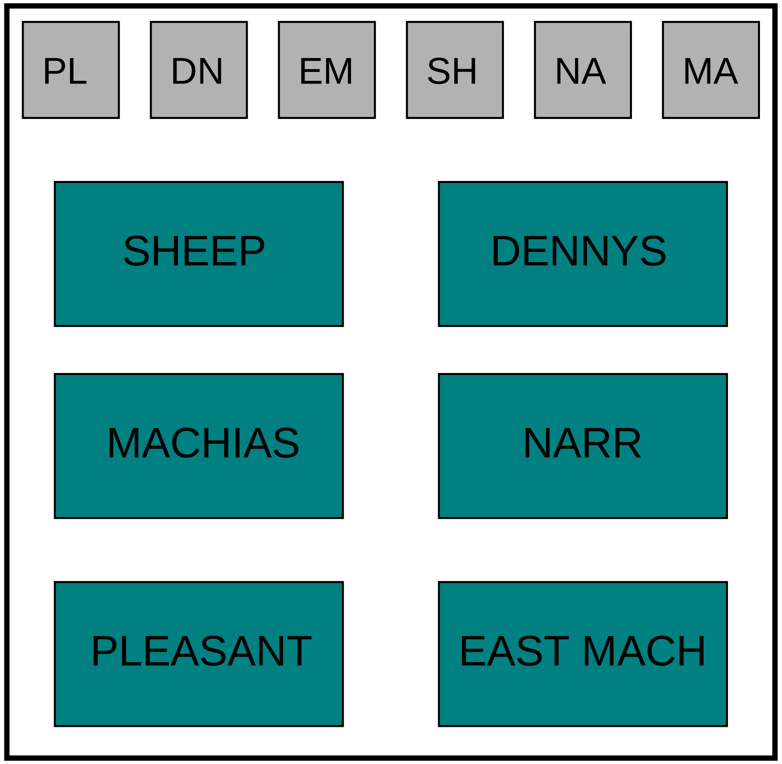
1. Assessment of DPS stock divergence

2. Assessment of loss of trait variation

3. Establishment of protocols for long-term monitoring

Salmon rearing and trait measurement

Craig Brook Hatchery



PL DN EM SH NA MA

SHEEP DENNYS

MACHIAS NARR

PLEASANT EAST MACH

⬜ = Incubation rooms

🟩 = Brood modules

Morphology

Numbers of Fish With Digital Photos for Morphology									
Spawn year	**Sex**	**Machias**	**Narrag.**	**Sheep.**	**E. Machias**	**Dennys**	**Pleasant**	**Penobscot**	**Totals**
2002	Male	100	147	95	93	68	19	109	631
	Female	108	148	90	92	67	19	132	656
	Total	208	295	185	185	135	38	241	1287
2003	Male	70	43	55	59	53	28	162	470
	Female	73	47	62	69	54	27	185	517
	Total	143	90	117	128	107	55	347	987
2004	Male	No pics	48	No pics	51	32	No pics	93	224
	Female	No pics	46	No pics	34	34	No pics	57	171
	G	No pics	0	No pics	0	0	No pics	16	16
	Total	0	94	0	85	66	0	166	411
2005	Male	No pics	63	38	No pics	No pics	81	No pics	182
	Female	No pics	92	107	No pics	No pics	99	No pics	298
	Immature	No pics	80	93	No pics	No pics	50	No pics	223
	Unidentified	No pics	182	79	No pics	No pics	10	No pics	271
	Total	0	417	317	0	0	240	0	974
Grand totals		**702**	**1792**	**1238**	**796**	**616**	**666**	**1508**	**3659**

Adult Reproductive Traits

Number of families sampled for reproductive data (egg size, egg number, total ovarian mass)								
Spawning year	Machias	Narraguagus	Sheepscot	East Machias	Dennys	Pleasant	Penobscot	Total
2002	83	71	88	90	45	43	128	548
2003	64	46	56	70	52	6	62	356
2004	NA	57	NA	47	30	NA	86	220
2005	NA	63	69	NA	NA	99	NA	231
Totals	147	237	213	207	127	148	276	1355

