Understanding Genetics for Successful Conservation and Restoration of Resilient Chesapeake Bay Brook Trout Populations



STAC Workshop Report September 28-29, 2021 Virtual



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Table of Contents

Introduction	1
Workshop Overview	4
Presentation Summaries	5
Major Processes that Shape Brook Trout Genetic Structure	5
A Review of 'What We Know'	6
Hatchery introgression Local adaptation Planning for future adaptation	10
Tools for Studying Genetics	14
Using Genetics to Identify Robust and At-Risk Populations	15
Genetic Rescue	16
Role of Genetics in Supporting Reintroductions	17
Environmental DNA: Utility and Application	18
Communicating to the Public and Decision Makers	19
Lightning Talks Local Adaptation Adaptation to Warming Temperature YY Brown or Rainbow Trout for Suppression of Non-native Trout	21 22
Participant Feedback and Summary of Breakout Discussions	
Key Findings	
Recommendations	
Programmatic recommendations	38
Research recommendations	20
References	39
Appendix A: Workshop Agenda	45
Appendix B: Workshop Participants	47
Appendix C: List of Figures	
Appendix D: List of Tables	
Appendix E: Participant Responses to Mentimeter Introduction Prompt	

Introduction

Traditionally, fisheries management has focused on the abundance, distribution, and size structure of populations. Although these factors remain key management interests, a large and growing body of evidence highlights the importance of genetics in conserving wild populations, especially when populations are small and isolated (Frankham et al. 2017). Local adaptations are widespread among fishes and help populations cope with specific conditions in their local environment (Fraser et al. 2011). The field of conservation genetics is highly technical and has advanced rapidly in recent years, offering a wealth of information to support Brook Trout (*Salvelinus fontinalis*) conservation and restoration. A major impediment to successfully incorporating these advances into conservation outcomes is that most fisheries managers have only a basic understanding of fish genetics and its relevance to their management decisions. Moreover, additional communication between genetics researchers and the management community may help identify outstanding needs and guide future research and management efforts.

A two-day workshop held on September 28th and 29th, 2021, brought experts in the field of fish and Brook Trout genetics together with fishery managers and practitioners to share general knowledge of fish genetics and recent scientific advances. The main objectives were to:

- 1. communicate the importance of genetic information for Brook Trout management and review key conservation genetics concepts, and
- 2. explore available genetics datasets and explain how they can be used to support management.

To achieve the Chesapeake Bay Program Brook Trout Outcome of increasing occupied habitat by 8% by (Rummel et al. 2024), genetics can be incorporated into conservation and restoration decisions. to ensure that efforts are restoring resilient populations with the genetic diversity and adaptations to survive in a future landscape that will likely be more disturbed with increased habitat stressors, including increasing stream temperatures. Communicating this information in a timely manner can support programmatic priorities and restoration decisions that are being made each day. The workshop helped to inform many current and planned restoration activities and maximize our likelihood of success through application of science-based approaches.

The workshop agenda can be seen in Appendix A and a list of participants in Appendix B.

Table 1. Management applications and associated questions related to Brook Trout genetics which were identified prior to the STAC workshop.

Management Application	Identified Management Needs/Questions
Reintroduction Efforts	What role does genetics play in identifying source stocks?
	How many fish need to be translocated?
	Should source stock be multiple cohorts?
	Should source stock be from a single population?
	Should stocking occur over multiple years or a single effort?
	What genetic metrics should be considered in source stock selection? Would mixing two populations with low genetic diversity be a viable option?
Genetic Rescue	What are the metrics needed to determine whether a population is a candidate?
	How should source stocks be identified?
	How do we determine success of genetic rescue?
	Is there a tradeoff between outbreeding and inbreeding depression?
eDNA for Monitoring	What genetic techniques are available for determining Brook Trout occupancy?
	How effective is eDNA at determining occupancy? Spatial, temporal.
	Do we need specific genetic markers in highly fragmented populations?
	Can we infer density from eDNA samples?
	What is the most cost-effective approach for eDNA collection and processing?
Managing for Persistence	What metrics are best for determining genetic population "health"?
	Can we determine the adaptive potential of populations? How do we identify local adaptations and their benefits?
	Can we solely use genetics to manage Brook Trout populations, or is demographic data still the driving force for management?
	What role should genetics play in determining "stronghold" populations? What metrics should be used?
	At what point do you declare small, isolated populations as the "living dead" and stop devoting valuable resources to them?
	Have we lost populations due to genetic issues? Is habitat loss a more imminent issue that supersedes genetic concerns?

	Have we lost genetic traits, particularly in the mid-Atlantic and southeast, due to patch isolation from logging, mining, road construction, land use, etc.?	
Hatchery Introgression/Support	Is hatchery introgression a problem?	
	Does hatchery introgression happen at a significant scale?	
	Can hatcheries be used to strip spawn wild fish and bolster cohort survival in a wild population?	
	Do consecutive years of year class failure have negative genetic impacts on the population?	

Workshop Overview

This workshop was initially planned to convene in-person at ThorpeWood in Thurmont, Maryland (https://thorpewood.org/). ThorpeWood is a 155-acre mountain retreat with 18,000 acres of adjacent public and private lands with a native Brook Trout stream. Due to COVID-19 travel restrictions, this workshop was reformatted into a fully remote activity, and participants joined via video conferencing.

The workshop started with members of the steering committee and Lori Maloney (Eastern Brook Trout Joint Venture) welcoming participants, presenting the expertise in the room, and outlining the overall objectives for the workshop. Following this introduction, Day-1 speakers spoke on the following topics: major processes that shape Brook Trout genetic structure; review of what we know; tools for studying genetics; using genetics to identify robust and at-risk populations; and genetic rescue. Three half-hour discussions were scattered throughout, which were later synthesized by workshop chair, Dr. David Kazyak (U.S. Geological Survey). Kazyak opened the second day by providing an overview of Day 1 and began the workshop by co-leading a presentation with Jake Rash (North Carolina Wildlife Resources Commission) and Shannon White (Contractor to the U.S. Geological Survey) on the role of genetics in supporting reintroductions. Other talks centered around environmental DNA and its application, strategies for communicating study results and new information to the public and decision makers, a series of short 10-minute lightning talks on the future of Brook Trout research, and a panel discussion on translating genetics to the general public.

Workshop talks are summarized in the following section. Workshop presentation slides are available on the STAC *Understanding Genetics for Successful Conservation and Restoration of Resilient Chesapeake Bay Brook Trout Populations* workshop webpage, accessible using the following link: https://www.chesapeake.org/stac/events/understanding-genetics-for-successful-conservation-and-restoration-of-resilient-chesapeake-bay-brook-trout-populations/.

Presentation Summaries

All workshop talks and panels are summarized below, with slides and recordings linked if available. At the start of the workshop, Dave Kazyak (U.S. Geological Survey), Eric Hallerman (Virginia Tech), Lori Maloney (Eastern Brook Trout Joint Venture) and Steve Faulkner (U.S. Geological Survey) provided an introduction to the effort and reviewed workshop objectives.

Major Processes that Shape Brook Trout Genetic Structure

Presenters: Eric Hallerman (Virginia Tech [VT]) and Dave Kazyak (U.S. Geological Survey [USGS])

Presentation Slides

Dave Kazyak and Eric Hallerman presented the major processes that shape Brook Trout genetic structure. At all points in their natural history, Brook Trout would have been subject to population genetics processes, i.e., mutation, migration, selection, random genetic drift, inbreeding, and coadaptation. The signatures of these population genetic processes are superimposed upon deeper population genetic patterning that arose from their natural history. North America has been subject to a cycle of glaciation and deglaciation. Glaciation caused contraction of the species' distribution into three glacial refugia. Deglaciation led to recolonization of the landscape. During post-glacial dispersal, Brook Trout crossed drainage divides through stream capture, local inland flooding, and coastal dispersal. Regarding the latter, Brook Trout are marine dispersers, and some now-separated rivers were joined during the Pleistocene, including all rivers entering Chesapeake Bay.

