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How do Age-of-release and Genetic Matching of Parents impact Return Rates of Connecticut River Atlantic Salmon?

By

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This paper was prepared
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for eight credits.

This paper is dedicated to my family.

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ABSTRACT

The Connecticut River Atlantic Salmon Restoration Program (CRASRP) has stocked the Connecticut River with Atlantic salmon (*Salmo salar*) fry and smolts since 1967. In 1996, the CRASRP began using microsatellite DNA loci to genetically match unrelated fish for parental pairs in order to preserve maximum genetic variation in the population and increase survival. This study compared historic return rates of fry and smolts to see which life-stage produced higher return rates. The cost of fry and smolt returns was calculated to determine cost-effectiveness. To see if genetic breeding improved survival, return rates and percent eye-up were compared before and after 1996. Eye-up was also correlated with parental relatedness among family groups to see if more distantly related parents produced better embryo survival. Smolt stockings yielded significantly greater return rates than fry stockings and smolts were more economically efficient. Return rates of smolts and fry were significantly lower after 1996. Eye-up was slightly better after 1996, but more distantly related parents did not result in better eye-up. In order to obtain higher return rates, hatcheries should put more effort into stocking smolts. The analysis of the genetic breeding program was inconclusive. Low return rates likely result from poor marine survival.

INTRODUCTION

Anadromous fishes, like Atlantic salmon (*Salmo salar*) hatch in rivers, mature at sea, and then return to the river to spawn (Appendix A). In order to complete their life cycle, they must have access to riverine spawning grounds. Dams impede fish passage despite devices like fish ladders, which are imperfect and are not installed at all dams. Atlantic salmon were extirpated from the Connecticut River in 1798 following construction of the first dam across the main stem of the river (CRASC, 1998). Prior to extirpation, Atlantic salmon runs numbered in the thousands. The current Connecticut River Atlantic Salmon Restoration Program (CRASRP) was started in 1967 to restore Atlantic salmon to the Connecticut River (Appendix B). Members of the CRASRP include the four basin states, Connecticut, Massachusetts, New Hampshire, and Vermont, and three federal agencies: the National Marine Fisheries Service (NMFS), U.S. Fish and Wildlife Service (USFWS), and U.S. Forest Service (USFS). The goal of the program is to create a recreational fishery and it has successfully established an annual run numbering in the low hundreds (CRASC, 1998).

Juvenile stocking is commonly used in Atlantic salmon restoration programs (Aprahamian et al., 2003; Jonsson & Jonsson, 2009). Smolts were stocked as part of the CRASRP from 1967 to 1994, when smolt stocking was stopped due to budget constraints, although in 2000 the Dwight D. Eisenhower National Fish Hatchery, USFWS, North Chittenden, Vermont again began to stock smolts (CRASC 1998). Fry stocking was initiated in 1987 and is currently the main practice of the CRASRP. Fry stocking was started in order to add stream-reared smolts to the program since stream-reared smolts, or smolts produced from fry stocking, were predicted to yield higher return rates than hatchery-reared smolts (Jokikokko et al., 2006). Jokikokko et al. (2006) showed that post-smolt survival was higher for salmon stocked as parr than for those stocked as smolts. Also, the post-smolt survival of European wild smolts has been shown to be greater than that of hatchery-reared smolts (Jonsson et al., 2003; Jutila et al., 2003; Kallio-Nyberg et al., 2004). The most likely mechanism behind the decrease in survival experienced by hatchery-reared fish is domestication selection (Olla et al., 1998; Jonsson & Jonsson, 2006).

Domestication selection is evolutionary selection for traits that improve survival and reproduction in the hatchery environment (Waples, 1999). Hatchery-reared fish experience phenotypic and genotypic changes that often maladapt them for survival in the wild (Jonsson & Jonsson, 2006). Phenotypic changes critical to survival upon release include inhibited predator-avoidance and feeding behavior (Olla et al., 1998). One advantage of fry stocking is that hatchery-reared fry should experience less domestication selection than do hatchery-reared smolts because the time that fry spend in the hatchery is less than that of smolts (Fleming et al., 1994). Thus, adults produced from fry stocking resided longer in the natural environment under the pressures of natural selection and should have higher fitness. However, there are also advantages to smolt stocking. When smolts are stocked, they migrate out of the river almost immediately and avoid the high density-dependent mortality (Whalen & Labar, 1994; McMenemy, 1995) and predation risk (Henderson & Letcher, 2003) experienced by fry and parr in the river.

Due to the inherent tradeoff between fitness and survival, choosing which life-stage to stock depends on the goal of the program (Aprahamian et al., 2003). Smolt stockings should produce higher return rates, but fry stockings should produce higher quality, or more fit, adults. Cost is also an important consideration. Smolts, because they are reared longer in the hatchery, are more expensive to raise than fry (Ken Sprankle, USFWS, personal communication). However, the higher return rate of smolts could compensate for their initial greater expense. In order to assess which life-stage is more efficient to stock, this study compared the return rates from fry and smolt stockings and calculated the cost per return of each life-stage.

Another goal of Atlantic salmon restoration programs is the preservation of genetic variation in the population in order to avoid founder effects and population decline. Inbreeding has been shown to decrease genetic variation within populations and thus make them more susceptible to decline and extinction (Frankham, 1995; Frankham, 2005). Since their extirpation from the Connecticut River, there has been essentially no natural Atlantic salmon reproduction in the river. CRASRP staff spawn broodstock in hatcheries. In 1996, a genetic breeding program was incorporated into the CRASRP in order to prevent inbreeding and preserve genetic variation. Microsatellite loci are used to determine the relatedness of broodstock and match parents that are the most genetically different.

Since lack of genetic variation could lead to population decline and extinction, breeding for maximum genetic variation may help improve survival. However, Neiman and Linksvayer (2006) reviewed evidence from several insect species that inbreeding could actually increase adaptive potential by preserving adaptive gene complexes. Such complexes are groups of two or more genes that work together to produce a phenotype that affects fitness. Inbreeding tends to increase a population’s homozygosity, which in turn stabilizes gene complexes by decreasing the chance of introducing a new allele. The purpose of breeding for maximum genetic variation (outbreeding) is to increase heterozygosity and would therefore likely disrupt any adaptive gene complexes in the salmon genome.

