

Northeast Fisheries Science Center Technical Memorandum 457

A Primer on Salmonid Conservation Genetics for Managers with a Focus on Recovery of the Gulf of Maine Distinct Population Segment of Atlantic Salmon

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ABSTRACT

Recovery programs for protected stocks of US Atlantic and Pacific salmon are often reliant on conservation hatchery programs for preventing extinction and rebuilding depleted populations. But broodstock management and hatchery rearing practices can result in both loss of genetic diversity and domestication selection, thereby reducing population fitness. A broad suite of tools for conservation genetic management is available for monitoring effects of hatchery breeding and rearing to minimize negative effects and maximize success. However, programs inconsistently apply new genetic tools and best practices across regions. Decision-makers are confronted with an expanding knowledge base, while funding/staffing limitations and programmatic inertia delay the use of newer genetics in recovery programs. We developed this primer of genetic tools for managers by synthesizing relevant studies, reviews, and focused conversations with a broad group of salmon geneticists. As a case study, we summarized management of the federally endangered Gulf of Maine Distinct Population Segment (GOM DPS) of Atlantic salmon, *Salmo salar*. The conservation hatchery program has prevented extinction of the GOM DPS, but status-quo conservation measures have not rebuilt abundance nor significantly mitigated extinction risk and there is concern over extant genetic diversity. Given the rapid transformation of river and ocean habitats, rebuilding genetic diversity would help the GOM DPS persist through challenges (e.g., climate change) and leverage opportunities (greatly improved river-ocean connectivity) to increase abundance. Moving the GOM DPS into a recovery-oriented program will require co-managers in state, federal, and Tribal partners actively evaluate and implement new approaches to broodstock management, stocking, and genetic assessment. We recommend framing this process in a resist-accept-direct approach in an open, transparent, and collaborative environment that allows academic, industry, and agency experts to modernize and innovate.

EXECUTIVE SUMMARY

Hatchery propagation of salmonids in North America has occurred for more than a century to increase fishing opportunities, enhance depleted natural populations, or prevent extinction (Mahnken et al. 1998, Fraser 2008, O'Reilly and Kozfkay 2014). But there is increasing awareness of the reduced fitness of hatchery fish relative to wild-spawned individuals, resulting from loss of genetic diversity and domestication selection imparted by broodstock management, demographics, and hatchery rearing practices. Therefore, recent recommendations have been to limit use of hatchery supplementation **except** when restoring imperiled or reestablishing extirpated populations (Lennox et al. 2021).

Anthropogenic-driven declines in both Atlantic and Pacific salmonids have transformed habitats and once commercially harvested populations, resulting in extinctions and listing of many distinct population segments as threatened or endangered. Many conservation efforts are heavily reliant on captive breeding and hatchery supplementation for restoration and recovery. Concurrently, a rapid expansion of tools for conservation genetic management empowers managers to monitor and understand the effects of hatchery breeding and rearing programs to minimize potential negative effects and maximize success of conservation-oriented programs (Fraser 2008, Fisch et al. 2015, Frankham et al. 2017, Waples et al. 2020). However, across programs (and regions), genetic tools are inconsistently applied. Decision-makers are confronted with a complex and expanding literature base, which often requires specialized training in conservation genetics to interpret. Additionally, funding and/or staffing limitations and programmatic inertia create barriers to modernization of conservation genetics in hatchery programs. Therefore, we developed this paper to synthesize relevant studies, reviews, and in-depth conversations with salmon geneticists to provide managers with a primer of salmon conservation genetic resources. We focus specifically on approaches for maintaining and metrics for evaluating reproductive fitness and evolutionary potential, which will be essential for salmon population recovery in transformed rivers and oceans, using the federally endangered Gulf of Maine Distinct Population Segment (GOM DPS) of Atlantic salmon, *Salmo salar* as a case study.

Effective population size (Ne) is the global standard for reporting genetic diversity for management and policy (Fedorca et al. 2024). There is a consensus that conservation breeding programs should strive to maintain an Ne $\geq$ 100 over the short term (i.e., $\leq$ 5 generations) and 1,000 in the long-term to both reduce inbreeding and maintain evolutionary potential (Frankham et al. 2014). These are higher numbers than previously thought. The toolkit for conservation genetic management has grown more powerful and cost-effective over the last 20 years. New tools offer benefits for managing genetic diversity needed to maintain or increase reproductive fitness, which will be integral for rebuilding self-sustaining populations of commercially important salmonids in rapidly changing ecosystems (Frankham et al. 2014, Waples et al. 2020). For example, the development of SNP panels, containing hundreds to thousands of markers, has greatly improved the precision and accuracy of estimates of Ne, heterozygosity, inbreeding, and

relatedness relative to the use of microsatellites (Waples et al. 2020). Genomics data can also lend powerful insights into genetic quality and is useful for planning interventions to increase diversity and reproductive fitness, such as genetic rescue (Fitzpatrick et al. 2023).

The hatchery program that supports GOM DPS Penobscot River population predates the federal listing as endangered by more than a century, exceeding the long-term intervention metric. Additionally, the five-generation short-term benchmark for other GOM hatchery populations initiated in the mid-1990s has passed. Consistently low genetic diversity in some of these seven-river specific stocks and recent decreases in others are concerning, particularly because at least one, and possibly all the founding populations exhibited low genetic diversity and Ne (Lage and Kornfeld 2006, USASAC 2024). We echo the recommendations of Lage and Kornfield (2006), that DNA extraction studies to compare historic and contemporary genetic diversity across the GOM DPS using archival scale and tissue samples would provide useful context for management of the DPS. This information would also help decision makers differentiate between "thousands of years of natural selection" or "five to six generations of genetic drift as noted by NRC (2002)" and inform genetic rescue efforts with the extant populations.

The conservation hatchery program has prevented extinction but current strategies are not rebuilding populations (USASAC 2024). Our assessment and conversations with specialists suggests more intensive intervention (e.g., genetic rescue) would help rebuild diversity and adaptive potential to better attain the demographic abundance and self-sustaining population criteria required for downlisting, delisting, and sustainable populations. Concurrently, expanding genetic monitoring and embracing genomics approaches to include markers for adaptive traits and measures of reproductive success would help secure genetic diversity to maintain/enhance reproductive fitness in the transforming GOM DPS ecosystem.

Within the Northeast, Atlantic Salmon is one of the most highly vulnerable species to impacts of climate change in the wild (Hare et al. 2016). A warming climate also poses challenges to hatchery propagation, assessment, and monitoring of these populations (USASAC 2024). Recovery programs rely on continued conservation hatchery programs and more consideration of innovative technical approaches now available. Evaluation and implementation of new approaches to broodstock management, stocking location and life-stages, and genetic assessment could expand both the demographics of hatchery supplementation but importantly the effective population sizes and rebuilding potential across the entire DPS. Our evaluation suggests that implementing a resist-accept-direct framing of conservation hatchery efforts for the GOM DPS of Atlantic Salmon would facilitate adaptation and persistence in rapidly transforming ecosystems. Such evaluations would inform both approaches that mitigate and reduce challenges from land use, pollution, and climate change and leverage opportunities from current investments in improved river-ocean connectivity and increased hatchery production to increase abundance.

Moving the GOM Atlantic Salmon DPS into a recovery-oriented program will require cross-agency collaboration among state, federal, and Tribal partners and would benefit from discussion and evaluation of projected change in an open, transparent, and collaborative process in a forum, such as the Center for Independent Experts. Given the genetic assessment toolkit of 2025 compared to 2002, it is time for an open and transparent peer-reviewed broodstock management plan that is forward thinking and provides fish with the adaptive scope necessary for persistence and recovery within the Gulf of Maine DPS of Atlantic Salmon.

INTRODUCTION

Biologists have used hatcheries for propagation of salmonids in North America for more than a century (Kostow 2009, Maynard & Trial 2014, Fisch et al. 2015). Propagation of salmonids in hatcheries can increase fishing opportunities, enhance depleted natural populations, or prevent extinction (Mahnken et al. 1998, Fraser 2008, O'Reilly and Kozfkay 2014). In recent decades, range-wide declines in Atlantic and Pacific salmonids due to human actions have led to an increasing reliance on captive breeding and hatchery supplementation for population persistence. However, the demographic benefits provided by hatchery propagation may be countered by reduced fitness associated with loss of genetic diversity and artificial selection, resulting in increased susceptibility to environmental perturbation (Ryman and Laikre 1991, Waples and Drake 2004, Schindler et al. 2010, Christie et al. 2014). Consequently, more recent recommendations have been to limit the use of hatchery supplementation except in cases of restoration of imperiled/reestablishment of extirpated populations (Lennox et al. 2021). Science has expanded the suite of conservation genetic management tools available to monitor and understand the effects of hatchery breeding and rearing programs to minimize potential negative effects and maximize success of conservation-oriented programs (Ryman and Laikre 1991, Fraser 2008, Fisch et al. 2015, Frankham et al. 2017). These developments are especially relevant for highly endangered populations with potential high risk or rewards for conservation efforts.