"Deep" population genetic patterning of Brook Trout comes from natural, historical processes, onto which recent population genetic processes add their signatures, including anthropogenic impacts. European colonization of North America — with consequent human population growth, overexploitation of Brook Trout, habitat alteration, and introduction of non-native species — led to fragmentation of Brook Trout habitat, loss of populations, and isolation of remaining populations. These anthropogenic effects have impacted the distribution and genetic structure of Brook Trout. The Eastern Brook Trout Joint Venture estimates that Brook Trout has been extirpated from 21% of the historic range. Many local populations are now isolated and subject to heightened random genetic drift and inbreeding. Smaller populations have less capacity for evolutionary adaptation.

Widespread stocking began in the mid-1800s to reverse the decline of the species. Most of the widely stocked Brook Trout strains are from the northeastern United States, and before genetic differentiation was recognized, Brook Trout of a few narrow origins were widely stocked. In addition to any ecological impacts from competition between native and introduced Brook Trout, the effects of interbreeding can range from loss of hatchery background, to stable introgression, to replacement of native populations. Could there be genetic impacts from introgression, perhaps including loss of local adaptation? Results from Rainbow Trout – including a rapid fitness decline and reduced reproductive fitness of wild-born descendants in the wild – suggest that genetic impacts of stocking hatchery Brook Trout are plausible. This raises the question of whether introgression could compromise the viability of native Brook Trout populations.

From a genetic viewpoint, Kazyak and Hallerman stated that potential goals for managing Brook Trout could be to maintain short-term viability and long-term adaptive potential, especially in the face of anthropogenic impacts, including climate change.

A Review of 'What We Know'

Presenters: David C. Kazyak (U.S. Geological Survey), Shannon L. White (Contractor to the U.S. Geological Survey), Eric Hallerman (Virginia Tech)

Presentation Slides

The current genetic structure of wild Brook Trout populations represents a snapshot in time in a long history of continual change. The genetic structure seen today is a direct result of both natural and anthropogenic forces – some of which occurred long ago and others which are much more recent (Kazyak et al. 2022). In turn, the forces which are at play today will shape the future genetic characteristics of wild populations and their capacity for adaptation. Here, presenters review the major processes that have shaped the current genetic structure of Brook Trout populations and will continue to play an important role as wild populations contend with ongoing environmental change.

Glaciation

One of the deepest signals that can be detected in the population genetics of many North American fishes, including Brook Trout, relates to the history of glaciation (Power 2002). North America has experienced a series of ice ages that have shaped our landscapes and the genetic structure of many populations (Burg et al. 2006, Zanatta and Murphy 2008, Shafer et al. 2010). The most recent glacial maximum in eastern North America occurred towards the end of the Pleistocene, approximately 20,000 years before present. Brook Trout are thought to have survived in refugia scattered around the margins of glaciers (Danzmann et al. 1998, Power 2002). There are still distinct signals of those glacial refugia manifest in the genetics of wild Brook Trout today.

Within the Chesapeake Bay watershed, thick glaciers extended into Pennsylvania during the Pleistocene (Braun 2011). Sea levels were about 100 m lower than they are today, and the Chesapeake Bay did not exist (Newell et al. 2004). Instead, the Susquehanna River flowed south through a broad valley, which likely offered greater opportunity for connectivity when compared with the present-day watershed. As glaciers retreated, they sometimes changed major drainage patterns and opportunities for connectivity (Hocutt 1979). Because of this history of glaciation, one can also infer that populations in formerly glaciated areas were founded much more recently than those in watersheds without such a history.

In the eastern United States, the signatures of past glaciation are reflected in the major genetic clusters observed in wild Brook Trout (Kazyak et al. 2022). The inferred number of genetic clusters and their boundaries vary to some degree based upon molecular markers screened, sampling distribution, and data analytical techniques (Danzmann et al. 1998, Morgan et al. 2021, Kazyak et al. 2022). However, all methods provide strong evidence that supports the presence of major genetic clusters across the range, with each cluster having been separated for a very long time, placing each on an independent evolutionary trajectory. In the largest genetic analysis of wild Brook Trout to date, Kazyak et al. (2022) presented evidence for three eastern refugia: (1)

an Acadian refugium associated with populations now found in New England, (2) a Mid-Atlantic refugium corresponding to the majority of populations in the Chesapeake Bay watershed, and (3) a Mississippian refugium in the southern Appalachians. Despite the suggestions of some authors that these regional clusters may warrant taxonomic revision, the current body of knowledge does not support such a distinction (White et al. 2021, Denys et al. 2022). Nonetheless, it is important to remember that these clusters have been separated from one another for extended periods of time and have faced different evolutionary pressures.

Watershed topology

In addition to the impacts of past glaciation, there are also clear influences of watershed topology (i.e., the layout of stream networks) on the population genetic structure (i.e., the number of populations and levels of differentiation) of wild Brook Trout (Aunins et al. 2015, Morgan et al. 2021, Kazyak et al. 2022). In general, populations within a watershed are more likely to exchange genetic material and tend to remain similar to one another. As watershed size increases and populations become separated by increasingly larger distances, reduced connectivity leads to populations that are more genetically distinct. Thus, the nested nature of watersheds leads to patterns of hierarchical genetic structuring that reflect past and present connectivity.

The Eastern Continental Drainage Divide is one of the largest such physical breaks, separating populations of the interior basin from those on the Atlantic slope (Hall et al. 2002, Morgan et al. 2021, Kazyak et al. 2022). Despite being in close spatial proximity, populations on either side of the Eastern Continental Drainage Divide are typically genetically dissimilar because they have usually been separated for a very long time (exceptions are typically associated with stream capture or human-mediated transfers).

At finer spatial scales, there are clear patterns of hierarchical genetic structure which generally correspond to watershed structure and concomitant patterns of dispersal connectivity (Beer et al. 2019a, Beer et al. 2019b, Bruce et al. 2019, Weathers et al. 2019, Morgan et al. 2021). However, the spatial scale of genetic structuring often varies across the species' range. Brook Trout show evidence of less genetic structure at higher latitudes, which were recolonized more recently following glacial retreat and where larger patches of interconnected coldwater habitat are more prevalent (Kazyak et al. 2022, Torterotot et al. 2014). In the southern Appalachians, where a lack of glaciation has allowed populations to persist for millions of years, Brook Trout typically exhibit a tremendous amount of genetic variation at fine spatial scales (Weathers et al. 2019, Kazyak et al. 2021, Kazyak et al. 2022). In other words, genetic composition varies considerably from site to site even within relatively small watersheds. In fact, over half of the observed variation in microsatellite markers occurs at the scale of 10-digit hydrologic unit code (HUC10) watersheds or smaller – i.e., very small spatial units relative to the overall range of Brook Trout (Kazyak et al. 2022). Although many Brook Trout populations are physically isolated from nearby populations, Kazyak et al. (2016) reported fine-scale population structuring in a small stream network, the Savage River watershed of western Maryland, with each tributary hosting a genetically distinct population despite an absence of physical barriers to migration. We note that there are some locations where stream capture events have bridged across watersheds (including across the Eastern Continental Drainage Divide; Hocutt et al. 1979), and in these instances, the contemporary genetic signals may reflect ancestry from the adjacent watershed (Kazyak et al. 2022).