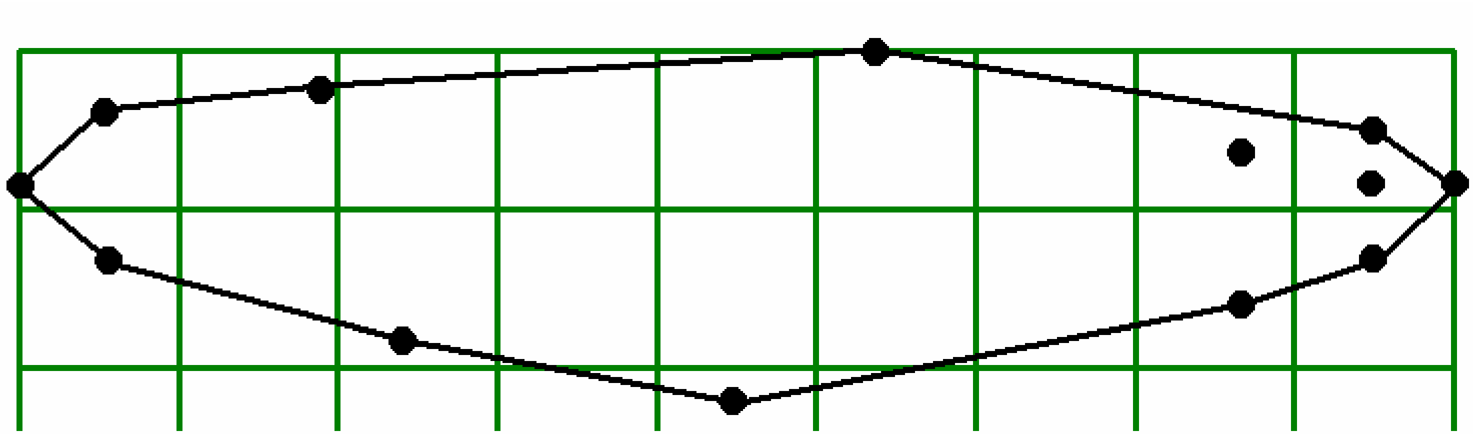
Juvenile Development

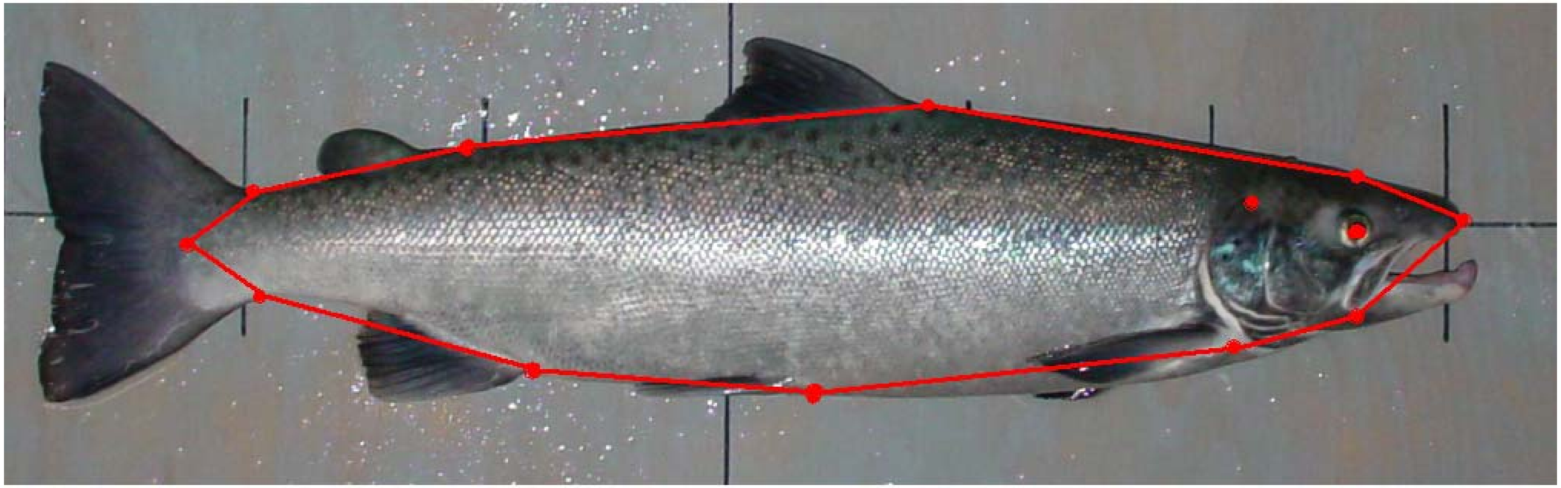
Number of families sampled for larval development and/or hatch									
Spawning year	Machias	Narrag.	Sheep.	E. Machias	Dennys	Pleasant	Penob.	Totals	Data collected
2002	26	49	19	29	30	30	26	209	Develop. and hatch
2003	30	38	30	29	30	27	18	202	Develop. and hatch
2004	NA	NA	NA	NA	NA	NA	NA	NA	NA
2005	NA	62	47	NA	NA	92	NA	201	hatch
Totals	56	149	96	58	60	149	44	612	

Morphology – Geometric Morphometrics

Consensus – “Average”