A vast body of literature has emerged over the last four decades related to genetic management of hatchery programs and whole textbooks have been devoted to the issue in single species alone (e.g., Verspoor et al. 2007). Managers charged with making decisions regarding salmonid supplementation or captive rearing are confronted with an ever-expanding number of papers, which often require specialized training in genetics to interpret. In this paper, we synthesized relevant peer-reviewed studies, reviews, and conversations with geneticists to provide managers with a primer of genetic management as it applies to salmonids. In the development of this document, we interviewed 16 leading experts (see acknowledgements) in salmon conservation genetics. These conversations helped enrich our literature search and provide expert interpretation and dialogues related to emerging issues, opportunities, and best practices. Their contributions were exceedingly valuable and provided broader viewpoints. This work serves as an extension to Fisch et al. (2015), who summarized hatchery genetic management techniques and how they have been applied to salmon (broodstock selection, spawning, rearing and release). Our goal was to

empower non-geneticists to better use and evaluate information as they develop hatchery propagation programs for salmonid species of concern and direct those wishing to take a deeper dive to the relevant references.

Our approach was to first summarize key terms and metrics used to measure genetic quality in supplementation and captive rearing programs and synthesize how these metrics may or may not predict population fitness in the wild. We italicized key terms at first mention, defined, and further explored in the Annotated Glossary. We then provide information to inform selection of the appropriate *effective population size (Ne)* for captive rearing programs and explore methods of increasing Ne. Finally, we provide an overview of current management of GOM DPS genetic diversity. Within the Northeast region, analysis suggests that this is one of the most highly vulnerable finfish species to impacts of climate change, across both the freshwater and marine habitats required to complete the life cycle (Hare et al. 2016, Borggaard et al. 2019). Moving toward recovery requires improving habitat suitability while rebuilding greatly diminished and extirpated stocks using conservation hatcheries. To this end, we present a suite of recommendations for genetic monitoring of the GOM DPS as it progresses through the four phases of recovery outlined in the Final Recovery Plan for the 2009 ESA Listing (USFWS and NMFS 2018). We conclude with suggesting implementation of a resist-accept-direct approach to conservation hatchery efforts for the GOM DPS of Atlantic Salmon to facilitate adaptation and persistence under a rapidly changing climate.

Monitoring Genetic Diversity in Conservation Programs

Captive breeding programs are designed to prevent imminent extinction of (generally small) populations that are unable to survive in the wild, usually because of some adverse habitat conditions or other threats to survival, e.g., poor survival through dams or in the marine environment, mixed-stock commercial fisheries, degraded spawning or rearing habitat, or high predation by non-native species (Fraser 2008, Sheehan et al. 2010, USFWS and NMFS 2018). The end goal of such a program is the reestablishment of a self-sustaining population once threats to survival have been ameliorated (Fraser 2008). Successfully attaining this end goal requires not only that habitat restoration and/or threat abatement to allow enough individuals to complete all parts of the life cycle in the wild but also that captive rearing protocols maintain sufficient genetic diversity and fitness for survival.

Captive breeding programs inherently deal with small populations, which are susceptible to genetic deterioration that can affect the ability to reestablish a self-sustaining population. Loss of genetic diversity in small populations can reduce the population's ability to adapt evolutionally to changes in the environment, such as those associated with climate change (Frankham et al. 2017). Compounding this, loss of variation before the establishment of captive programs can be substantial, further limiting adaptive capacity (Lage and Kornfield 2006). Small populations are also subject to inbreeding depression, i.e., the reduction in reproductive fitness resulting from

mating with close relatives (Charlesworth and Charlesworth 1987, Keller and Waller 2002, Frankham et al. 2002, Huisman et al. 2016). Large, non-threatened populations generally have greater genetic diversity, (when measured by *allelic richness* and *heterozygosity*), than smaller, inbred populations (Frankham et al. 2017). For example, *allelic richness* was 4.5 and *heterozygosity* was 0.63 in population of non-threatened gray wolves (*Canis lupus*), whereas values were 2.7 (allelic richness) and 0.42 (heterozygosity) in a threatened population of Mexican wolves (*Canis lupus baileyi*; Frankham 2017, Table 2.2).

Consequently, measuring genetic diversity is a critical component of captive breeding programs to ensure that genetic deterioration is minimized (Fraser 2008, Frankham et al. 2017). The primary methods used to assess genetic diversity in conservation programs are to genotype individuals for several neutral genetic loci, then compute: 1) the number of alleles at each locus and 2) the proportion of individuals that are heterozygous for each locus (Frankham et al. 2017). The mean genetic diversity for the population is then presented as *allelic richness*, *observed heterozygosity*, and *expected heterozygosity*, which are commonly reported by conservation hatchery programs for salmonids (e.g., Wither et al. 2001, Christie et al. 2012, Conrad et al. 2013).

The markers used to measure standing genetic diversity have evolved over time, but in general, the markers used in population genetics measure neutral or near neutral variation - loci that are not linked to adaptive traits (NAS 2002, Hansen et al. 2007, Pearse 2016). The primary *neutral markers* that have been used to assess genetic diversity and genetic differentiation in Atlantic salmon are *allozymes*, *maternal mitochondrial DNA*, *microsatellite DNA*, and *single nucleotide polymorphisms*, i.e., SNPs (NAS 2002, Hansen et al. 2007). In the rapidly changing climate, managers will be increasingly confronted with the question of whether these neutral markers should remain the criteria by which we assess conservation programs for small populations (particularly those at the more southernly range extents), if their current or historical allelic richness is not adaptive to emerging climate conditions (Lage and Kornfeld 2006, Kocik et al. 2022, Gillis et al. 2023).

Does Genetic Diversity Equate to Genetic Quality?

Supplemental breeding programs that maximize genetic diversity may not maintain long-term genetic quality, because measures of genetic diversity (allelic richness, heterozygosity) may not adequately reflect genetic quality (Hansen et al. 2007, Pitcher and Neff 2007). There are a few reasons for this. There has largely been a lack of empirical support for the links between genetic diversity and both fitness and evolutionary potential, although recent advances in *genomics* are beginning to make some of these links (reviewed in Waples et al. 2020). Additionally, populations can change at an adaptive or quantitative level without any evident change in neutral genetic diversity (D. Fraser, pers. comm).

Despite best efforts to prevent directed selection for traits, domestication selection is an inherent risk in captive rearing programs, because traits that allow salmonids to thrive under

hatchery conditions differ from those that maximize survival in the wild (Fraser 2008). Because sexual selection has been shown to increase expression of fitness-related traits in offspring among different taxa, the absence of sexual selection (including mate choice and male-male competition) in most salmonid breeding programs is likely to negatively impact offspring fitness (Neff et al. 2011). This lack of sexual selection can also prevent the purging of maladaptive alleles or genotypes from hatchery populations (Neff et al. 2011).

Results of quantitative genetic modeling indicated that selection in captivity may significantly reduce a wild population's fitness during supportive breeding (Ford et al. 2002). Lower reproductive success has been observed for hatchery-origin salmonids relative to wild-origin individuals, even when the parents of hatchery-origin individuals were of natural-origin (Christie et al. 2014). This pattern has been observed in steelhead, Coho Salmon, Chinook Salmon, and Atlantic salmon (reviewed by Christie et al. 2014). Reduced relative reproductive success appears to be a consistent outcome of hatchery propagation and even continually introducing wild individuals into the captive population will not eliminate this effect entirely, potentially due to the loss of sexual selection (Ford 2002, Neff et al. 2011, Christie et al. 2014).

Neff et al. (2011) urge that more emphasis needs to be placed on understanding the genetic architecture underlying fitness, as well as on increasing capacity for natural reproduction in supplementation programs. There is also increasing recognition that including measures of genetic quality or *adaptive genetic variation* in addition to measures of genetic diversity is an important component of monitoring for conservation breeding programs. Although not routinely used at this time, measures of genetic quality can be incorporated into captive breeding and supplementation programs via use of markers for adaptive traits, genomics approaches, *epigenetics*, and direct evaluation of *fitness-related measures* of population productivity (e.g. reproductive success, or population growth rate). Beyond strict genetics approaches to assessing fitness, evaluating physiological characteristics (e.g., aerobic scope, Eliason et al. 2011) can provide insights into adaptation to current climate and habitat conditions. Although ultimately reflected in reproductive success and population growth rate, such physiological characteristics can provide a direct measure of why some individuals survive and reproduce whereas others fail.

Monitoring Effective Population Size for Captive Breeding

In genetic management of captive breeding programs, the *effective population size* (Ne) is considered a more important metric than the *census population size* (Nc) because the genetic problems that arise in small populations are typically thought to be linked to Ne, not to Nc (Hansen et al. 2007, Frankham et al. 2017). Indeed, Ne was adopted as one of the key metrics for reporting genetic diversity for governmental and policy purposes (Fedorca et al. 2024). Ne is inversely proportional to heterozygosity, allelic richness, rate of inbreeding, and the levels of fluctuation in allele frequency (Jordan et al. 2007). The Ne of a population is indicative of the potential for the population to persist long-term (Fedorca et al. 2024); large non-threatened populations generally

have greater Ne and genetic diversity than smaller, threatened populations (Frankham et al. 2017). The rate of genetic change will be low in a population with a high Ne, thus will lose less genetic variation over the long term. Consequently, a population with greater Ne would have more genetic diversity on which natural selection can act, and as a result, should be able to adapt more readily to environmental changes than a population with lower Ne (Fedorca et al. 2024).