Furthermore, in species that often exhibit local adaptation, like Atlantic salmon, outbreeding could disrupt the evolution of locally-adapted traits (Dionne et al., 2009). Atlantic salmon often home precisely to their natal streams, which can create strains of salmon that are genetically distinct from each other even within the same river (Taylor, 1991; Dionne et al., 2009). Distinct genetic profiles at the tributary scale are indicators of local adaptation to specific streams.

Breeding practices that prevent the development of local adaptation could actually make it more difficult for salmon to adapt and survive. This study examined the success of the genetic breeding program since its implementation in 1996. Two measures of success were used: embryo survival and sea-run return rates.

The purpose of this study was to evaluate the effectiveness of two key components of the CRASRP: 1) the life-stage used for juvenile stocking and 2) the genetic matching of adults to preserve maximum genetic variation.

MATERIALS AND METHODS

Restoration Program Procedures

Information about the CRASRP procedures was obtained from the CRASC (1998) report and personal communication with Mickey Novak, USFWS. See Appendix B for more details.

Juvenile stock is released into the Connecticut River and its tributaries every spring. Adult sea-run salmon are captured at the Holyoke Dam, Holyoke, Massachusetts in the spring. Ten percent of returns are released above the dam to reproduce on their own. Sea-runs are tagged with a passive integrated transponder (PIT-tag), which is inserted under the dorsal fin and read by a receiver. The PIT-tag can be used to track fish movements in the river and identify fish in the hatchery. Sea-runs are categorized as coming from either fry or smolt stockings. This can be discerned because smolts have their adipose fin clipped before they are stocked whereas fry do not. Scale samples are taken to determine the age of the returning fish and the number of winters it spent at sea. A sample of the anal fin is sent to the Northeast Fishery Center, USFWS, Lamar, Pennsylvania for genetic analysis. Genetic analysis was previously done at the Silvio Conte Anadromous Fish Laboratory, U.S. Geological Survey (USGS), Turners Falls, Massachusetts, and is where the genetic information for the present study was obtained. Using microsatellite loci in the genome, the fish is matched to its parents and grandparents. This information is used to determine which stream the fish was stocked into and to prevent the fish from being mated with another from the same family. The tagged sea-runs are held over the summer at the Richard Cronin National Salmon Station, USFWS, Sunderland, Massachusetts.

In the winter, the sea-runs are spawned by hatchery staff. Prior to 1996, four random males were used to fertilize the eggs from each female. In 1996, the genetic breeding program was started whereby, with the use of microsatellite loci, parental matches are made by crossing the most unrelated fish in order to preserve genetic variation. Because of low numbers of returns and the need to prevent inbreeding, two males are used to fertilize each female's eggs. Parental pairs are assigned a relatedness value, which is the negative natural log of the proportion of shared alleles between the parents. Therefore, the fewer alleles the parents share, or the more unrelated they are, the higher their relatedness value.

The fertilized eggs are placed into individual holding containers for each family group (the offspring from one parental pair) and incubated at 38°F (3.3°C). Prior to incubation, an initial count of the eggs is done using water displacement. A sample of 50 eggs from an individual family group is put into a graduated cylinder that is partly filled with water. The volume of water that those eggs displace is then used in a ratio: number of eggs per volume of water. The entire batch of eggs from the family group is then put into the graduated cylinder and the total number of eggs is estimated from its water displacement and the ratio obtained from the sample of 50 eggs.

During the incubation period, eggs that were not fertilized and eggs that die turn white, distinguishing them from healthy, orange-colored, eyed eggs. “Dead” eggs are removed and a second count of the remaining eyed eggs is taken (also using water displacement) to determine the percent eye-up, or embryo survival, for each family group. After reaching the eyed stage, eggs are transported to several federal and state hatcheries along the Connecticut River where they are either kept as broodstock for the next year or stocked into the river as juveniles (Appendix B). Fry are currently released from several hatcheries. In the past, high numbers of smolts were released, but now limited numbers are stocked only from the Dwight D. Eisenhower National Fish Hatchery, USFWS, North Chittenden, Vermont (see Appendix C for historic stocking numbers of fry and smolts).

Data analysis

Smolt and fry return rates were obtained from the Office of the Connecticut River Coordinator, USFWS, Sunderland, Massachusetts. Eye-up data for sea-run family groups were provided by the White River National Fish Hatchery, USFWS, Bethel, Vermont. Parental relatedness data came from the Silvio Conte Anadromous Fish Laboratory, USGS, Turners Falls, Massachusetts.

Return rates of smolts and fry from the year-classes 1972 to 2007 were compared using a Mann-Whitney rank sum test in order to determine which life-stage yielded the highest return rate. This test was used because return rates were not normally distributed. Four years when fry were not stocked (1972-1973) and when fry had not yet returned (2006-2007) were not included in the analysis as they would yield false zero return rates. Seven years (1995-1999 and 2001-2002) when smolt-stocking was extremely low were also not included since that level of stocking would not be expected to yield any returns. This resulted in 32 years (1974-2005) of fry return rates and 29 years (1972-1994, 2000, and 2003-2007) of smolt return rates.

Percent eye-up for sea-run families and return rates of fry and smolts were both compared before and after 1996 to see if the genetic breeding program improved Atlantic salmon survival. Return rate data were the same as described for the life-stage comparison. Percent eye-up data were from the years 1988 to 2006, excluding 2000 and 2001 when the data were lost due to recording error. Pre- and post- 1996 return rates for both fry and smolts were compared using a Mann-Whitney rank sum test because the data were not normally distributed. A Mann-Whitney rank sum test was also used for the pre- and post- 1996 eye-up comparison because the data had unequal variance.