In the Chesapeake Bay watershed, intermediate levels of genetic structure have been reported, and major drainages are associated with genetic clusters (Kazyak et al. 2022). In Maryland, nearly all wild Brook Trout populations were clearly partitioned into major drainages based on microsatellite genetic markers, providing clear evidence of the influence of watershed structure on genetic connectivity (Morgan et al. 2021). Similar patterns were reported by Beer et al. (2019a) for the Susquehanna River Basin in New York, where populations in HUC12s clustered with one another. This general pattern of hierarchical spatial structure has been observed repeatedly in studies of eastern Brook Trout (Aunins et al. 2015, Buonaccorsi et al. 2017, Weathers et al. 2019).

Hierarchical genetic structure reflects patterns of past and present connectivity. In general, populations with a regular exchange of genetic material are expected to remain similar to one another. Prior to widespread anthropogenic impacts, which created physical barriers and resulted in the extirpation of local populations, gene flow among Brook Trout populations was likely much more prevalent (Weathers et al. 2019). Brook Trout metapopulations (groups of populations linked by some gene flow, but not enough to homogenize the entire group) in interconnected watersheds would have regularly exchanged genetic material (Kazyak et al. 2015, Aunins et al. 2015, White et al. 2020). However, landscape alterations, barrier construction, and the introduction of exotic fishes have fragmented most formerly interconnected watersheds to the point where most wild Brook Trout populations south of Pennsylvania (and many further north) are completely isolated from one another with no opportunity for natural gene flow (Weathers et al. 2019, Morgan et al. 2021, Kazyak et al. 2022).

Isolation and drift

Small, isolated populations present special management considerations (Weathers et al. 2019). From a demographic perspective, these populations are more likely to become extirpated, and there is no opportunity for natural recolonization (Hilderbrand 2003). For those populations that persist, access to downstream habitats (which are often more productive) is limited, which may impact both individual fish size as well as population dynamics and carrying capacity (Petty et al. 2014., Huntsman and Petty 2014). Isolated populations may also be more vulnerable to extreme weather conditions, as access to thermal refugia and deeper pools may be restricted.

In addition to those demographic concerns, there are also important genetic considerations for isolated populations. Although gene frequencies may change as a result of natural selection, they also change randomly through time. This process is known as random genetic drift (Wright 1931) and can lead to disappearance of genes from a population and differentiation among populations. At a fundamental level, genetic drift occurs because offspring inherit but a sample of all gene variants from spawning adults.

Although ubiquitous, the rate at which gene variants are lost due to genetic drift varies widely among populations. This variability reflects how well-sampled genes are from parents to their offspring. Geneticists use the concept of "effective population size" to describe an idealized population which would experience genetic drift at the same rate as the population of interest. Although the technical considerations around effective population size are complex, for most purposes, effective population size can be simply thought of as a way to describe the rate of

genetic drift expected in a population of finite size. In large effective populations, the amount of fluctuation in gene frequencies between generations is usually very small (Kimura and Crow, 1963) because genes from the parental generation are sampled many times to produce a cohort of offspring. However, the rate of random change increases exponentially as population size declines. As a result, effectively small populations are more prone to loss of genetic diversity through genetic drift (Figures 1-3). Small effective population sizes and low levels of genetic diversity have been widely reported in many populations of Brook Trout (Beer et al. 2019b, Weathers et al. 2019, Kazyak et al. 2022; Figures 4-5), raising concerns about the viability of these populations over the long term.

In general, populations that are large and demographically stable tend to have lower rates of genetic drift, but other factors such as the sex ratio, evenness of reproductive success, variation in population size, and the extent to which generations overlap also play important roles. In many stream-dwelling wild Brook Trout populations, abundance regularly fluctuates by an order of magnitude (Kanno et al. 2016), which is expected to increase the rate of genetic drift. Reproductive success often varies considerably among individuals (Blanchfield et al. 2003, Kanno et al. 2014), further accelerating genetic drift. The ratio between census and effective population size varies widely among populations, and can be much less than one (Frankham 1995). Even populations that outwardly appear secure (e.g., those which are numerically abundant and occur in forested, protected watersheds) can still be experiencing rapid genetic drift (i.e., have a small effective population size) and associated losses of genetic diversity (Morgan et al. 2021, Kazyak et al. 2022).

The increased rate of genetic drift observed in populations of smaller effective size has major implications for conservation. Genetic drift may contribute to inbreeding depression and increase the retention of harmful mutations (Frankham et al. 2009). Genetic drift also leads to a loss of alleles (i.e., gene variants) and a loss of heterozygosity (i.e., the presence of multiple gene variants within an individual). Rare alleles are typically lost more rapidly than common ones. Compounding the challenges of managing small effective populations, the random genetic shifts in small, isolated populations reduce the effectiveness of natural selection (Reed and Frankham 2002). Such an ongoing loss of genetic diversity and reduction in the effectiveness of natural selection is problematic in the context of evolutionary potential — these populations are expected to have reduced ability to adapt to change.

Hatchery introgression

The widespread release of millions of hatchery-reared Brook Trout across the eastern United States, including within the Chesapeake Bay watershed, has had an important impact on some wild populations (Pregler et al. 2018). In many instances, stocking released large numbers of propagated Brook Trout into habitats that already supported endemic populations (Kazyak et al. 2018, White et al. 2018). Although many jurisdictions have scaled back or eliminated stocking of Brook Trout into wild populations, the genetic impacts of past stocking can be pervasive if gene flow has occurred between hatchery and wild fish.

The genetic mixtures that result from interbreeding between wild and stocked trout (i.e., introgression) can have negative impacts on local populations through outbreeding depression (i.e., reduced fitness as the result of mixing dissimilar lineages; Hindar et al. 1991, Currens et al.

1997, Rosenthal et al. 2022). Native populations have experienced selective pressures specific to their local environments and, as a result, are expected to have developed local adaptations (refer to discussion below). However, most hatchery stocks were derived from wild populations in the northeastern United States and Canada – i.e., far from the Chesapeake Bay watershed (Pregler et al. 2018). Thus, hatchery-derived Brook Trout are unlikely to possess appropriate adaptations for local waterways. Previous studies have shown ecological differences between hatchery-derived and wild Brook Trout in key traits such as growth, survival, and diet (Wesner et al. 2011). Introgression between native and hatchery-derived trout could disrupt locally adapted gene complexes and/or introduce maladaptive genes.

Although previous studies have identified populations with strong signals of introgression, the majority of Brook Trout populations in the eastern United States seem to retain much of their native genetic characteristics (Kazyak et al. 2018, White et al. 2018; Beer et al. 2019a, Bruce et al. 2019). This observation suggests that hatchery Brook Trout and/or their offspring may be poorly suited to wild habitats. Alternatively, pre- or post-zygotic mating barriers may limit introgression between native and hatchery populations. At this time, the mechanisms limiting introgression between hatchery and wild Brook Trout in many populations are not well understood. Although there are some reported correlations between stocking history and level of introgression (Bruce et al. 2020, Hargrove et al. 2022), the patterns are generally inconsistent. Moreover, introgression rates appear to vary across a range of habitats and demographic scenarios (White et al. 2018).

Local adaptation

Local adaptation occurs when natural selection acts upon heritable traits to increase the fitness of individuals in their environment. Local adaptations are widespread in fishes, and particularly well-studied in salmonids (Fraser et al. 2011). Species in the genus *Salvelinus* appear to be particularly prone to undergoing rapid evolution and adaptation, giving rise to populations with extreme morphological and physiological variation (Muir et al. 2016). Specific to Brook Trout, studies have shown local adaptations associated with morphology, spawning phenology, habitat use, growth rates, maturation age, migratory behavior, and physiological tolerance of stressors (Letcher et al. 2007; Fraser and Bernatchez 2008; Stitt et al. 2014).