Maintaining a high Ne in supportive breeding programs is crucial to avoid inbreeding depression and preserve genetic variation necessary for long-term evolution. Sudden decreases in Ne during bottlenecks, such as in captive rearing, can lead to the fixation of harmful recessive alleles (Hansen et al. 2007). Although supportive breeding increases the population size, it may decrease Ne if wild and hatchery populations have different fitness, leading to faster inbreeding and genetic drift (NAS 2004). Furthermore, increases in inbreeding and genetic drift can occur in supportive breeding programs if the effective population size does not consistently and significantly increase (Wedekind & Müller 2004). Continuous substantial increase in Ne is essential, with recommendations suggesting a Ne/N ratio close to 2.0 for Atlantic Salmon broodstock programs (NASCO CNL(24)61 2024).

Techniques commonly employed in supportive breeding programs to maintain or enhance Ne include increasing the number of breeders and equalization of family size (Hansen et al. 2007). Equalization of family size reduces rates of inbreeding and drift to half of what they would have been under random mating, consequently reducing the probability of random loss of alleles (Fernandez and Caballero 2001). There is, however, an important tradeoff between maintaining Ne and maintaining heritable variability (Wedekind and Müller 2004). Equalization of family sizes reduces the intensity of natural selection and increases the chance that mild deleterious alleles will accumulate in the population (Fernandez and Caballero 2001, Robert 2009). In the short term, the risk of accumulating deleterious mutations from equalizing family sizes is not vastly different than in unmanaged populations over the short term (≤ 20 generations), and equalization of family sizes effectively preserves genetic variation, so the procedure can "seem warranted". Population management may be improved by maintaining heritable variability (Wedekind & Müller 2004), due to a "demography-genetic trade-off" (Robert 2009). That is, if a population requires long-term captive rearing, then the demographic benefits of such a program may be lost to the negative effects of domestication selection, absence of sexual and natural selection, and inbreeding (Robert 2009).

Overlapping year-classes can also buffer Ne within populations with a small number of individuals (Jorde & Ryman, 1995). For this reason, it is important to maintain the flexibility to cross year-classes in captive-breeding programs when possible. In Atlantic Salmon, precocious parr can be used to increase the number of breeders and incorporate multiple year-classes within a broodstock. Martinez (2000) found that precocious parr sired a considerable proportion of offspring and Ne was greater than would have been observed if precocious parr had been absent. Retaining breeders from one year to the next also allows for overlapping generations in a captive breeding program and can be accomplished by reconditioning Atlantic Salmon kelts after

spawning. This practice essentially adds a repeat spawning component to the hatchery broodstock for this iteroparous species, as would be observed in nature.

Duchesne & Bernatchez (2002) demonstrated via modeling that, as expected, replacing very few breeders (0 to 25% replacement) in a captive program led to large increases in the *inbreeding coefficient* (F). Surprisingly, replacing between 75 to 100% of breeders did not substantially reduce F relative to replacing 25-50% (Duchesne & Bernatchez 2002). That is, it was not necessary to replace all (or even most) breeders to minimize inbreeding in a captive breeding program. Furthermore, the effect of replacement rates on F became even less important as the number of breeders in the program increased (Duchesne & Bernatchez 2002). Consequently, Bernatchez (2009) recommended that >33% of breeders be replaced annually in supplementation programs for Atlantic Salmon populations in the Province of Quebec (Canada).

Effective Population Size for Maintaining Diversity

> *"Maintaining genetic diversity to allow populations to evolve in response to changing environments is theoretically plausible, but it is not yet supported empirically." (Neff et al. 2011)*

Supportive breeding programs that produce many individuals can result in a false sense of program success if the number of breeders that actually contribute to production of the following generation is low and unchanging, as this will result in a much lower Ne than Nc size (Ryman and Laikre 1991, Ryman et al. 1999). If the Ne of a population maintained through supportive breeding does not increase at a continuous and substantial rate, inbreeding and genetic drift will increase (Wedekind and Müller 2004, Consuegra & Nielsen 2007). The '50-500 rule of thumb' is often cited to represent the effective population sizes needed to: 1) avoid the effects of inbreeding (Ne > 50) and 2) maintain the ability of the population to evolve and adapt to environmental change (Ne > 500; (Franklin 1980, Ryman et al. 1995, Wedekind and Muller 2004, Consuegra & Nielsen 2007, Jamieson and Allendorf 2012).

The minimum Ne of 50 has been considered necessary to avoid the effects of inbreeding in the short term (i.e., over 5 generations) and is based on a rate of increase in the inbreeding coefficient of 1% per generation (Jamieson and Allendorf 2012). This rate is thought to be counteracted by natural selection (Franklin 1980, reviewed in Consuegra & Nielsen 2007). When Ne < 50, it is less likely that natural selection can counter the fixation of deleterious alleles, a process termed "mutation meltdown" (Consuegra & Nielsen 2007). An Ne of 500 has been considered necessary to prevent loss of genetic variation needed to adapt to environmental change (i.e., the variation needed to retain the "evolutionary potential" of the population). This is because at Ne = 500, mutation should replace variation lost to genetic drift for traits that are selectively neutral (Franklin 1980, Consuegra and Nielsen 2007, Jamieson and Allendorf 2012). According to Jamieson and Allendorf (2012), an Ne of 500 should (in general) equate to a census population of c. 5,000 individuals. For Atlantic Salmon, an Ne = 500 should be maintained by 100-167

effective breeders (Nb) per year, based on mean generation time of 3-5 years (p. 247, Consuegra & Nielsen 2007).

There has been debate in the literature recently regarding the continued use of the 'the 50-500 rule of thumb' for guiding captive breeding and supplementation programs, especially for species like Atlantic Salmon that are highly fecund and experience large variance in population size among generations (Consuegra & Nielsen 2007, Jamieson and Allendorf 2012, Frankham et al. 2014). The papers that first proposed 'the 50-500 rule-of-thumb' were published more than 30 years ago and came from studies with domestic and laboratory animals, as well as from the opinion of animal breeders (Franklin 1980, Soulé 1980, Jamieson and Allendorf 2012, Frankham et al. 2014). Jamieson and Allendorf (2012) argue that the 50-500 rule is still relevant to avoiding inbreeding depression and maintaining adaptive potential. However, they do indicate that these numbers should be used as a guiding principle to identify populations that are likely to suffer adverse genetic problems, rather than as "specific numeric goals" to which managers must be held accountable or used as thresholds to predict risk of extinction.

Frankham et al. (2014) counter that because the effects of inbreeding depression are generally more pronounced in stressful environments (i.e., in the wild) than in benign environments (i.e., in captivity), the findings from studies using captive animals may not be applicable, and worse, may even underestimate the risk of inbreeding depression for imperiled wild populations with Ne = 50 (Fox & Reed 2010). In a relevant study from the salmonid literature, Thrower and Hard (2008) observed that the more stressful marine rearing phase was associated with substantial inbreeding depression for inbred (full sibling) relative to control (non-inbred) anadromous *O. mykiss*, despite no apparent inbreeding depression during the freshwater rearing stages.

Frankham et al. (2014) stress that any population that has an inbreeding coefficient greater than zero is at risk for negative impacts on fitness, and inbreeding depression has been observed even in laboratory populations with Ne ≥ 50 (Frankham et al. 2014). Their review also draws on empirical and theoretical studies to conclude that maintaining "initial evolutionary potential in perpetuity" requires an Ne of 1,000 instead of 500 (Frankham et al. 2014). The authors make several recommendations regarding the 50-500 rule, among which are: 1) that an Ne of 100 replace the minimum Ne of 50 (in order to reduce the risk of inbreeding over the short term); 2) explicitly defining the "short term" as five generations; 3) that an Ne of 1,000 replace the Ne of 500 for maintaining evolutionary potential; and 4) genetic diversity should be managed to maintain reproductive fitness (Frankham et al. 2014).

Despite the different conclusions reached by Allendorf and Jamieson (2012) and Frankham et al (2014) regarding the continued validity of the 50-500 rule, they do seem to agree that these minimum values represent a starting point, not a hard and fast rule for managing genetic diversity in imperiled populations. Determination of the Ne needed to maintain evolutionary potential in a

population may better be informed using genetic models based on mutation, selection, drift, and empirical data from the population in question (Frankham et al. 2014).

Extant Genetic Diversity in the Gulf of Maine Atlantic Salmon

Historically, the North American range of Atlantic Salmon extended from Ungava Bay (Quebec, Canada) in the north to the Housatonic River (Connecticut, USA) in the south. Southern populations have been extirpated, and the only remaining viable Atlantic Salmon runs within the United States occur in Maine. The Gulf of Maine Distinct Population Segment (GOM DPS) of Atlantic Salmon was federally listed as endangered on December 17, 2000 (65 FR 69459). Under the 2000 listing, the DPS included all naturally reproducing populations from the lower Kennebec River to the mouth of the St. Croix River, as well as hatchery broodstock and their captive progeny. Within these boundaries, Atlantic Salmon occurred in the Dennys, East Machias, Machias, Pleasant, Narraguagus, Ducktrap and Sheepscot Rivers and Cove Brook and were extirpated from at least 14 small coastal rivers. Following subsequent status review (Fay et al. 2006), the range of the GOM DPS was expanded in 2009 to encompass all naturally reproducing anadromous Atlantic Salmon from the Androscoggin River northward to the Dennys River (and associated hatchery populations). This expansion to the DPS included the Penobscot, Kennebec and Androscoggin Rivers that were not part of the 2000 listing (74 FR 29300, 2009). Currently, the DPS is being maintained by a conservation hatchery program, which has succeeded in preventing extinction but has not led to establishment of self-sustaining populations (USFWS and NMFS 2018), the attainment of which is a critical measure of success for salmon restoration (Lennox et el. 2021).