Linear regression analysis was used to directly compare parental relatedness to percent eye-up among sea-run family groups. The years 2003 and 2006 were used because they had good data series for both parental relatedness and eye-up.

Cost per return

The costs of rearing one fry and one smolt were obtained from hatchery managers. The cost of smolt rearing at the Dwight D. Eisenhower National Fish Hatchery, USFWS, North Chittenden, Vermont was estimated to be $0.24 per smolt. Two estimates of fry rearing were obtained and averaged. The cost per fry at the White River National Fish Hatchery, USFWS, Bethel, Vermont was estimated to be $0.08. At the Kensington State Salmon Hatchery, CTDEP, Kensington, Connecticut, the cost per fry was estimated to be $0.17. The average of these estimates was $0.125 per fry.

To calculate the cost per return, the cost of rearing one fish was divided by the return rate and averaged across all year-classes. Year-classes were the same as those used for the life-stage comparison.

RESULTS

Smolts consistently yielded higher return rates than fry by as much as one order of magnitude (Fig. 1). The median smolt return rate ($3.96 \cdot 10^{-4}$ smolt returns/smolts stocked) was significantly greater than the median fry return rate ($2.10 \cdot 10^{-5}$ fry returns/fry stocked; U=57.0, P<0.001). For fry, the mean cost per return was \$10,153.74. For smolts, the mean cost per return was \$1,298.51. The difference between these figures was \$8,855.23. The cost per fry return was 7.8 times greater than the cost per smolt return.

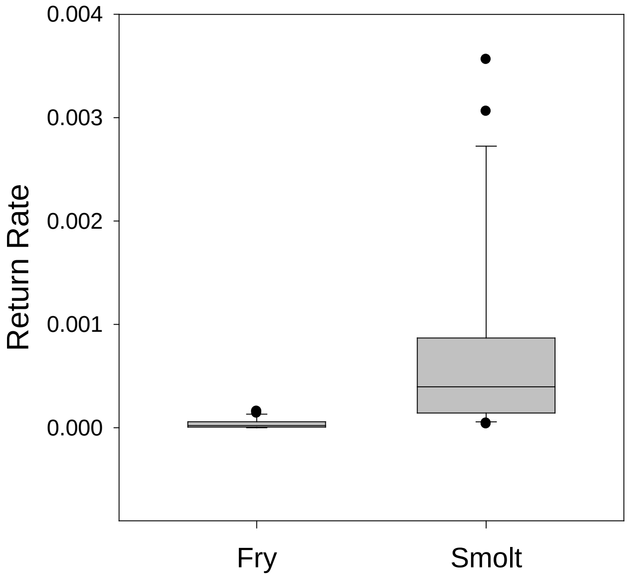


Fig. 1. Return rates of fry and smolts, 1972-2007. Return rate is equal to the number of returns divided by the number of fish stocked at that life stage. Years when return rates were zero were omitted when they were due to no or extremely low stocking. See text for the specific years omitted from the analysis.

Before the start of the genetic breeding program in 1996, both smolts and fry showed higher return rates (Fig. 2). Pre-1996 (n=23) smolt return rates were significantly higher than post-1996 return rates (n=6; U=23.0, P=0.014). Pre-1996 fry return rates (*n*=22) were significantly higher than post-1996 return rates (*n*=10; *U*=55.0, *P*=0.027).

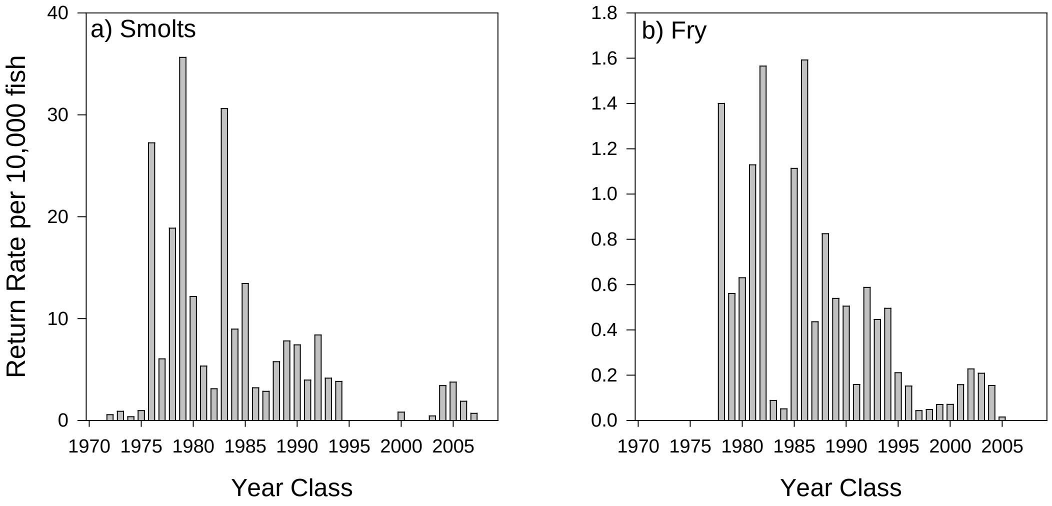


Fig. 2. Return rates by year-class of a) smolts and b) fry. Return rates were multiplied by 10,000. Note that the scale of the y-axis for smolts is more than one order of magnitude greater than that of fry. See text for the years omitted from the analysis for fry and smolts.

There was no significant difference in percent eye-up before and after 1996 (*U*=16.5, *P*=0.067; Fig. 3). From 1988 to 1995 (*n*=8), the median eye-up was 66.85%; from 1996 to 2006 (*n*=9), median eye-up was 80.20%.