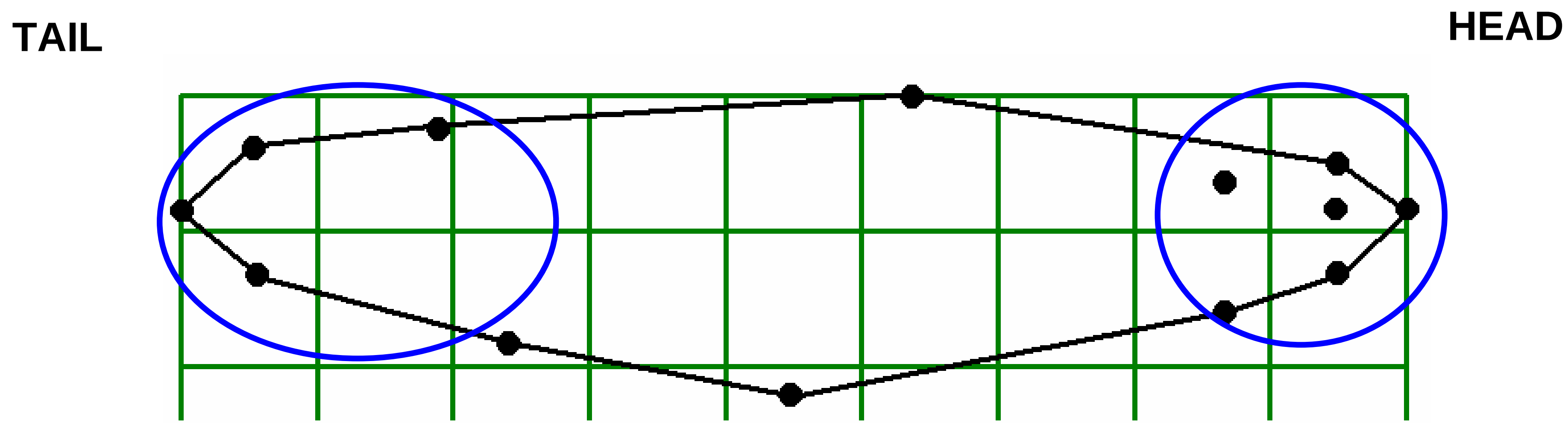
TAIL **HEAD**

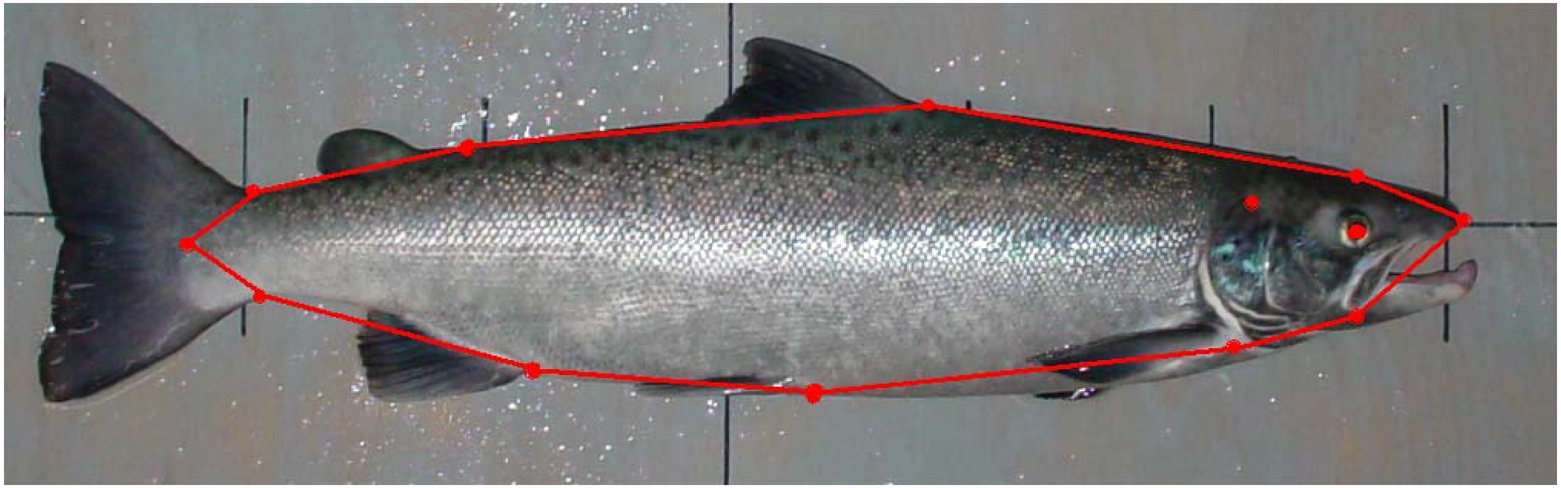




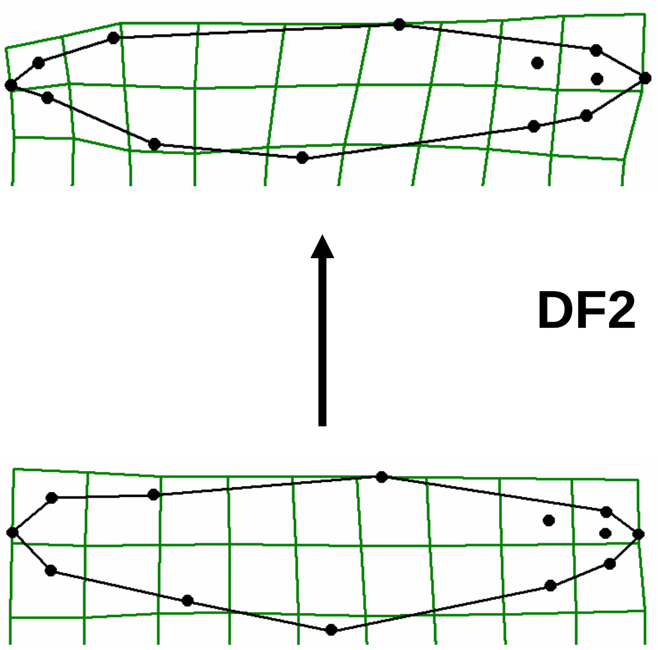
Morphology – Geometric Morphometrics

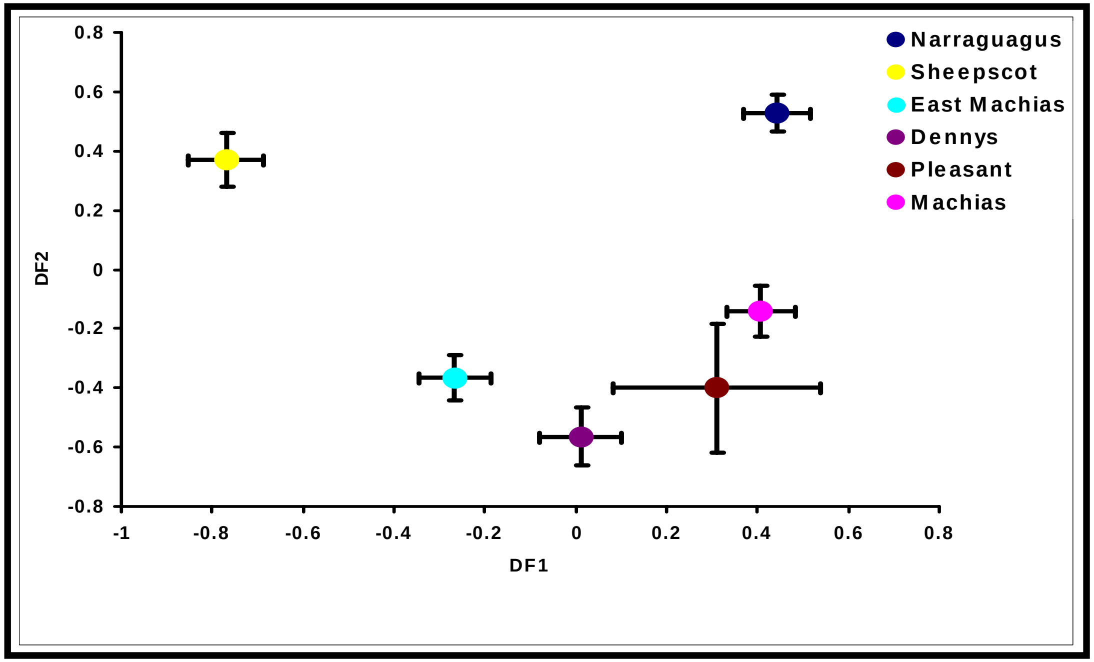
Consensus – "Average"