Hatchery supplementation of Atlantic Salmon in Maine has a long history - in fact, the first public salmon hatchery in the United States, USFWS Craig Brook National Fish Hatchery (CBNFH), opened in Maine during the late 1800s (Fay et al. 2006). CBNFH is one of two federal hatcheries used to this day for propagating GOM DPS salmon. Hatchery supplementation took on a variety of forms over the intervening years, and in 1991, a river-specific fry-stocking program was implemented to allow natural selection to operate on all but the earliest life-history stages in an attempt to protect genetic integrity and population structure (Moring et al. 1995, Fry et al. 2006).

Direct evidence indicates that in addition to being genetically distinct, local adaptation remains within the river-specific stocks of the GOM DPS despite generations of hatchery intervention (Wilke and Kinnison 2011; Sheehan et al. 2005). Sheehan et al. (2005) found evidence of a genetic basis for phenotypic differences between stocks from three DPS rivers (Dennys, Machias, and East Machias) when reared to maturity under common garden conditions. When reared in marine sea cages, the three stocks exhibited differences in growth potential. Further, Machias-origin individuals exhibited a different morphological phenotype than Dennys and East Machias individuals did. Specifically, Machias-origin salmon had a deeper head, elongated trunk, and caudal peduncle that was both shortened and narrower compared with Dennys and East Machias-origin salmon (Sheehan et al. 2005). The authors hypothesized that this fusiform body

shape may be an adaptive response to “Bad Little Falls”, the challenging waterfalls that salmon must pass to reach spawning grounds in the Machias River, a feature that is absent from other DPS rivers (Sheehan et al. 2005).

In a subsequent common garden experiment, Wilke and Kinnison (2011) demonstrated adaptive diversity in fitness-related traits among DPS rivers. Female salmon from the Machias, East Machias, Narraguagus, Dennys, Sheepscot, Pleasant, and Penobscot rivers were spawned at CBNFH and their eggs reared in a common garden setting and observed until 275-degree days post-hatch. The authors observed significant differences among populations in the relationship between egg size and ovarian mass (Wilke and Kinnison 2011). Time to hatching (measured in degree-days) also varied among populations, with the Narraguagus River stock requiring the fewest and the East Machias stock the most degree-days. Further, size-at-age varied among populations, even when maternal effects were accounted for (Wilke and Kinnison 2011). The results of these two studies have formed the basis for continued river-specific management of the GOM DPS.

Within the GOM DPS, nine river-specific distinct individual populations were recognized as remaining at the time of the expanded listing (74 FR 29300). The remaining seven are currently supported by the conservation hatchery program run by USFWS (USASAC 2022), all of which currently operate in a river-specific broodstock program, in alignment with contemporary stocking recommendations (NASCO 2024; Lennox et al. 2021). That is, broodstock originate from the same river where offspring are ultimately stocked as eggs, fry, parr, or smolts. Salmon in the other two river-specific populations (Cove Brook and the Ducktrap River) are not supported by hatchery supplementation.

The Penobscot River distinct population is maintained by a sea-run broodstock program, whereby reproductive adults returning from sea to spawn are collected at a facility located at the first hydroelectric facility encountered on their return migration (previously Veazie Dam, now Milford Dam). They are then transferred to CBNFH where they are spawned in single paired matings, occurring within the same broodstock year class using a spawning optimization program designed to avoid breeding close relatives and limit the accrual of inbreeding (Coombs et al. 2004). Offspring of sea-run broodstock are stocked in the Penobscot River at the eyed-egg, fry, parr, and smolt stage and are used to create a domestic broodstock line at GLNFH. The majority of returns originate from smolt stocking (USASAC 2023, Stevens et al. 2019).

The remaining six river-specific populations currently supported by the conservation hatchery program (Dennys, East Machias, Machias, Pleasant, Narraguagus, and Sheepscot rivers) are maintained by parr-based broodstocks (USASAC 2023). Biologists collect broodstock as 1+ or older parr stage via backpack electro-fishers and transfer them to CBNFH where they are reared to maturity. Adults are then spawned in single paired matings using the spawning optimization

program (Coombs et al. 2004), but across year-class crosses are prioritized as is possible based on mature individuals.

Currently, 18 microsatellite loci are used to monitor allelic diversity and Ne for each of the seven river-specific broodstock within the GOM DPS (USASAC 2018). This is an increase over the 11 loci previously used for this population and is at the high end of the range reported in the literature from other salmonid conservation programs. The Ne for all GOM DPS river-specific stocks is below the lower threshold considered necessary for adaptation to environmental change (Ne = 500; Franklin 1980, Consuegra & Nielsen 2007, Jamieson and Allendorf 2012), and none are close to the upper threshold of Ne = 1000 proposed more recently by Frankham et al. 2014). Only the Penobscot River sea-run broodstock has been managed for an Ne approaching 500, and although this stock may be less at risk for inbreeding than the parr-based broodstocks, it is at risk for loss of genetic diversity due to challenges meeting broodstock collection targets in recent years (USASAC 2023, 2025).

Lage and Kornfeld (2006) analyzed genetic samples collected in the Dennys River from 1963 to 2001 and found a significant decrease in genetic diversity and Ne over a 40-year period (Lage and Kornfield 2006). Given our knowledge of the demographics of all river-specific stocks in the DPS, diminished genetic diversity and Ne in each founding population is expected as well. But to date, similar analyses incorporating historical and current samples have not been conducted to identify loss of genetic diversity for the other GOM river-specific stocks. Therefore, we concur with the recommendations of Lage and Kornfield (2006), that use of banked scale samples and ancient DNA extraction techniques should be applied to compare historic and contemporary genetic diversity across the GOM DPS. As discussed previously, there are methods that can be used to increase Ne in captive breeding programs (i.e., genetic rescue, breeding across year classes) and there has been success in increasing Ne within river-specific GOM DPS broodstocks when targeted efforts (e.g., pedigree lines) were employed (Bartron et al. In Press).

Moving Towards Recovery for Gulf of Maine Atlantic Salmon

The final Recovery Plan for the 2009 listing of the GOM DPS of Atlantic salmon identifies four phases of recovery needed to downlist the DPS from endangered to threatened and ultimately move the DPS off the US Endangered Species List (USFWS and NMFS 2018). But moving forward through the phases of recovery will require shifts in the hatchery strategies and genetic management methods employed (e.g., Fish et al. 2015), as well as in the genetic monitoring methods used to assess the program. Therefore, in this section, we define each phase of recovery and identify some of the genetic monitoring techniques described within in this document that may help attain the objectives needed for reclassification and delisting.

PHASE 1:

In the first phase of recovery, actions focus on identifying threats to and habitat needs of the species, while the conservation hatchery program operates largely to prevent extinction (USFWS and NMFS 2018). Since the expanded listing for the GOM DPS in 2009, the conservation hatchery program has operated in this "prevent extinction" mode, with hatchery strategies (parr-based and sea-run based broodstock programs, as described previously) designed to increase redundancy, resiliency, and representation among the locally adapted stocks that constitute the DPS. Genetic monitoring approaches employed enable assessing and addressing risks associated with environmental variation, demographic variation, or changes in genetic diversity. Consequently, the current methods used for monitoring genetic diversity of the GOM DPS (observed and expected heterozygosity, allelic diversity, inbreeding coefficients, estimates of relatedness, Ne, and hierarchical comparisons of differences in allele frequencies) is appropriate for this phase of recovery.

PHASE 2:

The GOM DPS can be characterized as having moved into Phase 2, during which actions focus on abating the threats to the DPS identified in Phase 1 (USFWS and NMFS 2018). The role of the conservation hatchery program shifts to ensuring the persistence of the DPS, such that reclassification from endangered to threatened is possible at the end of Phase 2. Hatchery programs associated with Phase 2 must be geared toward creating sufficiently resilient wild populations to withstand foreseeable long-term stresses. Broodstock strategies employed are those that minimize the loss of adaptive traits, reduce the possibilities of domestication by ensuring some wild exposure/natural selection among brood-lines, and counter negative effects of small population size (see Figure 2 in Fisch et al. 2015). Given that the GOM DPS river-specific stocks have been maintained by the hatchery program for more than 20 years (more than five salmon generations), have been maintained at $Ne \leq 500$, intensive intervention may be needed to rebuild diversity and adaptive potential to attain a self-sustaining population. Implementing intensive strategies would require maximizing current capacity in existing hatcheries and necessitate building partnerships, collaborations, and new facilities to accommodate greater numbers of broodstock and/or employ new approaches to rearing (e.g., smolt-to-adult supplementation, Fraser 2008).