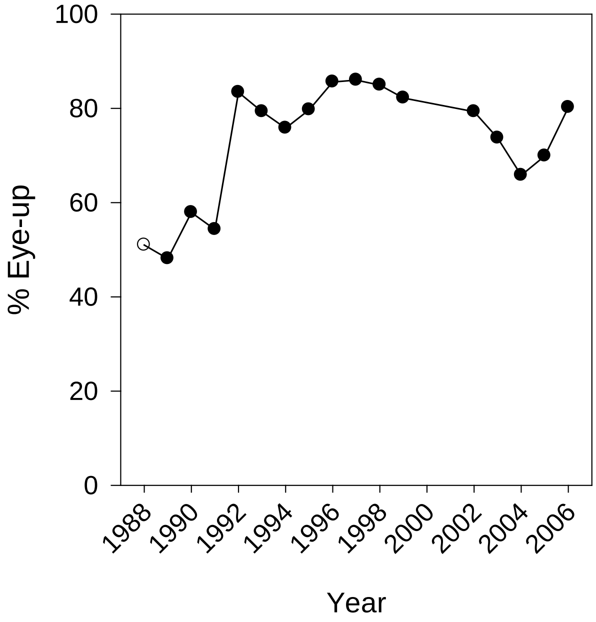


Fig. 3. Percent eye-up of sea-run salmon, 1988-2006. Values from 2000 and 2001 are missing due to recording errors.

Higher values of parental relatedness did not result in more embryos surviving for the years 2003 (R^2=0.0565, n=22, P=0.2869) and 2006 (R^2=0.0055, n=110, P=0.4414), although the test had low power (0.1830 in 2003 and 0.1167 in 2006; Fig.4). The method of selecting parental pairs resulted in distinct groups of pairs located at a few values of parental relatedness. Several eye-up values were higher than 100%, which most-likely resulted from error in the method of counting eggs.

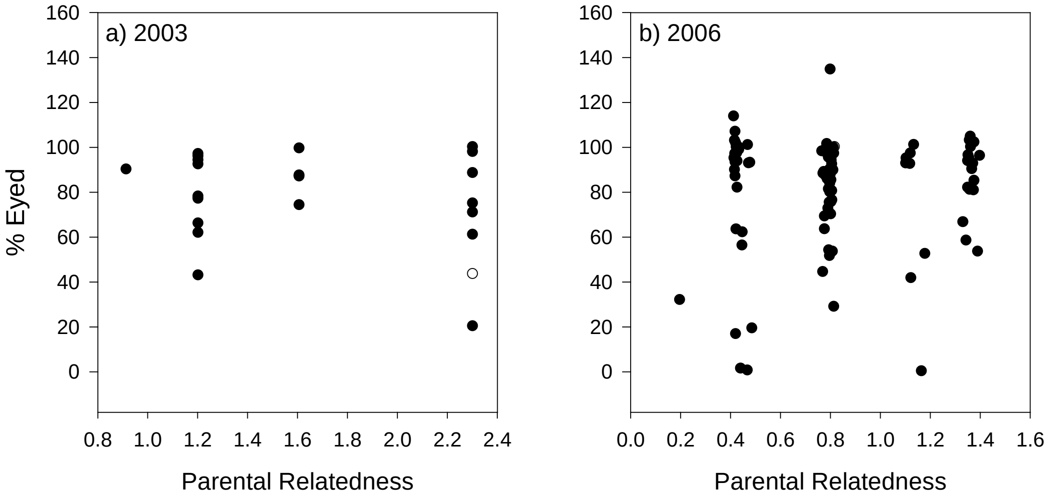


Fig. 4. Parental relatedness compared to percent eye-up among family groups for a) 2003 and b) 2006. Parental relatedness is equal to the negative natural log of the proportion of shared alleles between the parents. Eye-up values greater than 100% are most likely the result of counting error.

DISCUSSION

Age of release

Fry should produce higher-quality adults because they experience less domestication selection from the hatchery environment before they are stocked (Olla et al., 1998; Waples, 1999; Jokikokko et al., 2006; Jonsson & Jonsson, 2006). However, fry survival is extremely low. Predation on fry is intense during the first two days after stocking, eliminating as much as 49% of the fish stocked (Henderson & Letcher, 2003). Density-dependent effects are also strong at this stage, the carrying capacity of the stream being determined by habitat and food availability (Aprahamian et al., 2003). Increased stocking densities have been correlated with decreased fry survival directly (Whalen & Labar, 1994; McMenemy, 1995) and indirectly by decreasing individual growth (Grant & Imre, 2005; Ward et al., 2009). A decrease in growth negatively affects survival since survival is positively correlated with body size both at the time of stocking (Salminen et al., 2007) and in the post-smolt marine phase (Salminen et al., 1995; Jonsson & Jonsson, 2009; Kallio-Nyberg et al., 2009). All of these factors make survival from the fry to smolt stage very low and ultimately impact adult return rates.

In this study, smolts consistently produced higher return rates than did fry (Figs. 1 and 2). Although adults produced from fry stocking should have a higher fitness than those produced from smolt stocking, it appears that survival is more dependent on bypassing the high mortality at early life stages than avoiding domestication selection. Smolts avoid high density-dependent mortality because they migrate to the ocean, where survival is not density-dependent (Aprahamian et al., 2003; Jonsson & Jonsson, 2004), almost immediately after stocking. Therefore, if a restoration program aims to increase the production of adult Atlantic salmon past the carrying capacity of the river, then the program should stock smolts (Jonsson & Jonsson, 2009).

Smolts produced from fry stockings generally have higher survival rates than hatchery-released smolts, most likely as a result of avoiding domestication selection, which makes them better-adapted to the natural environment (Jonsson et al., 2003; Jutila et al., 2003; Kallio-Nyberg et al., 2004; Jokikokko et al., 2006; Jonsson & Jonsson, 2009). While the higher fitness attained by fry could lead to improved return rates in later generations (Fleming et al., 1996), it remains unlikely that a self-sustaining Connecticut River population would be created in the foreseeable future. This is because: 1) there are still dams that eliminate access to potential spawning and nursery habitat and 2) even if more adults were allowed to reproduce naturally, the current numbers of returning adults are so low that the amount of offspring produced from natural reproduction would be too low to sustain the population. At any rate, the primary goal of the CRASRP is not to produce a self-sustaining run of adults, but rather to produce large-enough runs to support recreational fishing (CRASC 1998). Therefore, since hatcheries will inevitably be supporting the Connecticut River Atlantic salmon population for some time, and because the interest of the program is to produce the highest number of sea-run returns possible, the CRASRP should focus less on fry stocking and increase the number of smolts stocked.