Local adaptations were discussed in greater detail in a later session of the STAC Workshop, but an important takeaway is that local adaptation is widespread and takes many forms. It also bears mention that wild Brook Trout are well-known for phenotypic plasticity (Wood and Fraser 2015), a process through which a single genotype can produce multiple phenotypes through a complex genetic-environment interaction. Although phenotypic plasticity is an important process that can increase individual survival, the correlation between genetic diversity and phenotypic plasticity is tenuous. As such, it is not covered in great detail in this document.

Planning for future adaptation

Stream habitats and the selective forces acting upon wild Brook Trout populations have continuously changed over time. However, human activities are now contributing to rapid environmental changes around the planet (IPCC 2023). Our management of wild Brook Trout is most likely to achieve lasting outcomes if conservation strategies are mindful of the biotic and abiotic stressors that present and future populations face.

A recent intergovernmental report presented a massive body of evidence related to climate change (IPCC 2021). In the last 150 years, dramatic increases in average temperatures have been documented around the world, and we are now on a trajectory to enter the warmest period in more than 100,000 years (IPCC 2021). North America is projected to warm by at least several degrees, with the exact outcome depending on decisions made in the coming years (IPCC 2021). The scientific consensus is that we are already locked into a period of increased warming beyond current conditions. Projections suggest that extreme heat events will become much more frequent (IPCC 2021), which presents a challenge for coldwater obligate species such as Brook Trout. The projections also indicate that precipitation is likely to increase somewhat in eastern North America. However, the timing and intensity may differ from historical patterns. Moreover, many other human activities (e.g., urbanization; Stranko et al. 2005) are occurring alongside climate change, further altering the selection pressures upon wild Brook Trout and threatening the persistence of many populations.

Given the pace and magnitude of projected environmental changes, the ability (or lack thereof) to adapt is likely to play a critical role in determining the fate of wild populations. Although there is broad scientific consensus in the general predictions surrounding climate change, there is much less certainty about local impacts and how Brook Trout might adapt. Although some work has been done to better understand genetic responses to heat stress (e.g., the presentation by Mariah Meek summarized later in this document), there is still great uncertainty as to what specific genes and allelic variants will be needed in the future or how much capacity there is to adapt. These research directions could help prioritize Brook Trout conservation efforts in the context of rapid, unprecedented environmental change.

Despite the uncertainty as to exactly what specific genes will be adaptive in the future, theory and empirical studies both indicate that standing genetic diversity is the foundation of rapid adaptation to changing environments (Barrett and Schluter 2008, Bitter et al. 2019, McCulloch and Waters 2022). Contrary to historical views, adaptation is not always a slow process (Koch et al. 2014). Three factors are required for rapid adaptation: (1) strong selective pressures, (2) populations must be large enough for selection to act efficiently, and (3) populations must possess sufficient standing genetic diversity (Barrett and Schluter 2008, Petit and Barbadilla 2009).

One of the most effective strategies to promote resilience in wild Brook Trout populations may be to preserve and enhance larger, interconnected populations (which are typically more diverse) in intact watersheds, as well as broadly working to improve connectivity and habitat quality. Wild Brook Trout populations in the Chesapeake Bay region are likely to face serious challenges in the upcoming century as rapidly changing conditions test their capacity to adapt. Management strategies that consider forecasts of environmental changes, local adaptations, and the underlying processes that facilitate rapid adaptation may be more likely to generate lasting conservation outcomes (Colton et al. 2022, Meek et al. 2023, Thompson et al. 2023).

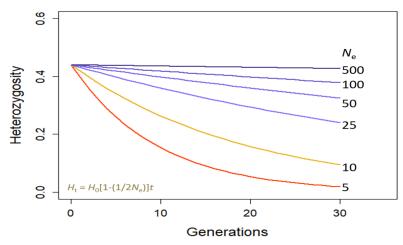


Figure 1. Theoretical loss of genetic diversity (as measured by heterozygosity) over generations in isolated populations. A range of effective population sizes (N_e) are presented.

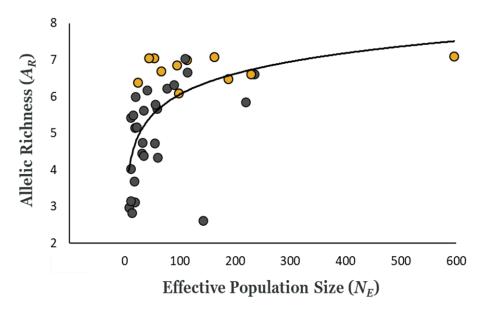


Figure 2. Relationship between one measure of genetic diversity (allelic richness) and effective population size in wild Brook Trout populations from Maryland. Isolated populations are shown with dark gray points. Populations from the interconnected stream network of the Upper Savage River watershed are shown in yellow. Data from Morgan et al. (2021).

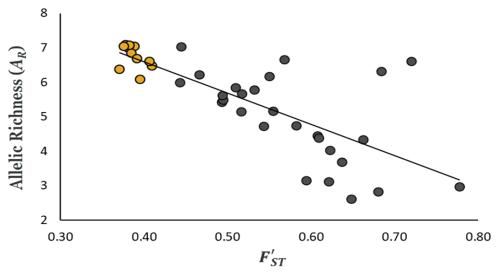


Figure 3. Relationship between one measure of genetic diversity (allelic richness) and the extent of differentiation (mean-population specific F'sT) in wild Brook Trout populations from Maryland. Isolated populations are shown with dark grey points. Populations from the interconnected stream network of the Upper Savage River watershed are shown in yellow. Data from Morgan et al. (2021).

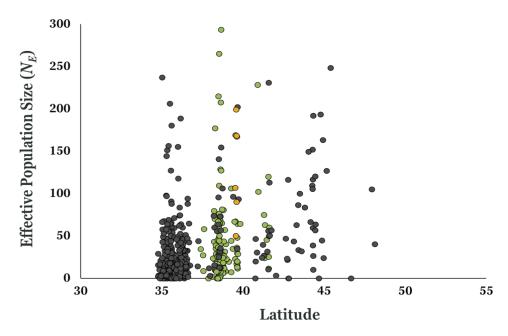


Figure 4. Latitudinal patterns of effective population size in wild Brook Trout populations. Populations from within the Chesapeake Bay watershed are shown in green, except those populations from the interconnected Upper Savage River watershed which are shown in yellow. All other populations are shown in dark grey. Data from Kazyak et al. (2022).

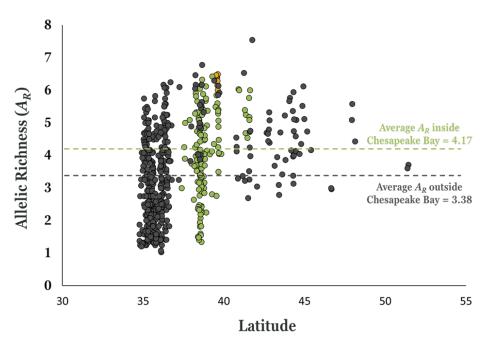


Figure 5. Latitudinal patterns of genetic diversity (as measured by allelic richness) in wild Brook Trout populations. Populations from within the Chesapeake Bay watershed are shown in green, except those populations from the interconnected Upper Savage River watershed which are show in yellow. All other populations are shown in dark grey. Data from Kazyak et al. (2022).

Tools for Studying Genetics

Presenter: Amy Welsh (West Virginia University)
Presentation Slides

The genetic markers used to study Brook Trout have evolved over time. Most Brook Trout genetic studies have used microsatellites (e.g., King et al. 2012), which are useful for evaluating the selectively neutral evolutionary processes of migration and random genetic drift. Microsatellites are short, tandemly repeated sequences of DNA that have high variability, and there are well-established baselines using microsatellites for Brook Trout genetics (Kazyak et al. 2021, Kazyak et al. 2022). However, microsatellites do not provide information on natural selection or adaptation because they are not associated with protein-encoding regions (i.e., they do not affect phenotype). Genomics methods provide a broader sample of the genome and allow us to assess adaptive variation in addition to neutral processes. Hence, the use of single nucleotide polymorphisms (SNPs) is increasing in the field of fish genetics. For example, Mamoozadeh et al. (2023) developed a SNP panel for Brook Trout to assess genetic diversity across the species' native range. This panel can differentiate populations, identify hatchery influence in populations, and identify genomic regions putatively associated with adaptation.