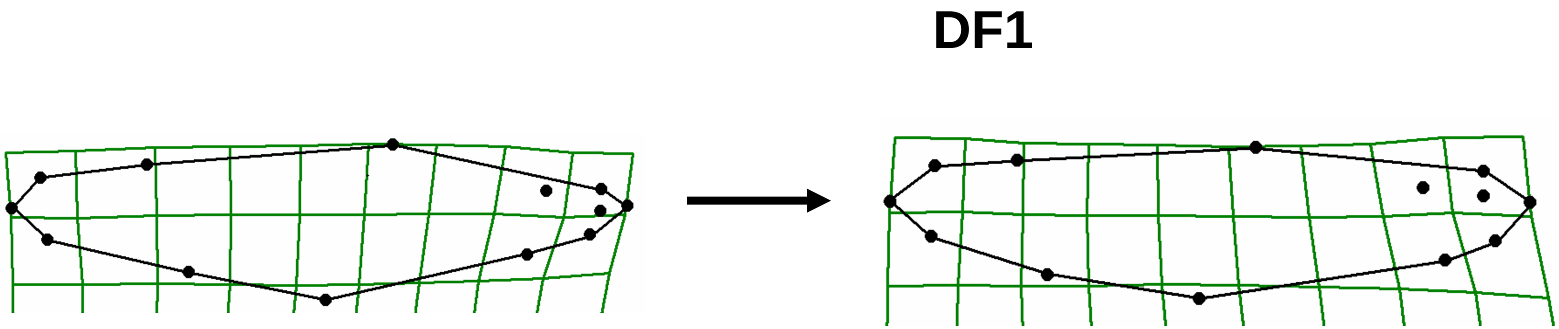




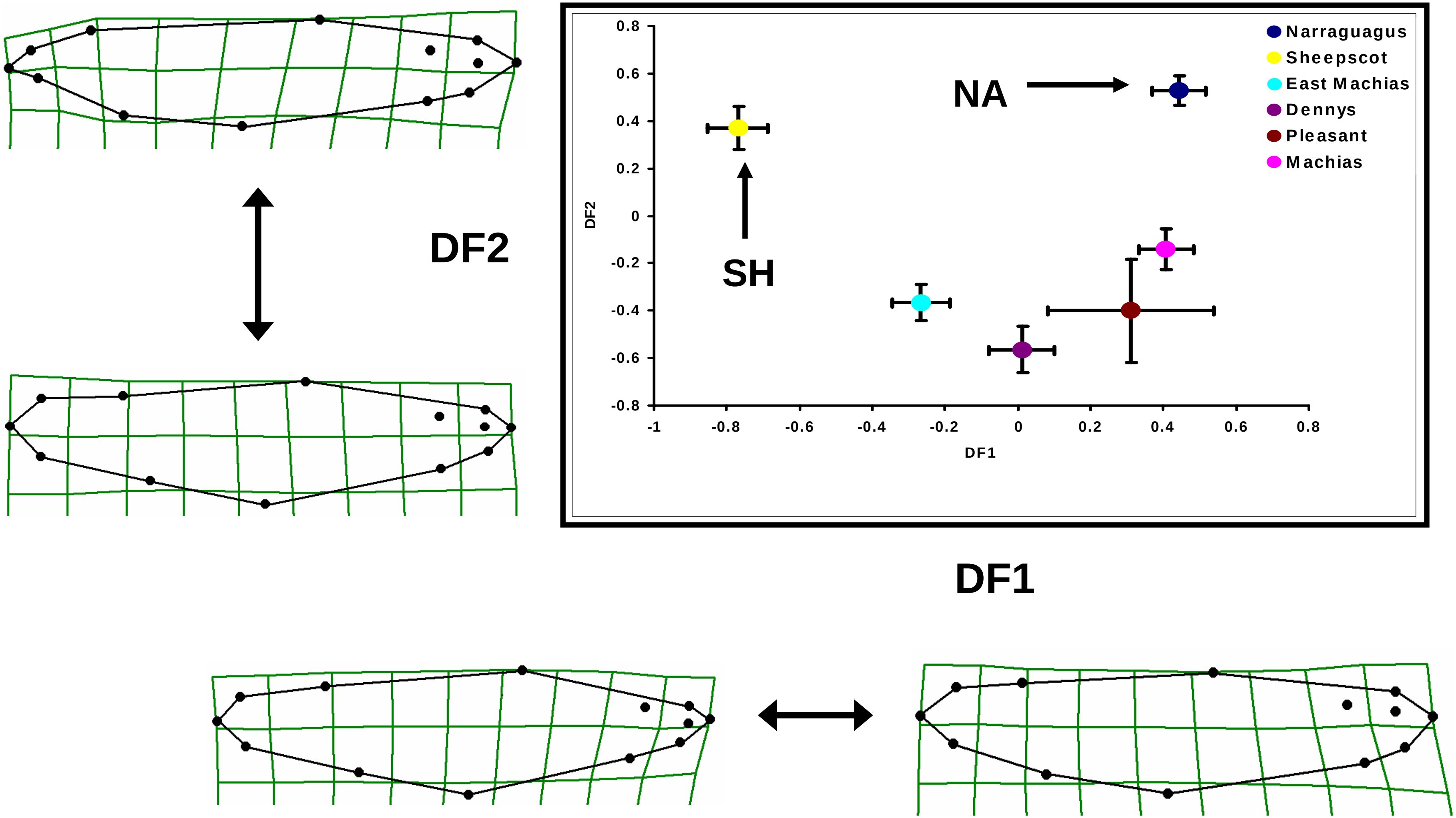
Morphology – Evidence of Divergence



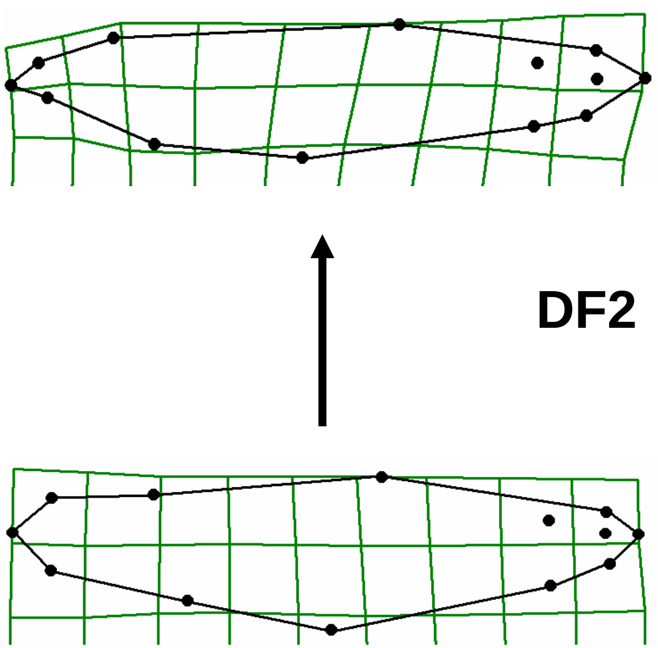


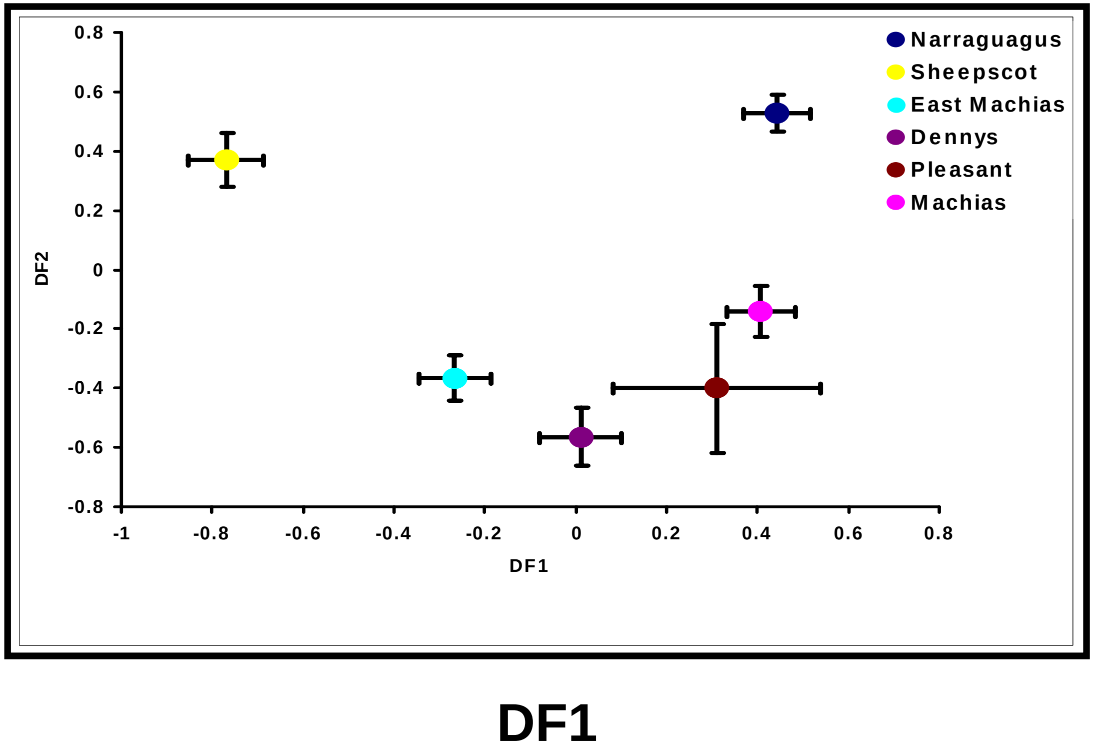


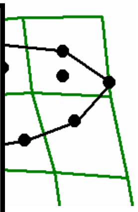
Morphology – Evidence of Divergence