Should the number of returns increase as a result of smolt stocking, it may eventually be possible to increase the number of adults released to spawn naturally, thus increasing the number of offspring produced by natural reproduction. However, the interactions between such offspring and hatchery-released juveniles could result in high mortality of the naturally-spawned fish (Jonsson & Jonsson, 2006).

In addition to the biological advantage incurred by stocking smolts, it was also shown to be economically more profitable. The cost per smolt return was 7.8 times less than the cost per fry return. Even though the upfront cost of smolt-rearing is nearly twice that of rearing fry, the cost per return shows that smolts yield a much greater return on investment. It is necessary to keep in mind that the cost per fish is difficult to estimate because it includes factors like labor hours, water costs, and energy use. Therefore, these numbers could contain a large amount of error. Nevertheless, considering the result of this cost analysis together with the higher survival of smolts adds even more support to the conclusion that it would be more beneficial to stock smolts.

Breeding for maximum genetic variation

In 1996, the CRASRP began genetically matching the most distantly-related parents in order to maintain genetic variation and thus increase salmon survival (Frankham, 2005). Since the Connecticut River Atlantic salmon population was established using fish from Maine's Penobscot River, there was some concern that the derivative population could have undergone a bottleneck event. Bottlenecks decrease genetic variation and, potentially, the population's chances of survival in the new environment (Frankham, 1995).

Martinez et al. (2001) did find differences in allele frequencies and concluded that a founder effect had occurred. However, their analysis was based on few microsatellite loci, which makes their result questionable (Letcher & King, 1999; Letcher & King, 2001), especially in light of later studies. Spidle et al. (2004) and Ayllon et al. (2006) found that the genetic profile of the Connecticut River had remained similar to that of its founder population. The Penobscot and Connecticut River populations were shown to have similar values of heterozygosity (Spidle et al., 2004; Ayllon et al., 2006) and only slightly different allele frequencies (Spidle et al., 2004), which was to be expected as a result of genetic drift. Additionally, Letcher and King (1999; 2001) found the Connecticut River population to have sufficient genetic variation to support parentage and grandparentage assignment (matching offspring with parents and grandparents). Letcher and King also reported in the 1998 USASAC report that genetic drift was zero even before the start of the genetics program. Although the results are mixed, the balance of evidence suggests that the Connecticut River population did not undergo a bottleneck event and that breeding practices, even before the start of the genetics program, have preserved the effective population size and genetic variation.

This does not necessarily make the genetic breeding program superfluous, however, as the program has slightly improved heterozygosities and genetic distance (USASAC 1998), which could improve chances of survival over time (Frankham, 2005). On the other hand, if this breeding practice disrupts adaptive gene complexes, the population's chances of survival could actually decrease (Neiman & Linksvayer, 2006). The disruption of adaptive gene complexes could decrease fitness and result in the loss of genetic variation as less-fit individuals are lost from the population. Thus, the breeding program would, in effect, be accomplishing the opposite of its intent. In order to detect any improvement in survival resulting from the genetic breeding program, this study looked at two measures of survival: 1) sea-run returns and 2) percent eye-up.

Return rates did not improve after the implementation of the genetic breeding program and both fry and smolt return rates were significantly lower after 1996 (Fig. 2). Therefore, it can be concluded that the breeding program has not improved returns. However, the program is probably not the cause of the lower return rates. Return rates have been dropping since the 1980s, well before the start of the program (Fig. 2). This drop in Atlantic salmon returns has been observed across the Atlantic Ocean – for both European and North American stocks (Jonsson & Jonsson, 2009), and is most likely due to poor marine survival (Chaput et al., 2005). Marine growth, age-at-maturity, and survival have been shown to correlate with ocean characteristics like sea surface temperature (SST) in both European (Friedland et al., 2000; Jonsson & Jonsson, 2004; Kallio-Nyberg et al., 2004) and North American (Friedland et al., 2003) stocks. However, the relationships and mechanisms behind these correlations remain poorly understood and seem to differ among stocks (Friedland et al., 2005). Whatever the mechanism, the trend of decreased return rates that began in the 1980s probably masks the influence, if any, of the genetic breeding program.

This makes it impossible to conclude anything about the effect of breeding for maximum genetic variation on the survival of Connecticut River Atlantic salmon after stocking, which is a question that is critical to answer for management purposes. This breeding practice could actually hinder the process of adaptation, not only by breaking up adaptive gene complexes (Neiman & Linksvayer, 2006), but also by disrupting local adaptation in Atlantic salmon populations that show distinct genetic profiles within one river system (Taylor, 1991; Dionne et al., 2009). Even though the Connecticut River Atlantic salmon population is still young in evolutionary terms and extensive local adaptation may not yet have developed, future local adaptation is prevented because of the

CRASRP breeding practices: salmon from the same stocking stream are not bred exclusively and all broodstock are bred to preserve maximum genetic variation. Preventing the development of local adaptation could actually make it more difficult for the salmon to adapt and survive. Dionne et al. (2009) stress the importance of managing Atlantic salmon at the proper spatial scale. However, the extent of tributary-scale adaptation in the Connecticut River will not be able to be determined for some time because exclusive breeding of sea-runs from the same stocking stream will be impossible until the number of sea-run returns increases dramatically. In the event that such a number is reached, the CRASRP should exclusively breed sea-runs from individual stocking streams in order to allow for tributary-scale adaptation.

Embryo survival, or eye-up, was another measure of the effect of the breeding program. In the hatchery, dead eggs could result from the egg not being fertilized or the embryo dying after fertilization. Fertilization success is most likely largely determined by hatchery spawning practices. After fertilization, eye-up depends on a number of variables in the incubation trays like temperature and concentration of dissolved oxygen (Mickey Novak, USFWS, personal communication). However, fertilization success and embryo survival could also be influenced by genetic variation since increased genetic variation could increase the chances of egg viability and embryo survival.