Regardless of the type of genetic marker that a study uses (microsatellites or SNPs), the genetic measures remain similar. For example, clustering approaches (using software like STRUCTURE [Pritchard et al. 2000]) are used to determine the number of populations based on the genetic data and to assign each individual an ancestry profile corresponding to different genetic source populations. Multivariate ordination, such as Principal Coordinates Analysis (PCoA), Principal Components Analysis (PCA), and Discriminant Analysis of Principal Components (DAPC), are

also used to cluster together genetically similar individuals into populations. Quantitative measures, such as fixation index (F_{ST}) , are used to measure the level of genetic differentiation between populations. There are also various measures for assessing within-population genetic diversity, such as heterozygosity and effective population size.

Using Genetics to Identify Robust and At-Risk Populations

Presenter: Jason Coombs (U.S. Fish and Wildlife Service)
Presentation Slides

With finite resources available for conservation, managers must prioritize where to allocate them. To help inform this process, genetic metrics such as allelic diversity, heterozygosity, and effective population size can be quantified for a population to assess its genetic signature. These genetic data can be coupled with measures of habitat size and quality to standardize and classify a population by its overall genetic health. One simple approach to classification involves looking at the effective number of breeders (N_b) as a function of population or patch size. One can then sort populations into four quadrats based on habitat area (small and large) and N_b value (susceptible and resilient). Depending on management goals, large, resilient populations in quality habitats may be the highest priority for conservation, as these populations have the potential to contain individuals with greater variation in life-history strategies and can serve as source populations for translocation actions. Alternatively, there may also be justification for conservation of small patches, as their presence spreads extirpation risk (portfolio effect) and preserves local adaptation, or populations with low N_b as these may be the most at risk of extirpation from the deleterious effects of inbreeding depression.

Genetic monitoring can also be used to evaluate the efficacy of demographic management activities (e.g., fish transplantation) or habitat restoration (e.g., barrier removal, riparian planting). Importantly, the use of a common genetic panel across the range of Brook Trout enables data to be pooled across studies for broad-scale analyses. One such example of this is the Brook Trout Explorer tool (bte.ecoshed.org; Figure 6), which allows users to interactively visualize genetic metric data along with population structure analysis results for populations across the native range and broodstocks from major hatcheries. One of the tool's principal functions is to allow resource managers to incorporate genetic information into the management action decision process.

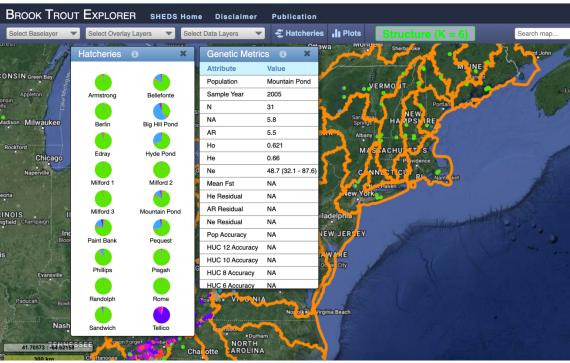


Figure 6. Screenshot of use of the Brook Trout Explorer tool with HUC 4 Watershed overlay.

Genetic Rescue

Presenter: Andrew Whiteley (University of Montana)

Presentation Slides

Small, isolated Brook Trout populations face many challenges, as continued erosion of population size, vital rates, and genetic diversity may result in elevated extirpation probabilities. To break the cycle, some have proposed translocating individuals among populations to achieve genetic rescue, which is defined as a decrease in population extinction probability owing to gene flow, as best measured by subsequent increase in population growth rates. Although genetic rescue programs have been implemented in other taxa, a concern that remains is the risk of outbreeding depression, particularly when working with species with high local adaptation (White et al. 2023). However, theoretical and experimental guidance suggests that the risk of outbreeding depression is likely minimal when moving individuals from populations that have limited genetic divergence and share similar life histories. Although this is encouraging, long-term, replicated studies are still needed to fully assess the risk of outbreeding depression, as most studies do not monitor demographic or genetic outcomes long enough to detect negative effects of outbreeding.

Genetic rescue has not been widely implemented or studied in wild populations of Brook Trout. However, one case study by Robinson et al. (2017) translocated 10 adult Brook Trout to each of four replicate populations in Virginia in 2011. The authors found that translocated individuals had high reproduction, and young-of-year that were produced by a cross between transplant and resident adults had larger body sizes. Although this suggests potential for 'hybrid vigor' (heterosis), increases in population size were also observed in a nearby control site, but recipient (rescue) populations increased at a relatively greater rate than the control. Initial results suggest a

positive effect of genetic rescue in these isolated Brook Trout populations. Monitoring of these sites is ongoing, and future analyses are planned to determine the long-term genetic and demographic effects of genetic rescue in these populations.

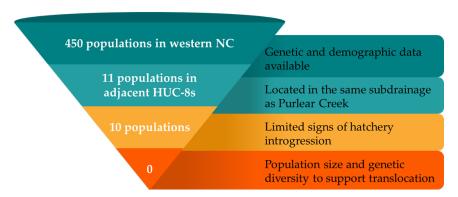
Overall, genetic rescue has potential to be an effective tool for the management of small, isolated Brook Trout populations. However, caution is warranted, as replicated studies with multigenerational monitoring of genetic and demographic outcomes are still needed to test for potential outbreeding depression. In addition, genetic rescue will not be a panacea; it will be best performed in concert with habitat management. It also may require repeated intervention for sustained effectiveness.

Role of Genetics in Supporting Reintroductions

Presenters: Shannon L. White (Contractor to the U.S. Geological Survey), Jacob Rash (North Carolina Wildlife Resources Commission), David C. Kazyak (U.S. Geological Survey)

In addition to genetic rescue, there is potential for genetic data to support Brook Trout reintroduction efforts. In particular, genetic screening of populations can help quantify processes such as genetic drift, local adaptation, inbreeding and outbreeding depression, as well as evolutionary potential that may be important when identifying source populations to use for reintroduction.

Genetic data have been used extensively in Brook Trout monitoring and management within North Carolina. For example, as reviewed by White et al. (2022), North Carolina has used genetics to guide decisions about source population selection (Figure 7) for Brook Trout reintroduction efforts. The state also quantifies post-translocation reproduction and admixture through genetic monitoring, allowing them to evaluate project success and adaptively manage the population.



Decision was made to use two sources in translocation effort

Figure 7. An example of a multi-step filtering process which highlights how demographic and genetic data were used in the design of a Brook Trout reintroduction effort in North Carolina.

Environmental DNA: Utility and Application

Presenter: Meredith Bartron (U.S. Fish and Wildlife Service)

Presentation Slides

Meredith Bartron (USFWS) discussed utilizing environmental DNA (eDNA) as a tool to study Brook Trout and presented of research at the USFWS Northeast Fishery Center that uses genomics to locate rare native species as well as invasive species. Unlike population and conservation genetics, which are focused on relationships among individuals and populations, eDNA is concentrated on protection of species and answering the associated questions that can arise in terms of abundance and distribution. eDNA can be a powerful tool for assessment and monitoring depending on the various fundamental aspects of how the tool is applied.