Morphology – Evidence of Divergence



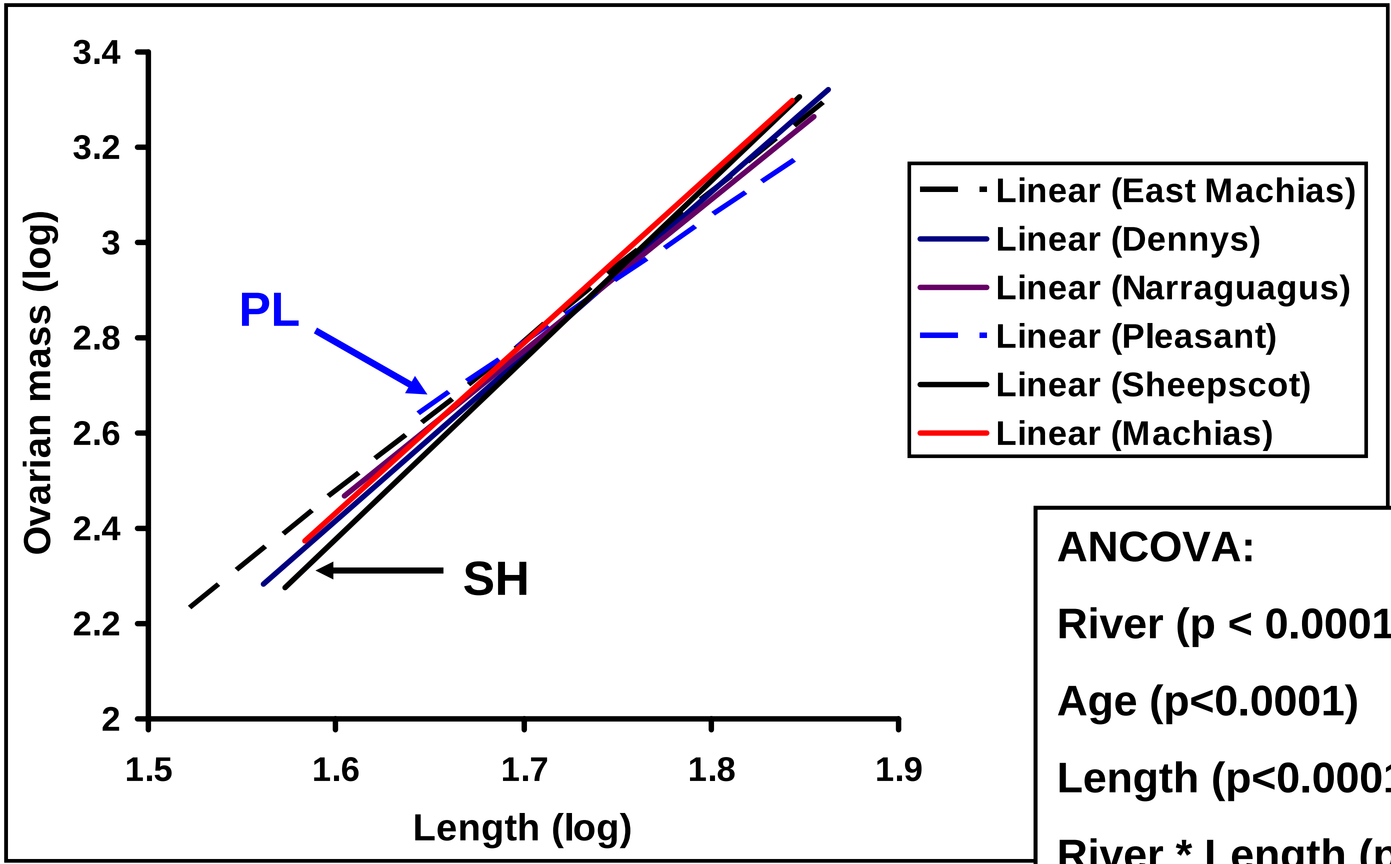




-- This suggests that body shape varies among rivers.

-- Such variation may reflect differences in hydrodynamic conditions of natural habitats.

Reproductive Data – Evidence of divergence



ANCOVA:

River ($p < 0.0001$)