The number of data points above 100% eye-up in Fig. 4 (*n*=14) brings the reliability of these data into question. The most probable cause of this error comes from the counting process (Dan Wong, USFWS, personal communication). Because there are so many eggs, there is not enough hatchery staff to count all the eggs individually. Therefore, water displacement is used to count the eggs before and after eye-up (see Materials and Methods for details). The error comes from the fact that there is often size variation within one batch of eggs. Therefore, if the sample of 50 eggs contained mostly eggs larger than the mean egg size, then the sample would displace a larger volume of water and the total number of eggs would be underestimated. Conversely, if the sample of 50 eggs contained mostly eggs smaller than the mean, the sample would displace a smaller volume of water and the total number of eggs would be overestimated. If the number of pre-eyed eggs was underestimated and the number of eyed eggs overestimated, then an eye-up rate greater than 100% could be achieved. This could also occur if there was higher mortality of large eggs than small eggs.

The question is whether or not this error is evenly-distributed throughout the data. While there is no reason to believe that it is not evenly-distributed (Dan Wong, USFWS, personal communication), this cannot actually be proven and the results should be interpreted with caution.

When percent eye-up was compared before and after 1996, no significant difference was found (Fig. 3). However, the difference was marginally significant (p=0.067) and the median eye-up before 1996 (66.85%) was apparently lower than the median eye-up after 1996 (80.30%). This increase is unlikely to be the result of increasing genetic variation as there was no relationship detected between parental relatedness and percent eye-up among family-groups (Fig. 4). The trend of eye-up seen in Fig. 3 probably reflects changes in spawning practices. Eye-up improved from 1988 to about 1992, then decreased slowly after 1997 until it rose again in 2005. It seems that eye-up had improved as hatchery practices developed, but then dropped right after the start of the genetics program. This could be purely coincidental since parental relatedness seemed to have no effect on eye-up (Fig. 4). However, the process of spawning the fish in order to match the appropriate parents involves a lot more handling of the fish, eggs, and sperm, which may affect fertilization success or embryo survival. It would be interesting to investigate the different spawning and incubation procedures used by the hatcheries over time to see if they can explain the eye-up trend in Fig. 3.

Since the effect of breeding to preserve maximum genetic variation on Atlantic salmon survival could not be discerned from this study, it is difficult to make a recommendation with regards to the continuation of the breeding program. The program was not necessarily beneficial because increased parental relatedness was not shown to improve eye-up or return rates. The program was also not shown to be detrimental as there was no significant decrease in eye-up since the implementation of genetic breeding (although the post-1996 drop in eye-up in Fig. 3 is a concern) and the decreased return rates after 1996 are most likely the result of other factors. Therefore, research is needed to determine whether the survival of Atlantic salmon populations is more dependent on preserving genetic variation (Frankham, 1995; Frankham, 2005) or adaptive gene complexes (Neiman & Linksvayer, 2006). This information will shed more light on the utility of breeding for maximum genetic variation.

General conclusions

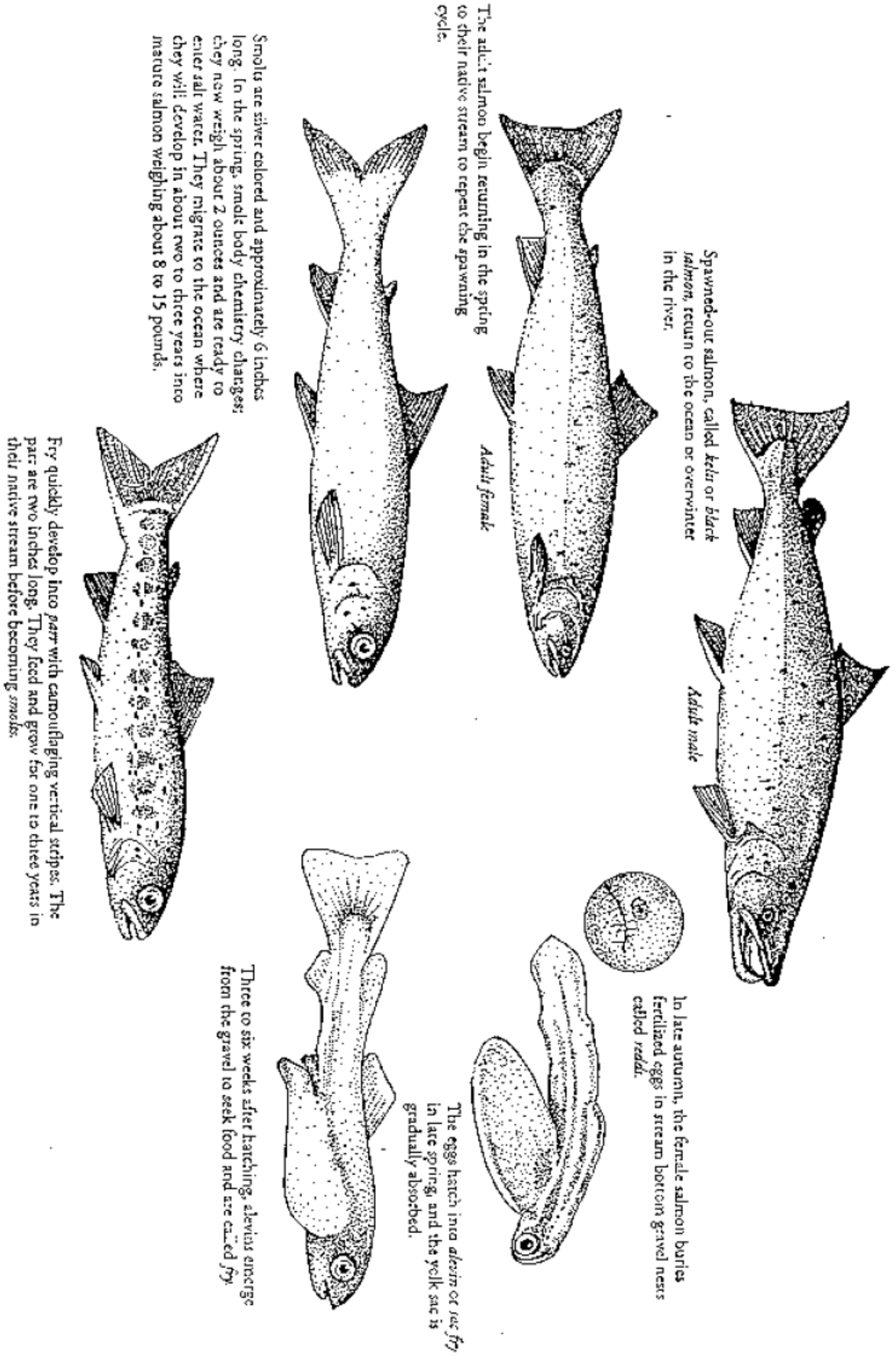
Breeding for maximum genetic variation could in theory either help or hinder an introduced Atlantic salmon population's ability to adapt to the natural environment. This study was not able to reveal the effect of such breeding on the survival of Atlantic salmon in the CRASRP, though parental relatedness was shown to have no effect on eye-up (Fig. 4).