Genetic rescue could be applied in Phase 2 to increase genetic diversity and reproductive fitness within the GOM DPS (Fitzpatrick et al. 2023, Frankham et al. 2017, Conrad et al. 2013, Thrower and Hard 2008). Comprehensive guidelines for the implementation of the genetic rescue approach have been outlined by Frankham et al. (2017), and genetic rescue could be prudently applied to the GOM DPS in experimental-scale controlled crosses that minimize risk to river-specific lines during Phase 2. Genetic rescue has been demonstrated to reduce relatedness and increase fitness for another critically endangered salmonid, the Central California Coast Coho Salmon *(Oncorhynchus kisutch).* In a long-term study of outcrossing between individuals from the

Russian River and Olema Creek, hybrids exhibited greater survival to eye-up, hatch, and swim-up stages and lower rates of deformities in the hatchery, while concomitant field-based survival studies showed increased survival for hybrids released into streams, without showing evidence of outbreeding depression (Pregler et al 2022). Shifting management practices to increase the target Ne closer to the "100-1000 rule" would reduce the risk of inbreeding and increase the chances of maintaining evolutionary potential. Increasing Ne could be accomplished by increasing the number of breeders or crossing year classes (e.g., through reconditioning and retaining breeders for more than once season or reconsidering the use of precocious parr). Retaining landlocked salmon that made an ocean migration in the Penobscot sea-run broodstock could also serve to increase Ne and increase genetic diversity (Eronen et al. 2022).

In Phase 2 of recovery, measuring genetic quality along with the currently assessed genetic diversity would facilitate monitoring for adaptive potential. Genomic datasets provide more information than the neutral variation measured by microsatellites (the current practice for the GOM DPS), and are more useful for informing the design, implementation, and assessment of genetic rescue approaches (Allendorf et al. 2010; Fitzpatrick et al. 2023). Specifically, genomic data would be useful to identify both which river-specific stocks would benefit the most from genetic rescue, and the best donor stocks (Fitzpatrick et al. 2023). Inclusion of markers for adaptive traits or adding measures of reproductive success would help to ensure that genetic diversity is being managed to maintain reproductive fitness in the GOM DPS. Genes with adaptive potential in Atlantic Salmon that have also shown heritability include resistance to infectious pancreatic necrosis virus (reviewed by Houston and Macqueen 2018), run timing (Cauwelier et al. 2018), sea at maturity (Johnston et al. 2014, Barson et al. 2015), thermal tolerance, and growth rates (Benfy et al. 2024). Further, better understanding the effects of the long-term hatchery supplementation that has been used to prevent extinction would benefit from fitness-based measures, for example studies of reproductive success or shifts in phenotype that would provide evidence of domestication selection (Fraser 2008). Given concerns over inbreeding depression, (particularly in the parr-based broodstocks), time-series analysis of phenotypic markers of reproductive capacity, particularly those related to maternal effects (e.g., fecundity, hatch rates, fry survival) offer a relatively low-coast approach that would aid in understanding the viability of these stocks going forward.

PHASE 3:

In Phase 3 of recovery, the recovery actions focus on increasing the naturally reared component of the DPS. Ideally, phase 3 should terminate with the delisting of the DPS. The role of the conservation hatchery programs shifts to supporting natural reproduction, with the goal of increasing abundance and distribution of naturally reared Atlantic Salmon. Monitoring reproductive success (e.g., relative mortality rates and relative reproductive success of hatchery and natural components of the population) becomes increasingly important to ensure that broodstock management has the desired outcome of maximizing natural production (e.g.,

Deschenes and Bernatchez 2002; Christie et al. 2014). As the population increases, standard productivity measures (e.g., smolt-to-adult survival) become more reliable as indicators of fitness and the capacity of the DPS to recover after hatchery supplementation ends and delisting occurs.

PHASE 4

By Phase 4, all freshwater and marine habitats have demonstrated potential to support a self-sustaining population of Atlantic Salmon and the role of hatcheries shifts to minimize the interactions between wild and hatchery-origin fish. Ford (2002) states that the only way to avoid reducing fitness of the wild population is to have a completely segregated program where all hatchery fish are recaptured and spawned in captivity and not allowed to mix with wild spawners. Thus, in Phase 4, hatchery supplementation for the purpose of recovery would be phased out, and hatchery programs could be re-prioritized to re-establish populations in rivers outside of the critical habitat of the DPS to further promote resiliency, conduct post-delisting assessment (e.g., fish passage studies) or rebuild a constituency for salmon through outreach activities (e.g., salmon in the classroom) that connect people with salmon (Liebich et al, 2018, Flye et al. 2025). Maintaining brood-lines within the hatchery program as a live gene bank would serve as an insurance measure to protect against unforeseen circumstances during Phase 4 (Lennox et al. 2021). Genetic monitoring in Phase 4 should prioritize measures of reproductive success, as well as the continued monitoring of genetic diversity and productivity measures; genomics and epigenetics approaches also may be incorporated as program needs and research questions necessitate.

CONCLUSIONS AND RECOMMENDATIONS

The 2002 National Research Council review concluded that “Maine salmon in DPS rivers are not mainly hatchery mixtures but rather show typical metapopulation structure that characterizes wild populations of salmon” (NRC 2002). Their conclusion was based on characterizations of neutral makers, which they stated was circumstantial and they were unable to differentiate between “thousands of years of natural selection” or “five to six generations of genetic drift.” Since this time, hatchery operations have prevented extinction and appear to have, for the most part, conserved existing genetic diversity as measured by 11-18 neutral markers (USASAC 2024). However, these conservation stocking programs have not rebuilt abundance (USASAC 2024) as projected by Legault (2004) and demographic risks and genetic/sampling drift related to small populations (NRC 2002, Lage and Kornfield) remain a concern. As highlighted in this review, the tools, techniques, and understanding of genetic impacts on recovery potential of imperiled salmonids have advanced considerably in the last 25 years. Threats to both DPS habitat and hatchery operations posed by a rapidly transforming climate require immediate evaluation and implementation of bold and creative strategies for broodstock management and stocking practices for managers to advance recovery. Kocik et al. (2022) explored conceptual options and opportunities for salmon in their southern range using the resist, accept, or direct decision-making

tool that is designed to aid resource managers in development of informed strategies for responding to ecological change.

In our assessment, current strategies for resisting changes in small populations may be compromised by the limited genetic scope at the river-specific level of founder hatchery populations (NRC 2002; Lage and Kornfield 2006). As such, efforts to direct population structure by implementing genetic rescue via crossing river-specific populations within the DPS deserve consideration given demonstrated benefits to survival and fitness in other highly endangered southern salmonine populations (Conrad et al. 2013, Pregler et al. 2022).

Moving the GOM Atlantic Salmon DPS into a recovery-oriented program will require cross-agency collaboration among state, federal, and Tribal partners. Status-quo conservation measures have not rebuilt abundance nor significantly mitigated extinction risk in wild populations. The rapidly transforming ecosystems of the Northwest Atlantic Ocean and Maine rivers pose immediate thermal challenges to both river temperature regimes and water sources at existing hatcheries. We believe it is time to fully evaluate these ongoing changes in an open, transparent, and collaborative process, in a forum such as the Center for Independent Experts. A willingness to evaluate and implement new approaches to broodstock management, stocking, and genetic assessment could expand both the demographics of hatchery supplementation but importantly the effective population sizes across the entire DPS. Given the genetic assessment toolkit of 2026 compared to 2002, it is time for a peer-reviewed broodstock management plan that is forward thinking and provides fish with the adaptive scope necessary for persistence and recovery within the Gulf of Maine DPS of Atlantic Salmon in a transforming climate with reconnected watersheds.

ANNOTATED GLOSSARY

In this annotated glossary, we define the terms presented throughout the preceding sections and provide examples of how their application in supplementation and captive rearing programs for North American salmonids.

Allelic Richness/Allelic Diversity

The allelic richness (also referred to as allelic diversity) of the population is obtained by computing the mean number of unique alleles per locus. Allelic richness is a sensitive indicator of whether genetic bottlenecks have occurred in the population and is useful for inferring the capacity for the population to adapt to future change (O'Reilly and Doyle 2007; Frankham et al. 2017). But allele frequencies can change considerably without any loss of alleles (Schwartz et al. 2006). Therefore, monitoring temporal changes in allele frequencies to track trends in allelic richness over time is a more sensitive measure for evaluating the success of captive breeding programs than simply monitoring loss of allelic diversity (Schwartz et al. 2006). It should be noted that populations with similar allelic diversities can differ in genotypic diversity depending on the family structure and degree of inbreeding in each population. Therefore, genotypic diversity is also worth considering, in addition to monitoring allelic richness/diversity (Stoddart and Taylor 1988).

Observed and Expected Heterozygosity

Observed heterozygosity is defined as "the number of heterozygotes as a proportion of the total individuals typed", whereas expected heterozygosity is "the proportion of heterozygotes expected from the allele frequencies under random mating, based on Hardy-Weinberg equilibrium" (Frankham et al. 2017). Heterozygosity is insensitive to the loss of rare alleles; thus, it is less sensitive than allelic diversity for indicating bottlenecks and loss of genetic diversity (Schwartz et al. 2006, Conseguera & Nielsen 2007).

Conseguera & Nielsen (2007) suggest that heterozygosity is correlated with fitness, thus has been used to infer the potential for a population to adapt evolutionarily. Conversely, Neff et al. (2011), argued that aside from cases involving small, inbred populations, links between heterozygosity and fitness were not well supported with empirical data. A recent review of the Inner Bay of Fundy Live Gene Banking Program for Atlantic Salmon reported that no significant relationship was found between offspring heterozygosity and survival to Age-1 following release into freshwater (DFO 2018). In a captive breeding program for Coho Salmon *Oncorhynchus kisutch* on the Russian River (California), the effect of parental heterozygosity (internal heterozygosity) did not consistently influence embryo survival to different developmental stages (hatch, eye-up, and swim-up) among brood years (Conrad et al. 2013).