Environmental DNA is an evolving technology with known and expanding applications. Initial studies used eDNA to detect invasive species (i.e., zebra mussels, northern snakehead, carp). For example, research completed by Barton et al. (2021) on expansion of round goby in New York examined various methods to detect invasives and compared the efficacies of different methods for early detection (trawl surveys, seining, minnow traps, and environmental DNA); results from the report found that eDNA was more accurate and informative for early-detection than traditional field methods.

Another application of eDNA involves rare or cryptic species that may be difficult to sample. An advantage to using eDNA is it can easily increase spatial coverage for distribution and/or monitoring assessments by evaluating whether the species' DNA is present in a water sample. eDNA can complement other field-area monitoring and management efforts to better understand what is occurring on the landscape. Still, there are several challenges with eDNA as it is a rapidly evolving tool:

- Sampling/Study Design: When and where to sample to maximize detection?
 - o Seasonality, which can influence eDNA detection or target species occupancy
 - o Depth of sampling
 - o Distance from target
 - o Stream flow
- Laboratory: *How can we ensure that lab methods are reliable and consistent?*
 - o Marker specificity & sensitivity
 - o Inhibition & marker efficiency
- Data Interpretation: What does a positive or negative detection mean? And will it tell me how many Brook Trout are there?
 - Strength of signal
 - Quantitative associations

When performing eDNA analysis, mitochondrial DNA is typically the focus because there are more copies available within a single cell and, in general, less variation within the species (i.e., mitochondrial DNA acts as an excellent 'barcode'; McCauley et al. 2024). The objective is generally to identify a conserved genetic region within the target taxon but distinct from non-target organisms. It is important to reduce uncertainty with both the sampling and laboratory processes and critical to understand and clarify the expectation of a positive detection. DNA does

not necessarily indicate the presence of live individuals of the target species, as eDNA can sometimes be found in systems through alternative sources. Similarly, if there are management actions resulting from eDNA studies, those actions should require a high level of confidence in results. Recent studies involving eDNA and Brook Trout have evaluated whether eDNA can be used to predict biomass. Yates et al. (2021) reported that eDNA did not scale linearly with biomass but is more strongly correlated with density. However, as suggested by Baldigo et al. (2017), there is still a high uncertainty in predictive ability.

Communicating to the Public and Decision Makers

Panelists: Jake Rash (North Carolina Wildlife Resources Commission), Matt Kulp (Great Smoky Mountains National Park), Shannon White (Contractor to the U.S. Geological Survey), Shawn Rummel (Trout Unlimited), led by David C. Kazyak (U.S. Geological Survey)

Panel Recording

There are numerous pathways through which information about Brook Trout genetics may be shared (Figure 8). Researchers generate raw scientific data, which may flow to managers and conservation practitioners through journal articles, reports, and presentations. In some cases, researchers engage directly with managers and conservation practitioners to share their science and expertise. However, it is generally the responsibility of managers and practitioners to interpret and incorporate raw science into conservation effort. Because managers generally need to be able to justify their activities to administrators, and perhaps even the public, they are most likely to apply new information that is available, accessible, and can be directly translated into positive management outcomes.

Across this diverse network of scientists, managers, practitioners, administrators, and the public, different parties will need different levels of information to operate most effectively. Researchers need to know what science is needed to support conservation, and those needs are often best identified by managers and conservation practitioners. Managers and practitioners may not need to know all of the nuances of genetics, but a working understanding of the major genetic processes and management considerations, as well as the tools and resources available can support management and their applications. Finally, since science and conservation activities do not happen in a vacuum, it may be helpful for managers to articulate the importance and utility of genetics for conservation to the public and to administrators.

Effective communication about genetics is essential if its benefits for conservation are to be fully realized. Communication about genetics can be daunting, as it is a highly technical, rapidly advancing topic and beyond the training of many fishery managers and conservation practitioners. Information may be difficult to access, particularly when published in journal articles that are not open-access. Diverse groups working within this space have their own priorities and end goals, which are not always in alignment. Some may perceive that genetic processes are important only over very long time scales or that other threats are more urgent. Given the technical nature of the science, lingering uncertainties, and near-continual stream of new information, there is a risk that the information can be challenging to communicate and act upon. However, the prevalence of small, isolated populations across the landscape, coupled with unprecedented environmental change underscores the importance of effective communication with decision makers.

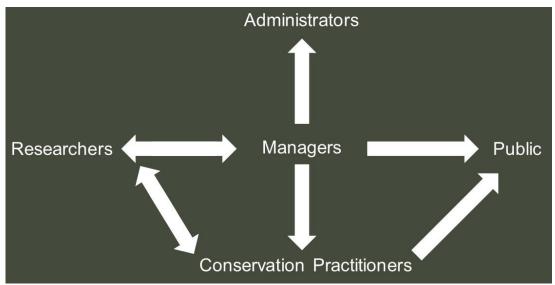


Figure 8. Conceptual diagram showing common pathways of sharing information related to Brook Trout genetics.

To improve the flow of information, Kazyak outlined a number of possible strategies to improve communications such as: 1) coordinating activities (i.e., workshops, conferences, training opportunities) with the purpose of reviewing key conservation concepts and data sets while building relationships between typically siloed groups, 2) building and updating public-facing tools to share available data across the landscape (e.g. <u>Brook Trout Explorer</u>, publication library, regional newsletter with publication alerts), and 3) supporting participatory science opportunities for researchers to engage with managers and practitioners, which can increase buy-in from managers (and could be facilitated through a public contact list of geneticists). Beyond current efforts, potential areas for further participatory science engagement could be an evaluation of reintroduction or genetic rescue activities under varying circumstances and/or the development of a more rigorous eDNA approach for monitoring conservation progress. For members of the public or in administrative roles, Brook Trout genetic outreach could be in the form of recorded and shared non-technical talks, an approachable short document or poster on the importance of genetics, a public lecture for a larger audience, an ArcGIS StoryMap on wild Brook Trout conservation and genetics, and/or a web site highlighting the major genetic considerations of Brook Trout management.

Despite this importance of communication, limited guidance and resources are available to support communication about Brook Trout genetics. To facilitate a discussion on Brook Trout genetics outreach, panelists Matt Kulp (Great Smoky Mountains National Park), Jake Rash (North Carolina Wildlife Resources Commission), Shannon White (Contractor to the U.S. Geological Survey), and Shawn Rummel (Trout Unlimited) were invited to share their experience in this space and ideas for improving communications moving forward. A synopsis of the questions posed and panel responses appears below.

What has worked well for you in communicating Brook Trout genetic information? Who was the audience?

- Targeting a general audience when presenting; mostly have communicated to local Trout Unlimited chapters and associated volunteers.
- User groups, university-affiliated groups and students, managers. Using analogies to discuss the concept of evaluating the health of a population better to speak in generalities than getting too technical.
- Knowing who your audience is can be a challenge, so being able to find the commonalities is important. Linking Brook Trout conservation to nature and its significance as an indicator for watershed health can help.
- Trout Unlimited hosts a <u>monthly webinar</u> from in-house science staff or outside researchers and works to increase access to relevant literature. Trout Unlimited has communications staff that work on products like <u>Trout Magazine</u>, <u>StoryMaps</u>, and <u>blog posts</u> which have found more traction than typical articles as they are easier to digest.

What challenges have you faced?

• Hard to understand what you cannot see, using figures or analogies has helped communicate genetic processes and concepts.

What ideas do you have for improving communication? Do you have specific suggestions?

- Continuing education periodic, short half-day seminars on emerging tools, terminologies, techniques so that we can better educate the public and colleagues.
- Support the need for basic training on genetics and how this relates to field work. Often run into landowners and the general public asking these questions.
- Pointing folks to a fact sheet on a website could be used by any of us while communicating some of these topics related to the public.
- Development of interactive web applications to aid data and model understanding and to facilitate natural resource management. Also, provides feedback for those who design these models and possible edits to make it easier to use or to establish curricula. More information can be found here: https://www.usgs.gov/apps/ecosheds/#/.