Although fry stockings produce potentially better-adapted fish than smolt stockings, smolt stockings yielded much higher adult return rates. The higher return rate does not necessarily indicate higher long-term survival of the population because smolt-stocking removes several early life stages from natural selection in the natural environment. Smolt-stocking could therefore result in eggs, fry, and parr that are maladapted to stream survival. However, to a program whose immediate goal is to increase sea-run returns in order to create a fishery, it may be more beneficial to put more effort into stocking smolts.

This analysis also showed that it was economically more profitable to stock smolts. Despite this result, there is the possibility that the cost estimates contained large amounts of error. In the case that smolt returns are actually more expensive, it might be beneficial to allocate less money to the genetic breeding program and more money to producing smolts. The effect of the breeding program was inconclusive and, in any event, genetic variation was shown to have been preserved even before the start of the program.

APPENDIX A: Atlantic Salmon Life Cycle

Image taken from the Connecticut River Atlantic Salmon Commission report (1998). The Connecticut River Atlantic salmon population migrates between the Connecticut River and the North Atlantic Ocean as far as Greenland (CRASC 1998).



The adult salmon begin returning in the spring to their native stream to repeat the spawning cycle.

Spawned-out salmon, called *kelts* or *black salmon*, return to the ocean or overwinter in the river.

In late autumn, the female salmon buries fertilized eggs in stream bottom gravel nests called *redds*.

The eggs hatch into *alevin* or *sac fry* in late spring, and the yolk sac is gradually absorbed.

Three to six weeks after hatching, alevins emerge from the gravel to seek food and are called *fry*.

Fry quickly develop into *parr* with camouflaging vertical stripes. The parr are two inches long. They feed and grow for one to three years in their native stream before becoming *smolts*.

Smolts are silver colored and approximately 6 inches long. In the spring, smolt body chemistry changes; they now weigh about 2 ounces and are ready to enter salt water. They migrate to the ocean where they will develop in about two to three years into mature salmon weighing about 8 to 15 pounds.

APPENDIX B: CRASRP history and detailed procedures

Information was obtained from the Connecticut River Atlantic Salmon Commission report (1998) and personal communication with Mickey Novak, USFWS.

Atlantic salmon have been extirpated from the Connecticut River for about 200 years, ever since the first dam was built across the main stem of the river in 1798. In the 1800s, efforts were made to reestablish the Connecticut River Atlantic salmon run, but were abandoned due to lack of organization. In 1967, the current CRASRP was established as a joint effort between the U.S. government and the four basin states, Connecticut, Massachusetts, New Hampshire, and Vermont. The government organizations involved are the U.S. Fish and Wildlife Service (USFWS), the National Marine Fisheries Service (NMFS), and U.S. Forest Service (USWS).

The first Atlantic salmon stocked in the Connecticut River came primarily from the Penobscot River in Maine. Since the early 1970s, the Connecticut River broodstock has produced enough offspring to sustain itself. The mission of the CRASRP is “to protect, conserve, restore and enhance the Atlantic salmon population in the Connecticut basin for public benefit, including recreational fishing.”

Currently, four federal and two state hatcheries work together to accomplish this mission. The Richard Cronin National Salmon Station, USFWS, Sunderland, Massachusetts takes sea-runs (adults that have returned to the river from the ocean) captured at the Holyoke Dam and provides eggs from this sea-run broodstock to three other hatcheries: the White River National Fish Hatchery, USFWS, Bethel, Vermont; the Kensington State Salmon Hatchery, CTDEP, Kensington, Connecticut; and the Roger Reed State Salmon Hatchery, MADFW, Palmer, Massachusetts. These hatcheries use the juveniles produced from the sea-run eggs for either domestic broodstock or for stocking into the river. Additionally, the North Attleboro National Fish Hatchery, USFWS, North Attleboro, Massachusetts takes the spent (already-spawned) sea-runs, known as kelts, and reconditions them for use as broodstock the following year. The Dwight D. Eisenhower National Fish Hatchery, USFWS, North Chittenden, Vermont is currently the only hatchery that stocks smolts. In addition to the production of juveniles for stocking, the installation of upstream and downstream fish passage devices at hydroelectric dams has also opened up new spawning and nursery habitat for the fish.