Neutral Genetic Markers

Early studies of genetic differentiation, (for example, the studies that showed genetic differentiation between North American and European Atlantic Salmon), were based on different variants of allelic enzymes, referred to as allozymes (NAS 2002, Waples et al. 2020). Allozymes have subsequently been replaced by nuclear DNA markers, e.g., microsatellite loci and SNPs (NAS 2002, Frankham et al. 2017).

Mitochondrial DNA (mtDNA) is the circular chromosome contained within the mitochondria of cells. Mitochondria are passed from mother to offspring, so mtDNA can be used to infer relationships within maternal lineages. Variation in mtDNA haplotypes supports the different genetic lineages between European and North American Atlantic Salmon reported using allozymes (NAS 2002). Mitochondrial DNA is more subject to drift than nuclear DNA, which produces stronger apparent differentiation between populations. However, because recombination does not occur in mtDNA, it is a poor measure of within-population genetic variation.

Microsatellites and SNPs are putatively non-coding (hence, neutral) segments of nuclear DNA that are commonly used for monitoring genetic diversity. Microsatellite loci are short segments of DNA comprised of repeating pairs of bases, e.g., CTCTCTCT (Hansen et al. 2007). Microsatellites replaced allozymes as the most used marker for measuring genetic variation because they are highly variable and the genotypes of individuals can be directly sequenced (NAS 2002, Frankham et al. 2017). Single nucleotide polymorphisms (SNPs) are single base substitutions in nuclear DNA that occur at a high frequency (Hansen et al. 2007). SNPs are replacing microsatellites for some population genetic applications because of several advantages they offer. While new microsatellite primers generally need to be developed for each locus in a species, SNPs can be screened using generic sequencing methods (Frankham et al. 2017). Further, because more loci can be included, use of SNPs can result in greater statistical power than use of microsatellite loci (Frankham et al. 2017). However, both microsatellites and SNPs are appropriate for measuring neutral variation and estimating allelic richness and heterozygosity. Large microsatellite panels (on the order of c. 100 loci) still represent a good return on investment for constructing pedigrees, individual assignment, and parentage-based tagging (I. Bradbury, *pers. comm*).

It should be noted that transitioning between the use of microsatellites and SNPs requires careful planning and can be costly. This is because of the need for a transition period when both maker types are used, to prevent the loss of baseline data collected with the previous method. Therefore, the costs associated with switching from microsatellites to SNPs involves not only the purchase of new equipment but also the costs of simultaneously conducting analyses with both marker types.

Adaptive Genetic Variation

Unlike the molecular markers discussed previously (allozymes, mtDNA, microsatellites, and SNPs), which are thought to be selectively neutral, genes under selection should confer an adaptive advantage to individuals, resulting in increased reproductive success or fitness when they are exposed to a specific environment (Hansen et al. 2007).

Major Histocompatibility Complex

Major histocompatibility complex (MHC) genes are involved in immune response and were among the first of the coding genes to be studied in salmonids (Hansen et al. 2007). In Atlantic Salmon, MCH genes have been linked to resistance to furunculosis and to mate choice (Langefors et al. 2001, Landry et al. 2001). Atlantic Salmon that were allowed free-mate choice had greater than expected MHC-dissimilar mating (i.e., individuals chose mates with different MHC genes), which is thought to be mediated through olfactory cues (Landry et al. 2001, Landry & Bernatchez 2001). They exhibited greater heterozygosity at MCH-loci, lower parasite loads, and resistance to a greater number of diseases (Landry and Bernatchez 2001, Consuegra and Garcia de Leaniz 2008, Neff et al. 2011). Consequently, heterozygosity at MCH loci could be used as a measure of fitness in captive breeding or supplementation programs.

Genomics

Recent advances in gene sequencing and genomics have given rise to the field of conservation genomics, i.e., the use of genomic techniques to solve problems in conservation biology (Allendorf et al. 2010). Waples et al. (2020) provide a useful review of the use of genomics approaches for salmon conservation. For neutral population parameters (e.g., SNPs), genomics simply provides (many) more markers, which can improve the precision and accuracy of estimates of Ne, heterozygosity, inbreeding, and relatedness (Waples et al. 2020).

Advances in genomics have also led to the identification of functionally important genes, i.e., genes linked to traits that directly impact fitness. Genomics, therefore, offers promise for shedding light on the genetic basis for local adaptation and inbreeding depression, predicting outbreeding depression, and identifying adaptation to captivity (Allendorf et al. 2010, Waples et al. 2020). Genomics also makes it possible to track changes in adaptive genetic variation and even target certain genetic variants for management action (reviewed by Pearse 2016). Genomics therefore provides the dual advantages of estimating neutral variation and identifying genome regions under selection (e.g., genes associated with local adaptation) simultaneously (Allendorf et al. 2010). However, conservation genomics approaches require a large investment in time and money for start-up and the bioinformatics needed for storage and analysis of such large volumes of data (Allendorf et al. 2010).

Run timing (Hess et al. 2016) and age at maturity (Barson et al. 2015) are two traits that have been linked to single genes in salmonids, which could serve as candidates for tracking changes in adaptive variation over time in managed salmonid populations. For example, a decrease in sea-age at maturity observed in a 36-year data set for Atlantic Salmon was evidenced genetically; over the same period, the population exhibited a significant decrease in an allele associated with older age at maturation (reviewed by Waples et al. 2020). Although these genes play a large role in the expression of a clear phenotype, environment, ecology, and other genetic or epigenetic influences also play a role (Pearse 2016). Additionally, genes that are adaptively important now may not be the genes that will be important in the future under different environmental conditions (Allendorf et al. 2010). Favoring specific adaptive loci for management purposes could also inadvertently cause loss of variation at other loci (Allendorf et al. 2010, Pearse 2016).

We still have much to learn when it comes to understanding the genetic basis for adaptive variation. Consequently, Pearse (2016) urges caution in the use of adaptive genetic variation for management and conservation purposes, and Allendorf et al. (2010) make the case for more long-term studies designed to assess links between genetic variation, fitness, and population growth. An integrated approach, whereby monitoring of neutral loci for genetic diversity is combined with monitoring a set of selected candidate adaptive loci for changes over time may represent a safer path forward than focusing solely on adaptive or neutral variation for conservation and management planning purposes (I. Bradbury pers comm.).

Epigenetic markers for genetic management of hatchery populations

Advances in the field of epigenetics may provide another means for genetic management of hatchery populations. Epigenetic mechanisms are those which do not change the DNA sequence itself but change how the DNA is expressed into proteins. This can occur by addition of methyl groups to the DNA sequence (alteration of methylation patterns) or modifications to the histone proteins that hold the DNA into its coiled chromatin form (Jablonka & Lamb 2002, Bannister and Kouzarides 2011). Consequently, epigenetic variation can result in phenotypic variation, even when measures of neutral genetic variation suggest the absence of population genetic structure (Allendorf et al. 2010, Fraser 2016, Le Luyer et al. 2018, Jonsson and Jonsson 2019).

Epigenetic changes can be environmentally induced. There is increasing concern that epigenetic modification of gene expression due to early life history spent in hatchery environments may contribute to the reduced fitness observed in hatchery-origin salmonids (Fraser 2016, Le Luyer et al. 2018, Jonsson and Jonsson 2019). Epigenetic differences have been documented between hatchery-origin and natural-origin Coho Salmon (Le Luyer et al. 2017) and steelhead (Gavery et al. 2018), despite a lack of significant genetic differentiation between wild and hatchery individuals originating from same river, indicating that the hatchery and natural-origin fish were indeed one population (Le Luyer et al. 2017, Gavery et al. 2018).

Hatchery-reared Coho Salmon exhibited hypermethylation of DNA in regions associated with ion homeostasis, synaptic and neuromuscular regulation, immune and stress response, and control of swimming functions, which could drive fitness reductions observed in hatchery populations (Luyer et al. 2017). Gavery et al. (2018) compared DNA methylation patterns in red blood cells and sperm of returning natural-origin and hatchery-origin adult steelhead. The observed difference in DNA methylation patterns between natural-origin and hatchery-origin steelhead were specific to cell type (Gavery et al. 2018).

In a follow-up study under controlled experimental conditions, Gavery et al. (2019) demonstrated that early-rearing environment (hatchery versus stream) had a strong effect on DNA methylation patterns in steelhead liver tissue. Once moved into a common hatchery rearing environment, DNA methylation patterns in liver cells became more similar, indicating that there is some plasticity in epigenetic modification of DNA: whereas some changes are reversible, others may persist and continue to affect gene expression (Gavery et al. 2019). The authors also found that relatedness had a greater effect on DNA methylation patterns in sperm than did the early-rearing environment. Consequently, the authors urged that studies designed to investigate links between the environment, phenotype, and epigenetics should account for relatedness among individuals in addition to accounting for tissue-specific and temporal differences in DNA methylation (Gavery et al. 2019).

The findings of Gavery et al. (2019) highlight the fact that research relating epigenetic modifications to phenotypic changes and fitness in salmonids are in the fledgling stage. As such, metrics are not developed enough to include among the standard measures used for genetic management of hatchery populations. Some of the factors that are known to differ between hatchery and wild rearing environments (e.g., temperature, diet, stressors) have been linked to differential DNA methylation patterns and may affect individual's capacity to adapt to changing conditions (Burgerhout et al. 2017, Moghadam et al. 2017, Jonsson and Jonnson 2019). Consequently, observed epigenetic differences between hatchery-origin and natural-origin Coho and steelhead support use of hatchery procedures that "naturalize" the rearing environment at the earliest life history stages.