Lightning Talks: Where are we and where are we going?

Lightning Talk Recording

The final set of workshop presentations was a series of three 'lightning talks', intended to be short presentations pertinent to the future directions of Brook Trout conservation and genetics. The steering committee invited speakers to discuss emerging areas of research, including local adaption, adaption to warming temperatures, and eradication of non-native trout using YY 'Trojan' Brook Trout males.

Local Adaptation

Presenter: Eric Hallerman (Virginia Tech) Presentation Slides

Allozyme, mitochondrial and microsatellite DNA variation are indicative of non-selective population genetic processes, but not of *adaptive* variation within and between populations. What do we know of adaptive genetic variation in Brook Trout? Some local-scale studies of

Brook Trout have inferred adaptive variation. All of these studies were conducted in Canada, and all investigated the relations among population fragmentation, effective size and putatively adaptive variation.

Fraser et al. (2014) investigated SNP variation at genes encoding different biological functions in 14 fragmented Brook Trout populations of variable sizes in Newfoundland. Putatively adaptive differentiation was greater between small and large populations or among small populations than among large populations, underlining the effect of random drift, suggesting that fragmentation does affect natural selection. Wood et al. (2017) examined additive genetic variation and quantitative trait differentiation for 15 traits among nine populations in Newfoundland that varied in census size (N = 179 - 8,416) and effective number of breeders ($N_b = 18 - 135$). Population size was only weakly related to quantitative genetic variation, suggesting that small populations may retain adaptive potential. Zastavniouk et al. (2017) examined relationships between mature adult traits and ecological variables among 14 populations of Brook Trout in Newfoundland. Body size, shape and coloration differed among populations, with a tendency for more variation among small populations in trait means and coefficient of variation than among large populations. Phenotypic differences were more frequently and directly linked to habitat variation or sex ratio than to population size, suggesting that selection can overcome genetic drift at small population size. Ferchaud et al. (2020) screened 14,779 SNPs among 1,416 fish representing 50 populations from lacustrine (isolated populations), riverine and anadromous (connected populations) life histories. They observed considerable accumulation of putatively deleterious mutations across populations, suggesting that genetic drift might be the main driver for accumulation of such variants. They identified genomic regions associated with anadromy.

This is a rather limited body of studies, with a limited scope in terms of geography and traits considered. We could benefit from a deeper understanding of genomic regions associated with adaptive traits. Investigation of adaptive genetic variation has not yet been extended to Brook Trout populations in the Chesapeake Bay region. Further understanding of adaptive genetic variation would inform management of regional Brook Trout populations and help conserve their long-term adaptive potential.

Adaptation to Warming Temperature

Presenter: Mariah Meek (Michigan State University) Presentation Slides

Over the past few decades, Adirondack Park has experienced mass die-offs of Brook Trout associated with extreme heat events, of which the most severe occurred in 2002 and 2005, particularly in unstratified lakes. This system presents a great opportunity to explore hypotheses associated with local adaptation and recovery following strong climate-associated bottlenecks. Brook Trout were collected from four geographically-proximate lakes (two stratified and two unstratified), which typically experience similar climatic conditions. These fish were brought into a common-garden experiment at Cornell University's Little Moose Field Station and raised in either thermally-optimal or thermally-stressful conditions. DNA and RNA were sampled at three different time points to examine similarities and differences with respect to how Brook Trout respond to heat stress.

This work determined that each of the study lakes supports a genetically distinct population. Hundreds of genes were up- or down-regulated in response to heat stress (Figure 9). Although

some of the genes were up- or down-regulated in all populations, the majority of up- or down-regulated genes were unique to a particular population. In a follow-up analysis, 1,192 SNPs were linked with the expression patterns of 292 genes. Using a survey of SNPs across the range of Brook Trout (Mamoozadeh et al. 2023), many of these genes showed strong correlation with the temperatures experienced by local populations (Figure 10). This approach may prove useful to help guide reintroduction and translocation efforts, as populations which are most (or least) vulnerable to projected temperature changes may be identified. Future work may link phenotypes related to heat stress (such as critical thermal maximum and respiration) to genotypes. This work may help conservation and management efforts by managing for heat stress using markers that respond similarly across populations, identifying populations at greatest risk due to decreased genetic diversity at important loci, and informing translocations and reintroductions.

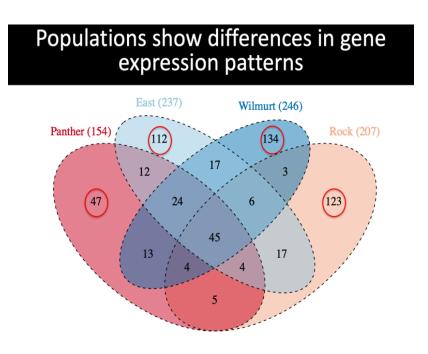


Figure 9. Venn diagram showing similarities and differences of gene expression patterns among four lake-dwelling populations of wild Brook Trout in Adirondack Park, NY. Although 45 genes were up- or down-regulated in response to heat stress in all four populations, the majority of up- or down-regulated genes were unique to a particular population. Adapted from "Adaptation to Warming Temperatures" [Presentation] by M. Meek (2021, September).

Genotypes are significantly correlated with temperature across the range Adj R2 = 0.2558 , P = 0.001855 Adj R2 = 0.2939, P = 0.0006639 0.9 1.0 Allele frequency Allele frequency 0.5 0.0 -0.3 -0.5 meanT Adj R2 = 0.2569, P = 0.002127 Adi R2 = 0.3603 , P = 0.0001682 1.0 Allele frequency Allele frequency

Figure 10. Sample relationships between average temperatures and allele frequencies of genes associated with thermal response in Brook Trout. Many relationships were statistically significant and showed reasonable strong correlation coefficients between genotypes and temperature. Adapted from "Adaptation to Warming Temperatures" [Presentation] by M. Meek (2021, September).

${\it YY Brown or Rainbow\ Trout for\ Suppression\ of\ Non-native\ Trout}$

Presenter: Andrew Whiteley (University of Montana) Presentation Slides

Typically, chemical or mechanical eradication is employed to remove an allopatric (occurring on its own) non-native species from the landscape. If the non-native species is sympatric with high-priority native species, mechanical suppression can be used to suppress or eradicate it, although this is not always successful. Species-specific tools for suppression could be very useful for managing non-native trout populations.

Recently, Trojan male Brook Trout have been developed by management agencies in the western United States that are seeking to suppress or eradicate non-native Brook Trout populations. Trojan males have two Y chromosomes and can only produce male offspring. Their introduction into non-native populations is expected to strongly skew the sex ratio of offspring towards males, ultimately reducing recruitment in the population. This process is used primarily to induce demographic changes and is different from biotechnology solutions that pass lethal alleles to offspring.

There are many outstanding questions (e.g., how many, how often and where to add YY males?) regarding the application of YY Brook Trout to successfully control invasive populations. Although studies are ongoing, some empirical and simulation research has been completed on

the introduction of YY male Brook Trout. Day et al. (2020) simulated the effects of fitness and dispersal on the use of Trojan sex chromosomes to manage invasive species and found that suppression and YY males should be used together to achieve extirpation most quickly (Figure 11). Simulations show that YY suppression is most likely to be effective when Brook Trout can move freely from release locations and at least 50% of the population are YY males. Overall, simulations have shown that the time to extirpation will likely vary with suppression effort and electrofishing mortality rate (Figure 12).

Because YY Brook Trout grow rapidly in hatcheries, many are mature at age-0. Early maturity in YY Brook Trout can help reduce the amount of time required for eradication. Relative fitness is also important – if hatchery fish have lower fitness than wild Brook Trout, over time, there is less of an effect on the wild population (Day et al. 2021). A relevant empirical paper (Kennedy et al. 2018) examined four streams in Idaho and reported that YY offspring were found in the same proportion as YY adult males, suggesting that YY males are reproducing in a manner proportionate to their abundance.