Sea-run salmon are caught in the spring at the Holyoke Dam, Holyoke, Massachusetts as they travel upriver. The majority is kept for broodstock and ten percent is radio-tagged and released further upriver with the hope that they will reproduce naturally. The sea-runs that are kept are PIT-tagged and a fin sample is taken for genetic analysis. A PIT-tag is a Passive Integrated Transponder that is inserted under the skin beneath the dorsal fin. The signal can be read by a receiver in the hatchery and is used later to identify the fish. They are held in the hatchery until spawning season in the late fall when they are injected with hormones to induce simultaneous spawning and thus produce more fertilized eggs. During breeding, geneticists use microsatellite loci to determine parental relatedness and cross males and females for maximum genetic variation. The eggs produced from spawning are incubated in the hatcheries and records are kept on the percent eye-up (embryo survival[1]) of each family group. The juvenile fish are raised in the hatchery to different ages, from months-old fry to two-year parr or smolts. The juveniles are then released into the Connecticut River and its tributaries.

After the sea-runs are spent they become kelts. The male kelts are sacrificed in order to determine if any contained diseases that could have been passed on to the offspring (this can be determined in females by sampling their ovarian fluid). The female kelts are reconditioned in the hatchery and used for broodstock the following year. A domestic broodstock is also maintained in order to ensure that the maximum number of eggs is produced each year. The domestic broodstock is a sample of the first generation (F1) offspring from sea-run parents that is not stocked into the river, but rather is kept for breeding. Only first generation offspring from sea-runs are used for the domestic broodstock in order to avoid too much domestication selection.

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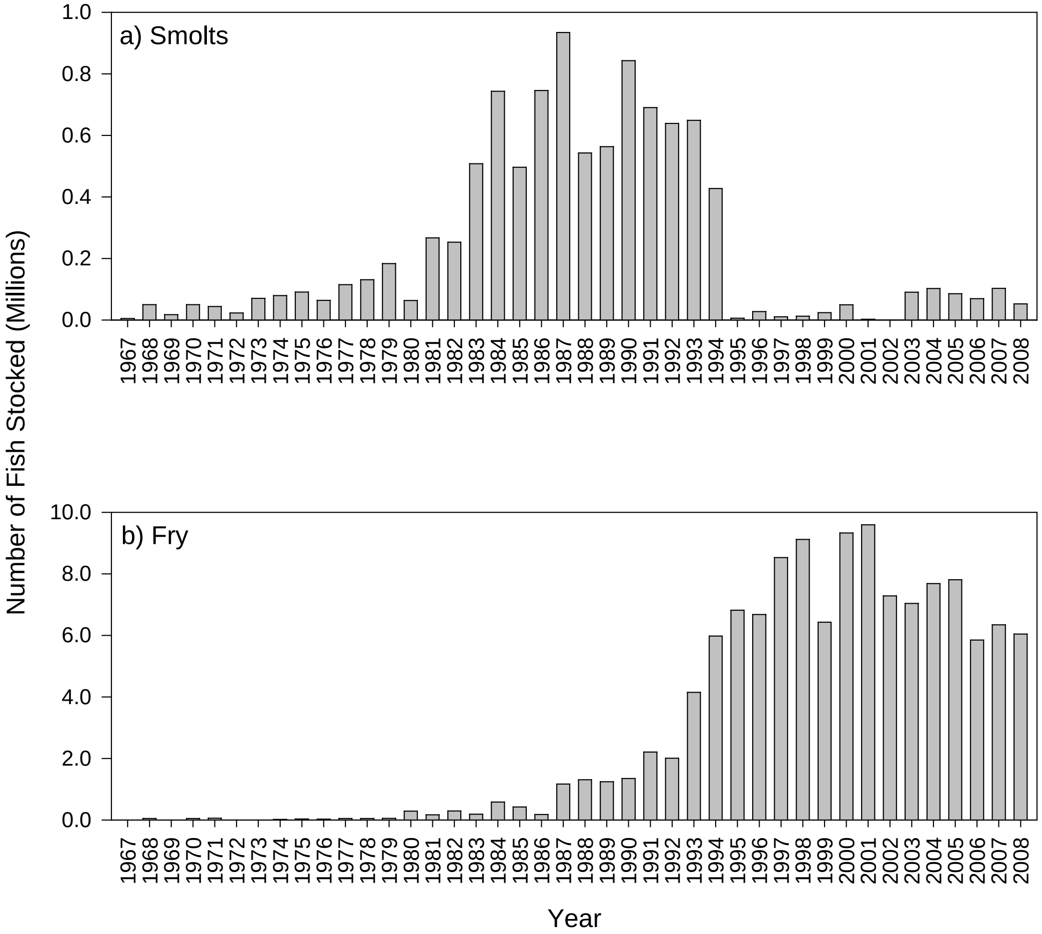
[1] Embryos that reach the eyed stage of development are considered to have survived because they have reached a less vulnerable stage of development and can better-withstand environmental fluctuations.

The juveniles that are released into the river will migrate to the ocean that spring or the following spring. Fish stocked as two-year-olds are either parr or smolts and usually migrate out immediately after stocking. Fish stocked younger than one year old are generally fry and usually reside for one year in the river before migrating. A small percentage will smoltify and migrate out as one-year-olds. Parr and smolts have their adipose fin clipped before stocking in order to identify them when they return. Fry are too small to have their fin clipped. In this way, the returns can be categorized into “hatchery” (parr or smolt) returns and “wild” (fry) returns. Parr and smolt returns cannot be distinguished and so, when return rates are calculated, they are all grouped into one category and called “smolts.” Therefore, return rates for a) “fry” and b) “smolts” are actually return rates of a) fry and b) smolts plus parr.

The fish return to the Connecticut River as adults in the spring. Over time, a consistent run of a few hundred adults has been established. However, according to historical records, runs used to number in the thousands. The CRASRP aims to once again establish annual runs numbering in the thousands. Once a large enough run is established, a higher percentage of fish will be released above the Holyoke Dam to give more opportunity for salmon to spawn naturally and create a better-adapted stock. The ultimate goal is to produce a run large enough to support fishing of Atlantic salmon in the Connecticut River.

APPENDIX C: Juvenile stocking numbers, 1967-2008

Stock numbers from 1967 to 2008 were provided by the Office of the Connecticut River Coordinator, U.S. Fish and Wildlife Service, Sunderland, Massachusetts.



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