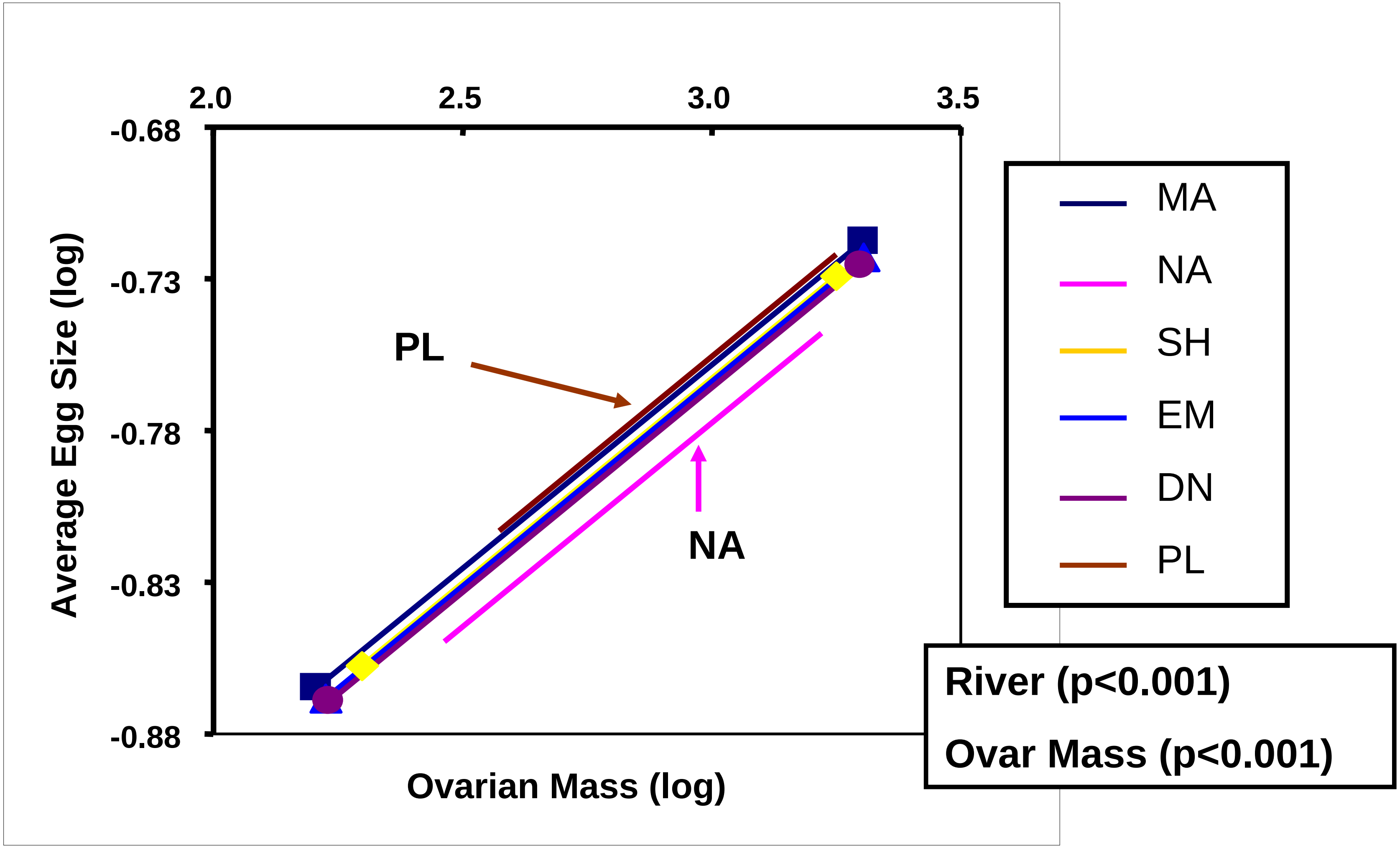
Age ($p<0.0001$)

Length ($p<0.0001$)

River * Length ($p<0.001$)

-- Populations differ in relative investment in reproductive versus body tissue.

Reproductive Data – Evidence of divergence



River ($p<0.001$)

Ovar Mass ($p<0.001$)

-- Some populations in relative egg number vs egg size investment.

-- Example: 5.16% difference between the Pleasant and Narraguagus.

Future Applications of Trait Monitoring Data

1. Assessment of domestication selection

2. Optimization of hatchery products

3. Models for evaluating genetic management outcomes

Recommendations for Near Future

1. Increase set of monitored traits (e.g., age at maturity)

2. Install digital thermographs in each incubation room

3. Increase monitoring of trait variation in Penobscot

4. Develop long-term monitoring with further FWS support

Acknowledgements

Kinnison Lab
Field crew

Tom King
and staff of
CBNFH Staff

John Kocik

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Attachment 2. Trait monitoring 4.17.2006

Maine Atlantic Salmon Trait Monitoring

Michael T. Kinnison and Nathan F. Wilke
University of Maine, Orono

Beginning in Fall of 2002, the Kinnison laboratory at the University of Maine began a program of systematic trait variation assessment at the Craig Brook National Fish Hatchery (CBNFH). The objectives of this work were 1) to assess whether the stocks of salmon comprising the Gulf of Maine DPS showed evidence of adaptive trait differences, 2) to assess whether there were signs of loss of potentially adaptive trait variation in any of the hatchery supplemented stocks, and 3) to establish protocols for continued trait variation monitoring at CBNFH as part of best hatchery practices.

1) Assessment of stock divergence: Based upon data collected for salmon spawned in 2002 and 2003 evidence suggests that the DPS stocks overlap considerably in morphological, reproductive and developmental trait variation, but some do indeed differ in heritable trait variation associated with their body shape (morphology) and reproductive investment (relative ovarian investment and egg size/number trade off). We are currently in the process of completing our assessment of variation associated with juvenile development traits (hatch timing, alevin development).

2) Assessment of loss of variation: Initial analyses of morphological and reproductive traits for salmon spawned in 2002 suggested a correlation between coefficients of variation for phenotypic traits and relative microsatellite diversity. A subsequent analysis of fish spawned in 2003 did not support that same trend. We suspect these analyses were confounded by a shift in microsatellite loci used in the hatchery genetics program. We hope to revisit this issue in the near future.

3) Establishment of protocols: We believe that our approaches for measuring morphology and reproductive traits are effective and efficient. Indeed, some of these approaches could potentially be implemented as part of normal hatchery operations. Our approaches for measuring hatch time and developmental were complicated by previously uncharacterized variation in water temperatures among the incubation bays of the different DPS populations. We have attempted to deal with that variation in some of our analyses but suggest that dedicated digital thermographs be installed in each incubation and rearing bay to ensure more accurate characterization of development and rearing conditions in the future. Furthermore, techniques for assessing development would be made much more efficient by developing approaches that allow for full-term monitoring at CBNFH (rather than having to move eggs or larvae off-site). At present we are willing to incorporate our data into current salmon management databases, though we request propriety in analyses and publication.

Future Directions and Recommendations

We believe it is essential that trait variation continue to be monitored at CBNFH as part of a comprehensive broodstock management and recovery program. Ultimately, the performance of populations in the wild is a function of their suitability for those habitats as determined by variation in their traits. We foresee three major future roles for trait monitoring related data in management of Maine DPS salmon.

1. *Assessment of domestication selection:* Domestication selection is currently considered to be one of the greatest potential threats to supplemented populations and is also thought to be pervasive in hatchery programs despite current best efforts. We suggest that one of the only ways to minimize the effect of such selection is to actively monitor for it.
2. *Optimization of hatchery products:* In addition to domestication selection, hatcheries may influence the traits of the fish they produce through environmental influences on rearing. Egg size, egg number, fry size, fry developmental stage and other important traits that might influence the number or quality of hatchery produced fish can be influenced by hatchery practices. By combining trait monitoring with studies of the performance of hatchery fish in the wild it may be possible to produce a better restoration product.
3. *Models for evaluating genetic broodstock management outcomes:* By quantifying population trait variation, as well as its underlying genetic basis we could obtain data that can be used to model the outcomes of various broodstock management practices. For example, by linking trait data to pedigree data we can estimate the heritability of various traits. Such heritability estimate allow us to approximate the influence of domestication selection, or could be used to estimate likely changes in the traits of DPS salmon should some degree of population mixing be desired.