Fitness-Related Measures

Standard genetic monitoring using neutral loci can identify changes in neutral variation but will not identify maladaptive changes, necessitating evaluation of measures of population productivity or other fitness-related measures (Neff et al. 2011, D. Fraser, pers comm.). Consequently, considerable value still lies in assessing measures of population productivity, as well as in measuring and tracking phenotypes over time, despite the availability of conservation genetic and conservation genomic techniques for monitoring hatchery supplemented populations. Pearse (2016) highlights the fact that maintaining the genomic complexity necessary to retain given phenotypes may be adequately accomplished without resorting to directed management for

specific adaptive loci. Application of well-established methods for conservation genetic management that maximize diversity (e.g., maintaining sufficiently high Ne, minimizing relatedness in mating, and reducing loss of local adaptation) combined with restoration of habitat and ecological functioning such that diverse phenotypes can be supported in nature is the simplest (and possibly best) approach to retaining phenotypic diversity in supplemented populations (Fraser 2008, Pearse 2016).

Tracking phenotypic data over time can help ensure that as much of the historic life history diversity as possible is maintained, which should inherently help preserve neutral genetic variation, whereas shifts in phenotype relative to the natural population (or a historic baseline) indicate problems that warrant more in-depth genomic tracking (D. Fraser, pers. comm). Relevant phenotypic data include but are not limited to size-at-age, size and age structure, sex ratios, fertility, fecundity, deformity rates (Fraser 2008, DFO 2018). Such metrics are relatively easy and inexpensive to obtain in the hatchery, and their effective use can even lead to better understanding of population demographic processes than monitoring of neutral diversity (Neff et al. 2011). Consistent collection and analysis of data in the hatchery is essential for identifying shifts in phenotype between natural-origin and hatchery-origin salmonids (Hard et al. 1999) and for linking individual phenotypes with population productivity measures such as reproductive success and population growth rate (D. Fraser, pers. comm).

Standard population productivity metrics, including smolt-to-adult returns and reproductive success can be used to assess fitness in captive rearing and supplementation programs, and are also useful for assessing different release strategies, rearing techniques within a program, or for evaluating the capacity of natural populations to recover after hatchery supplementation ends (Kline & Flagg 2014, Jones et al. 2018) For example, the mean SAR for Redfish Lake Sockeye salmon was found to be greater for smolts produced by adults that were released to spawn naturally (1.84%) than for smolts reared in the hatchery (0.60%) or from releases of pre-smolts (Kline & Flagg 2014).

Success of captive breeding or supplementation programs rest on the ability to produce individuals that can survive in the wild to replace themselves, leading to a growing and ultimately self-sustaining population (Evans et al. 2014; Lennox et al. 2021). Lifetime reproductive success, that is the number of returning adult offspring produced per adult provides not only an ideal metric for assessing program success but also serves as a measure of fitness.

Reproductive success has been used for evaluating several captive breeding and supplementation programs for steelhead and Chinook and Sockeye salmon (see references in Table 1). In a study conducted in Little Sheep Creek, Oregon (Snake River Steelhead DPS), where hatchery-origin and natural-origin steelhead interbreed, hatchery-origin individuals outnumbered natural-origin individuals by a ratio of 5:1. However, hatchery-origin individuals had lower reproductive success than natural-origin steelhead (Berntson et al. 2011). Similar results have been

obtained for Chinook Salmon, with hatchery-origin individuals exhibiting reduced reproductive success relative to natural-origin Chinook Salmon, when factors including return year and fish size were accounted for (Janowitz-Koch et al. 2018). Encouragingly, Janowitz-Koch et al. (2018) found that interbreeding between hatchery-origin and natural-origin Chinook was not associated with a reduction in fitness. They found that crosses between hatchery-origin and natural-origin individuals resulted in similar reproductive success to crosses between natural-origin males and females.

Reproductive success provides a useful way to evaluate tradeoffs between the demographic boost and the potential reduction in fitness provided between use of hatchery-origin individuals in salmon restoration programs (Anderson et al. 2012, Christie et al. 2012). For example, Anderson et al. (2012) compared per capita reproductive success of hatchery-origin and natural-origin Chinook Salmon spawners to assess the fitness cost of allowing hatchery-origin individuals to be included among the adults colonizing a stretch of river newly reconnected by installation of a fish ladder. They found that allowing hatchery-origin Chinook to ascend the fish ladder doubled the number of females in the initial recolonizing population, resulting in a large demographic boost, with no consistent loss of fitness as evidenced by lack of a relationship between reproductive success and origin (hatchery-origin vs natural-origin) in females (Anderson et al. 2012).

Reproductive success has also been used to identify life stages affected by fitness-related effects of hatchery propagation (Christie et al. 2014). Hatchery-origin Coho and Atlantic Salmon released as fry had greater relative reproductive success than individuals released at the smolt stage (Thériault et al. 2011, Milot et al. 2013). These findings have been used to suggest that adverse effects on fitness related to hatchery rearing become greater with the duration of hatchery rearing (Christie et al. 2014). Comparison of relative reproductive success among life stages also provided evidence that impacts of hatchery rearing on fitness may occur during fertilization or early rearing in steelhead and Chinook and Atlantic Salmon (Christie et al. 2014).

Effective Population Size (Ne) and Effective Number of Breeders (Nb)

The effective population size (Ne) was first described by Wright (1931), and is defined as follows:

> *"The number of individuals that would result in the same loss of genetic diversity, inbreeding, genetic drift, or coalescence if they behaved in the manner of an idealized population" (Frankham et al. 2017)*

where an "idealized population" is one in which the sex ratio is equal and the variance in reproductive success follows a Poisson distribution, such that the genetic drift and inbreeding is equal to the levels observed in the actual population of interest.

The effective number of breeders (Nb) is often reported instead of the effective population size. The effective number of breeders may be thought of as an annual Ne, where it represents the number of adults that contributed to a given cohort (Ruzzante et al. 2016). In species with overlapping generations and iteroparity, individuals breeding each year represent multiple age-cohorts. The effective number of breeders is generally less than the number of returning adult salmon each year, because only a fraction of adults spawning in a given year will produce surviving offspring (Waples 2002).

Census Population Size

The *census* population size (Nc) is the number of individuals in a population, generally obtained from counts, surveys, or mark-recapture methods.

Inbreeding Coefficient

The inbreeding coefficient (F) is used to measure the degree of inbreeding and represents the probability that two alleles at a locus are identical by descent. That is, the two alleles at a given locus within an individual come from a common ancestor. An individual that is not inbred will have F = 0, and an inbred individual will have F = 1 (adapted from Kalinowski et al. 2012 and Frankham et al. 2017).

Thrower and Hard (2008) found that in resident (Rainbow Trout) and anadromous (Steelhead) forms of *O. mykiss*, inbreeding at the level of full sibling did not influence fitness-related measures in freshwater (survival to age one, survival from age one to age two, weight, or length at smolting), relative to control (non-inbred) anadromous and resident *O. mykiss*. But relatedness did significantly impact survival and growth during marine rearing: for each 10% increase in the inbreeding coefficient, marine survival in was depressed by approximately 30% (Thrower and Hard 2008). Consequently, the effects of inbreeding on fitness should be evaluated across both freshwater and marine rearing phases in salmonids to fully understand the impacts and overall success of salmonid captive rearing programs (Thrower and Hard 2008).

Calculating Effective Population Size

Effective population size can be estimated either by demographic or by genetic methods. Demographic methods require high quality, detailed data that is fed into an equation depending on whether sex ratios are equal (Eqn. 1), where V_k is variance in family size (i.e., variance in reproductive success) and N is the number of individuals born each year, or not equal (Eqn. 2), where N_f is the number of females and N_m is the number of males in the population. The data needed to apply demographic methods are often not available for Atlantic Salmon (Wright 1931, Conseguera & Nielsen 2007).

Eqn. 1: $Ne = (4N-2)/(V_k+2)$

Eqn. 2: $Ne = 4N_fN_m/((N_f+N_m)$

Direct estimates of Ne can be obtained by using molecular markers to estimate random genetic drift (Conseguera & Nielsen 2007). Genetic methods are very sensitive to sampling variance, and genetic migration and non-random sampling of life stages or locations (due to subpopulation structure within catchments) can bias estimates (Conseguera & Nielsen 2007). The two primary techniques for estimation of Ne via genetic methods are the temporal method and the one-sample method. Estimation of Ne for a population is most commonly accomplished using the temporal method, which relies on changes in allele frequencies for a number of loci over time (Conseguera & Nielsen 2007). In the temporal method, a population is sampled at two points in time. There will be more random drift in smaller populations, and the random changes in allele frequencies over the interval or the loss of allelic diversity over time are used to estimate Ne (Waples 1990; Wang 2001; Wang & Whitlock 2003). The temporal method is sensitive to the number of generations between samples and the number of samples, so using several samples covering a longer time interval produces the best results (Conseguera & Nielsen 2007). However, the temporal method can be problematic for species that have overlapping generations (like Atlantic Salmon).