Future monitoring would benefit from considering a broader set of traits. Fortunately, database approaches at CBNFH may greatly facilitate theses data needs. For example, age at maturity could be obtained by a survey of the maturity status of all fish in the hatchery at spawning time. Database approaches could in-turn link the year of maturation with the age of fish based on juvenile scale pattern analysis or pedigree data.

Greater investment should be made to characterize trait variation in the Penobscot River population. Possible future listing of this population should be informed by knowledge of trait variation. Likewise, there is already some concern that current brood collection and offspring production approaches might currently contribute to unwanted selection (e.g., early brood collection from the wild, grading of juveniles, and accelerated rearing).

Finally, trait monitoring will likely need to become more of a shared responsibility between the University of Maine and the U.S. Fish and Wildlife service. The University of Maine does not presently have dedicated financial or staff resources to indefinitely devote to this monitoring program. Thus, future trait monitoring may be difficult, if not impossible, without some outside financial support or even more direct participation by CBNFH staff.

Table 1. Photos of adults taken at Craig Brook National Fish Hatchery 2002-2005. In 2002 and 2003 all fish were photographed during normal spawning operations. In 2004 and 2005 fish were photographed 1-2 weeks preceding actual spawning. Unidentified individuals represent photos of mature fish that were not sexed at the time photos (data will be available from Craig Brook Spawning Database).

Numbers of Fish With Digital Photos for Morphology

Spawn year	Sex	Machias	Narraguagus	Sheepscot	East Machias	Dennys	Pleasant	Penobscot	Totals
2002	**Male**	100	147	95	93	68	19	109	631
	Female	108	148	90	92	67	19	132	656
	Total	208	295	185	185	135	38	241	1287
2003	**Male**	70	43	55	59	53	28	162	470
	Female	73	47	62	69	54	27	185	517
	Total	143	90	117	128	107	55	347	987
2004	**Male**	No pics	48	No pics	51	32	No pics	93	224
	Female	No pics	46	No pics	34	34	No pics	57	171
	G	No pics	0	No pics	0	0	No pics	16	16
	Total	0	94	0	85	66	0	166	411
2005	**Male**	No pics	63	38	No pics	No pics	81	No pics	182
	Female	No pics	92	107	No pics	No pics	99	No pics	298
	Immature	No pics	80	93	No pics	No pics	50	No pics	223
	Unidentified	No pics	182	79	No pics	No pics	10	No pics	271
	Total	0	417	317	0	0	240	0	974
Grand totals		**702**	**1792**	**1238**	**796**	**616**	**666**	**1508**	**3659**

Table 2. Reproductive sampling at Craig Brook National Fish Hatchery 2002-2005. Data consists of mean egg size and estimated number of eggs per family collected at eyed-stage (shocking). These estimates include actual dead eggs (DPS) or the estimated number of dead eggs (Penobscot) in the count of egg size.

Number of families sampled for reproductive data (egg size, egg number, total ovarian mass)								
Spawning year	Machias	Narraguagus	Sheepscot	East Machias	Dennys	Pleasant	Penobscot	Total
2002	83	71	88	90	45	43	128	548
2003	64	46	56	70	52	6	62	356
2004	NA	57	NA	47	30	NA	86	220
2005	NA	63	69	NA	NA	99	NA	231
Totals	147	237	213	207	127	148	276	1355

Table 3. Larval development sampling at Craig Brook National Fish Hatchery 2002-2005. Data for 2002 and for 2003 include records of number of hatched alevins collected at 2-4 times per day during hatch period. Data for 2005 included percent hatched by family at set number of degree days. Development data consists of wet and dry weight of yolk and body tissue at three time points (measured in degree days) following hatching and preceding full yolk absorption.

Number of families sampled for larval development and/or hatch									
Spawning year	Machias	Narraguagus	Sheepscot	East Machias	Dennys	Pleasant	Penobscot	Totals	Data collected
2002	26	49	19	29	30	30	26	209	development and hatch
2003	30	38	30	29	30	27	18	202	development and hatch
2004	NA	NA	NA	NA	NA	NA	NA	NA	NA
2005	NA	62	47	NA	NA	92	NA	201	hatch
Totals	56	149	96	58	60	149	44	612	

Attachment 3. Penobscot Sea-Run Broodstock Information and Questions

Richard Dill, Maine Atlantic Salmon Commission

At the fall 2005 TAC meeting, it was presented that egg take per Penobscot sea-run female was above 9,000 eggs from 2002-2004. At the time, there was no reason to assume that the 2005 Penobscot sea run broodstock would be any different.

The question: At the higher fecundity rate, do we need a broodstock goal of 400 females to achieve 3 million eggs to support the hatchery smolt and fry programs?

* As it turns out, the average number of eggs per female salmon for 2005 was 7,613.

- Brood fish collection target for the Penobscot River is **currently** 600 Atlantic salmon**,** distributed in the following size groups:

  Multi-sea-winter (**large**; Fork length >63 cm) females N = 400
  Multi-sea-winter (**large**; Fork length >63 cm) males N = 160
  Grilse (**small**; Fork length <63 cm) N= 40

- Size group classifications are established from historical fork length and scale sample data for Atlantic salmon from Maine rivers. Within each size group, we do **not** select for any characteristics, such as size, weight, color, etc.

- The annual egg take required to support the Penobscot River fry and smolt stocking programs is:

Program	Target Numbers	Green Eggs needed (90% EU)
Fry	1,500,000	1,800,000
Smolts	600,000	1,200,000
	Total:	3,000,000

- The broodstock goal for females was based on an average fecundity of 7,500 eggs/female (7,500 eggs per female X 400 females = 3,000,000 eggs).

- Recently, fecundity has increased to an average of over 9,000 eggs/female (9,000 eggs per female X 400 females = 3,600,000 eggs)

- Should the number of females taken as broodstock be reduced to reflect the recent increase in fecundity?

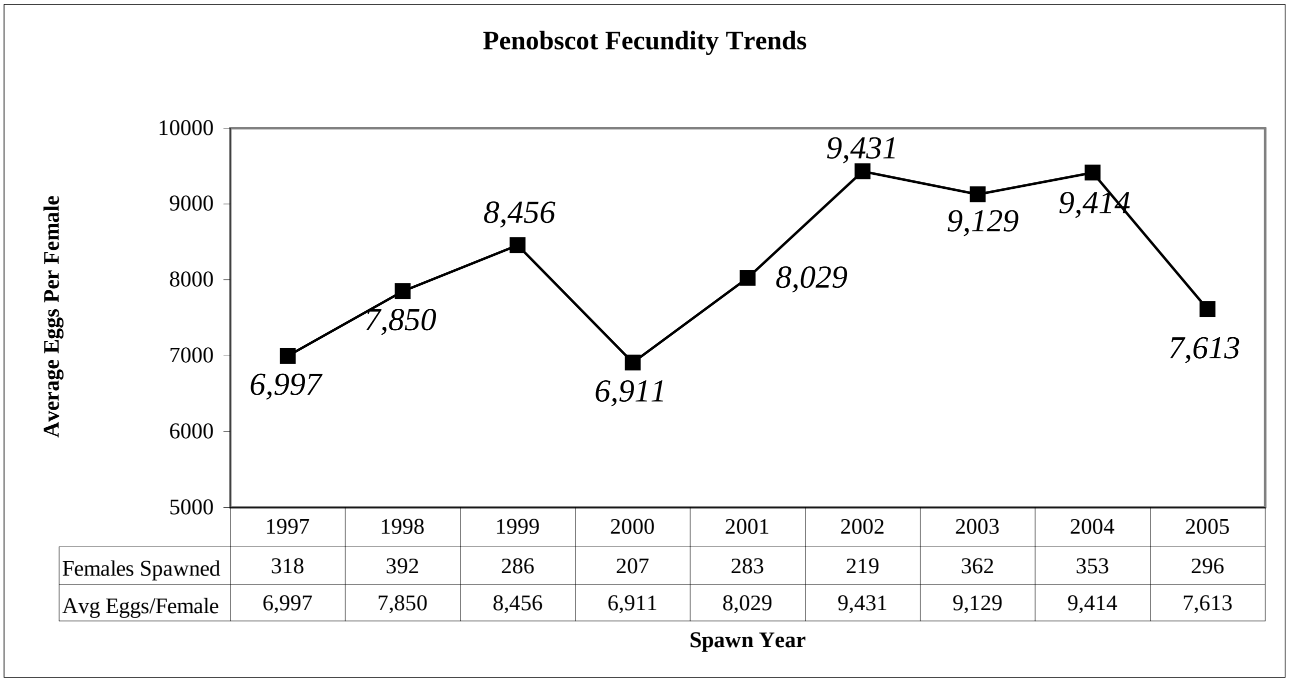
- 3,000,000 eggs / 9,000 eggs per female = 333.3 females; Call it 350 females to account for mortality, non-ripening fish, etc.

- Since 1997, we have only captured 350 or more females three times.

Year	Sex	# Females Captured	# Females Spawned	Avg Eggs/Female	Comments
1997	F	320	318	6,997	2000 and earlier, no pit tags, therefore no way to fix errors in gender calls at the trap.
1998	F	**389**	392	7,850	
1999	F	294	286	8,456	
2000	F	196	207	6,911	
2001	F	299	283	8,029	
2002	F	226	219	9,431	
2003	F	**368**	362	9,129	
2004	F	**361**	353	9,414	
2005	F	301	296	**7613**	

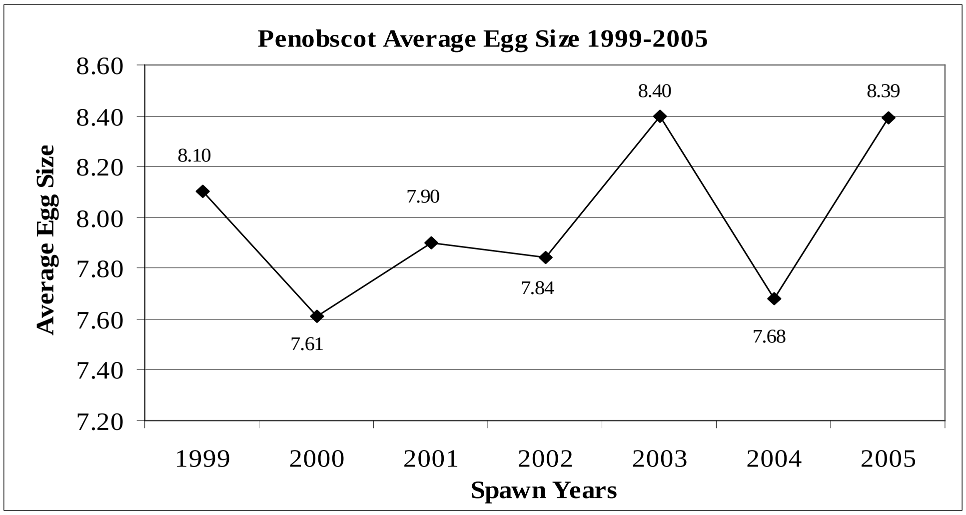
- If we were to reduce the number of females to 350, and in some years had a "normal" fecundity of 7,500 eggs (2,625,000 eggs), we have set ourselves up to fail to meet egg needs for the smolt and fry hatchery programs (3 million) even though we met the female brood goal of 350 fish.

- <u>Therefore,</u> we should keep 400 females as the broodstock goal. If fecundity is higher than normal, then CBNFH max capacity is 2 million Penobscot fry, ~ 500,000 more than ASC annual request. The ASC currently stocks less than 10% of total number of fry needed to saturate all of the available habitat in the Penobscot drainage at 100 fry per unit.



More questions:

- Should we be taking more males in order to bring the male to female sex ratio closer to 1:1? Meredith or Greg, you will have to explain the genetic side of this one?
- Why is the fecundity recently higher than the accepted norm of 7,500 eggs per female?
- Are eggs getting smaller in size?



- Are females getting bigger in size?

 # broodstock by year, avg fork length, and eggs per female

Year	DISPOSITION	SEX	Total # broodstock	# Repeat Spawners	# salmon w/fork length	Avg Fork Length - all female brood fish	Avg Fork Length - not including Repeat Spawners	Eggs per Female
1990	CBNFH	F	330	1	29	75.10	74.68	na
1991	CBNFH	F	335	6	86	73.13	72.33	na
1992	CBNFH	F	378	2	334	72.35	72.24	na
1993	CBNFH	F	299	2	274	74.17	74.11	na
1994	CBNFH	F	170	0	77	73.53	73.53	na
1995	CBNFH	F	393	3	393	72.39	72.27	na
1996	CBNFH	F	376	5	376	72.70	72.49	na
1997	CBNFH	F	320	5	319	73.04	72.86	6997
1998	CBNFH	F	389	7	389	72.80	72.62	7850
1999	CBNFH	F	294	5	294	73.23	73.00	8456
2000	CBNFH	F	196	7	196	72.96	72.37	6911
2001	CBNFH	F	299	1	299	73.05	72.99	8029
2002	CBNFH	F	226	11	226	74.48	73.98	9431
2003	CBNFH	F	368	2	368	72.41	72.34	9129
2004	CBNFH	F	361	11	361	73.46	73.12	9414
2005	CBNFH	F	475	8	475	71.67	71.42	7613

- Have the methods for measuring egg size and estimating eggs per female changed? Will have to ask Tom / Denise.