Although the temporal method was more common earlier on (1990-2008), the use of one-sample methods have become more prevalent (Palstra and Fraser 2012). One-sample methods are based on deviations from Hardy-Weinberg equilibrium and interlocus linkage disequilibrium, where deviations are expected to increase as Ne decreases (Conseguera & Nielsen 2007). Some of the populations in which the linkage disequilibrium estimator has been used to estimate Ne include Snake River steelhead and Chinook Salmon, and the Gulf of Maine Atlantic Salmon DPS. The effective number of breeders (Nb) has been calculated using the linkage disequilibrium method for steelhead in the Hood River canal (Berejikian & Van Doornik 2018). Wither et al. (2014) used pairwise linkage disequilibrium of alleles between loci to compute Nb for Sakinaw Lake Sockeye.

Several factors can result in an effective population size that is less than the census population size. First, when a subset of the population of reproducing adults is more fit (i.e., produces more offspring) than the remainder of the breeding population, the effective population size will be less than the total number of breeding individuals in the population (Wright 1931). Differences in sex ratios also serve to reduce Ne relative to the census size (N). The more that the sex ratio deviates from 1:1, the greater the reduction in Ne relative to N. The Ne will also be reduced in species with overlapping generations, for example, in Atlantic Salmon (Hansen et al. 2007; Conseguera & Nielsen 2007). Further, fluctuating population size (variance in Ne over several generations) will reduce Ne relative to the census size because the long-term Ne of a population is described by the harmonic mean over generations and will be less than the mean population size. The loss of genetic variation can't easily be regained when the population

increases (Hansen et al. 2007; Conseguera & Nielsen 2007). In the following example, the recorded population size over four years is 200, 250, 50, and 300. The mean census size would be 200, whereas the mean effective population size would be only 123:

$$\underline{N} = \frac{200 + 250 + 50 + 300}{4} = 200$$

$$\text{Ne} = \frac{1}{\frac{1}{200}+\frac{1}{250}+\frac{1}{50}+\frac{1}{300}} = 123.7$$

Relatedness

Relatedness metrics reflect the proportion of alleles that are identical by descent in two different individuals (Frankham et al. 2017). There are two main ways to infer relatedness: 1) pairwise methods using relatedness coefficients, and 2) likelihood approaches where pairs of individuals are classified by relationship (Blouin 2003). Relatedness measures can be derived from neutral alleles (e.g. using microsatellites) and increasingly are replacing use of pedigrees to avoid inbreeding as salmonid enhancement programs grow and maintaining pedigrees becomes a challenge due to time and available hatchery space (Kozfkay et al. 2007, Conrad et al. 2013). Estimates of relatedness are associated with high variance because there is a chance that alleles do not originate from a common ancestor, so they are not identical by descent, but simply are identical by state (Kozfkay et al. 2007).

Kozfkay et al. (2007) used empirical data from the Snake River Sockeye Salmon (*O. nerka)* Captive Broodstock Program, which was founded from fish collected as anadromous returning adults, residual adults, or smolts that were emigrating from Redfish Lake to assess the performance of four different pairwise relatedness methods and one maximum likelihood estimator. Among the pairwise relatedness methods, they found that relatedness was underestimated by Ritland's estimator (R_R), Lynch and Ritland's estimator (R_{LR}), and Queller and Goodnight's estimator (R_{QG}), whereas the allele sharing estimator (Mxy) overestimated relatedness. Ritland's estimator was associated with the lowest Type 1 error rate[1]. The lowest Type 2 error rates were found for Mxy and R_{QG}. Misclassification of individuals by the maximum likelihood method was predominantly

[1] Type I error occurs when we reject the null hypothesis when it should have been retained (i.e., stating there is a pattern in the data when the pattern/effect is due to random variation, in other words, a "false positive"). Type II error occurs when we retain the null hypothesis when it should have been rejected, i.e., incorrectly stating that a true pattern in the data originates from random variation, or a "false negative" (Gotelli and Ellison 2004).

due to classification of related fish to less related category, and overall, 74% of full-sibling pairs, and 80% of unrelated pairs were classified correctly (Kozfkay et al. 2007). Relatedness measures that minimize false positives (i.e., has lower Type I error) and underestimate relatedness may allow for greater genetic diversity in the population but increase the chance of spawning closely related individuals. Measures that minimize false negatives and overestimate relatedness (i.e., have a lower Type II error rate) reduce the risk of inbreeding at the potential cost of genetic diversity (Willis 1993, Kozfkay et al. 2007). Consequently, Kozfkay et al. (2007) concluded that relatedness estimators are best used in combination as relative measures to generate pairs for mating, rather than absolute measures of relatedness (Kozfkay et al. 2007). They suggested that maximum likelihood and R_R be used to rank individuals by genetic importance to the breeding program and Mxy and R_{QG} used to create spawning pairs (Kozfkay et al. 2007).

Matings between close kin (half-siblings or greater $R_{QG} \geq 0.25$) are avoided in the captive broodstock program for Russian River Coho Salmon using a spawning matrix based on pairwise relatedness (Queller and Goodnight's relatedness coefficient) generated from multi-locus genotype data (Conrad et al. 2013). Even though individuals were only mated if they were less related than half-siblings, there was a significant, negative relationship between R_{QG} and offspring survival at the eye-up, hatch, and swim-up stages; this indicated that less related parents had greater fitness (Conrad et al. 2013). Empirical analysis has further shown that full siblings can have Rxy below 0.3 and half-siblings can have Rxy below 0.1 (A.P. Spidle, Northwest Indian Fisheries Commission, unpublished data). In captive-rearing programs where relatedness is used mate-choice selection (e.g., Nooksack Chinook and Stillaguamish Chinook, Elwha odd-year pink salmon), crosses between individuals with Rxy>0 are avoided.

Parentage-Based Tagging

Parentage-based tagging (PBT) provides a method for marking a large number of individuals without the need for physical tags, and can be incorporated into existing programs that, like the broodstock program for the Gulf of Maine Distinct Population Segment of Atlantic Salmon (GOM DPS), which already genotype broodstock annually. The parental genotypes serve as the "marks", against which putative offspring can be compared (Anderson & Garza 2005). Offspring will have a genotypic tagging rate of 100% if all parents are genotyped and mating is tracked, which is a much greater rate than can be attained (and at a lower cost) than with other tagging methods (Steele et al. 2013). Parentage-based tagging can be used for a variety of assessments requiring the identification of individuals, including but not limited to, assessing survival of hatchery-derived offspring to different life history stages, estimating smolt-to-adult survival rates, and determining reproductive success (Steele et al. 2013).

It has been found that a panel of 96 SNPs has sufficient resolving power for assigning offspring to parents when both parents were genotyped (Steele et al. 2017). A minimum panel of 192 SNP loci and the Genotyping-in-Thousands (GT-seq) protocols developed by the Columbia

River Inter-Tribal Fisheries Commission (CRITFC) genetics lab make it possible to assign single parents to offspring, for situations where one parent was either not sampled or not successfully genotyped (Campbell et al. 2014). Columbia-basin-wide SNP panels for now incorporate 379 SNPs for steelhead and 298 SNPs for Chinook Salmon (Hasselman et al. 2018).

The challenge to start-up of a PBT program lies in identifying markers with sufficient variation to accurately assign parents when thousands of potential parents (from multiple spawn years and different populations) are being analyzed simultaneously (Steele et al. 2019). As few as 13 microsatellite loci or 150 SNP loci have been required to assign kinship in systems with three populations and the range of year-classes typical of salmonid populations (seldom more than 4 year-classes per cohort or per spawning population). With the same dataset, using PBT to tie generations together has proven more accurate than mixture modeling or individual assignment forms of genetic stock identification (A.P. Spidle NWIFC unpublished data).

Genetic Rescue

In genetic rescue, new genetic variation is introduced to a population (e.g., through crossing individuals from different stocks) that increases the fitness of the population (Frankham et al. 2017, Fitzpatrick et al. 2023). Genetic rescue can reverse loss of genetic diversity and inbreeding, increase Ne, increase survival and fitness, and reduce risk of extirpation (Frankham et al. 2017, Conrad et al. 2013, Thrower & Hard 2008, Garant et al. 2005). Benefits of genetic rescue have been observed for fitness measures including total fitness, lifetime reproductive success, survival, fecundity, population growth rates, fertilization success, and fecundity (Frankham et al. 2015). Such benefits may be particularly pronounced under the natural (i.e., stressful) rearing conditions experienced by Atlantic Salmon in the river and ocean relative to their hatchery-reared conspecifics. In a meta-analysis conducted by Frankham (2015), the median percent increase in composite fitness (reproduction and survival combined) was 148% for crosses conducted under stressful conditions in the wild and 45% under less stressful conditions in captivity.

Genetic rescue is underutilized as a tool in the recovery planning for species listed under the US Endangered Species Act (Fitzpatrick et al. 2023), potentially due the concern among resource managers of outbreeding depression (reduction in reproductive fitness) that can result from crossing the genetically impaired stock with individuals from a different genetic stock (Frankham et al. 2017). A growing body of evidence indicates that the risk of outbreeding is overstated, especially when compared with the elevated risk of extinction caused by loss of genetic variation at low population size (Allendorf et al. 2011, Frankham et al. 2017, Fitzpatrick et al. 2023). For example, in a recent meta-analysis examining the effects of crossing on various measures of fitness, Frankham (2015) found evidence of outbreeding depression in only nine of 156 examples evaluated. Furthermore, the grouping of populations into evolutionarily significant units or distinct population segments should reduce risk of outbreeding depression when crosses

occur within a unit (i.e., among rivers within the GOM DPS) as opposed to crossing individuals from different units (Frankham 2010).

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