In summary, various states have introduced YY male Brook Trout in some streams (i.e., Washington, Idaho, New Mexico), while other states are holding off for more research - Montana. Studies have shown that YY males can work in small isolated systems and that there is an opportunity to prophylactically add YY males after chemical or mechanical removal methods to gain extirpation. It is clear that there is a need to suppress the population before introducing YY males; it may take many years of effort for the strategy to work and that YY males are most likely to contribute to eradication in closed systems.

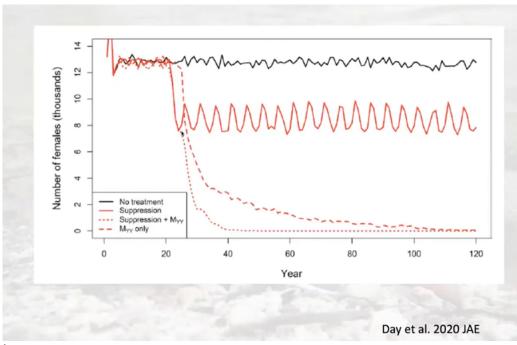
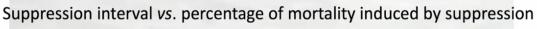


Figure 11. Figure showing number of females under various treatment scenarios over time. Suppression of the population in conjunction with the introduction of YY males is represented by a red, dotted line. Figure from Day et al. (2020).



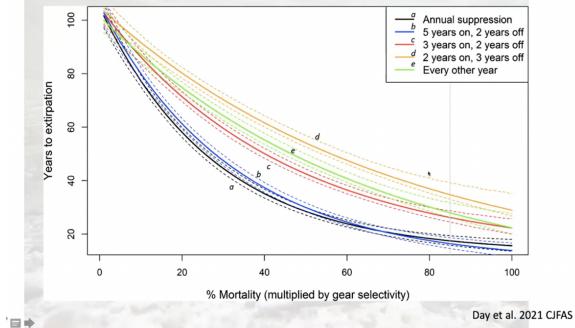


Figure 12. Comparison of the length of time required to achieve extirpation with varying treatment strategies and electrofishing mortality rates. Figure from Day et al. (2021).

Participant Feedback and Summary of Breakout Discussions

Throughout the workshop, participants were asked to respond to a series of questions aimed at gauging their current use of genetics, their interest in incorporating genetics into future conservation efforts, and the perceived limitations of using genetic data in their respective workplaces.

Individually, workshop participants were prompted to respond to the following questions. The responses to these questions are outlined in the following section, with each question hyperlinked for easy access to specific answers:

- How are you using genetics in your current management strategies/plans? Responses
- What obstacles exist to accessing existing genetic information relevant to your work (e.g., journal subscriptions, technical nature of information, etc.)? <u>Responses</u>
- What are the key knowledge gaps that you perceive as important? <u>Responses</u>
- Do you have access to sufficient genetic information to support management in the area where you work? <u>Responses</u>
- What are the biggest challenges you've found for reintroduction? Responses
- What is preventing us from doing more reintroductions (e.g., finding unoccupied habitat, source populations, logistics, etc.)? Responses
- Have you considered using genetic rescue? Responses
 - Would more publications of successful genetic rescue implementation help you make a decision to implement this in your management area? Responses
 - What is your most pressing concern about implementing genetic rescue? Responses
- What are the biggest challenges you've found for reintroduction? Responses
- If YY brown or rainbow trout were developed, would you consider attempting YY-based eradication? Responses
 - O Under what scenarios do you envision using YY-based eradication if it becomes an option (open/closed system, in conjunction with manual suppression)? Responses
- What other tools or actions would be helpful to facilities communications regarding Brook Trout genetics? Responses
 - o Rate how useful each of these platforms would be. Responses
 - What obstacles make it challenging to communicate about Brook Trout genetics?
 Responses
 - What topics are most important to communicate? Responses
 - Who do you communicate with most frequently? Responses

On the second day of the workshop, participants were divided into four small breakout groups, with 5-7 people in each group. These groups met for 30 minutes before reconvening in the main Zoom room to share their discussions. The responses from the breakout groups are also provided below, with hyperlinks to specific answers:

- How are you currently using eDNA within your conservation program? Responses
- What additional opportunities are there to apply eDNA to support your conservation program? What areas of uncertainty do you still see? Responses
- Roadblocks to implementation? What are the negative indications that would cause you to either not need it or not use it? How do we use eDNA to track Brook Trout restoration progress? <u>Responses</u>

• How do you we eDNA to track Brook Trout restoration progression? <u>Responses</u>

Responses to prompts and breakout group questions are listed below.

How are you using genetics in your current management strategies/plans? Please note if you are a researcher in your response.

- Painting a broad brush on genetics. We are limiting our stocking of our hatchery salmonids so not to stock hatchery species on top of our known wild populations. We are hoping to develop more on genetics.
- Detection of putative native populations as donors for repatriation; decisions on the presumed need for artificial population cross-breeding (of multiple acceptable donor populations) to enhance genetic diversity for start-up reintroductions.
- Using genetics to inform management and policy changes to try and prevent introgression of hatchery genes into wild populations, collecting baseline information across drainages, and to inform restoration projects.
- Research: pedigrees to understand what drives fitness in the wild, SNPs to understand population structure, thermal tolerance, and adaptation to isolation.
- Consideration of genetic connectivity for management. Projection of large, well-connect populations, and desire to re-connect disjunct populations. Protection of wild patches from stocking.
- Researcher: identifying native populations on the landscape, investigating how stocking practices (e.g., intensity, frequency, life stages) affect hatchery introgression, and the fate of hatchery-derived alleles in wild populations.
- It is foundational. Specific examples include population restorations, habitat projects, and land protection efforts.
- Researcher: mainly using eDNA to assess or monitor distribution and relative health/density of Brook Trout and other fish species in streams and rivers; also assessing introgression of wild/hatchery Brook Trout populations in remote streams.
- We are trying to prevent infiltration of hatchery genetics into our wild Brook Trout populations and trying to figure out the genetic history of our populations.
- Researcher: generating genetic data and information to inform conservation and management of Brook Trout (and other fishes).
- Identifying native, hatchery strain, and hybrid populations; determining the genetic health of various populations; using genetic health data to inform which source stocks to use to translocate into newly restored stream segment.
- We have utilized genetics to determine what fish from existing streams we may use to stock new streams that we are trying to restore.
- Researcher: primarily to determine where populations are located, whether domestic introgression has occurred, and prioritize populations for conservation/management. To a lesser extent, to inform restoration of specific phenotypes.
- Prioritizing conservation activities where southern Appalachian Brook Trout have been identified. Prioritizing specific populations for conservation work based on genetics.
- Use N_E >50 as a threshold for prioritizing Brook Trout patches for resiliency, <50 as future needs for genetic rescue.
- To identify hatchery introgression, unique populations, and source populations for restoration projects.

- Researcher: fish passage barrier assessment, population connectivity, and hatchery introgression.

What obstacles exist to accessing existing genetic information relevant to your work (e.g., journal subscriptions, technical nature of information, or not sure what exists)?

- Obstacles more exist with funding the genetic work and communicating need and benefits of this work.
- Information often too complicated and difficult to understand.
- Access to journals and electronic databases.
- I need to know what is relevant before I know what I need to access to accomplish management/conservation goals.
- Better integration among labs would help provide more consistent information and could lead to a central location for data and results.
- Funding to process existing samples. Universities and labs want larger projects to support students. States don't always have those resources. Regional effort/project (pooled funding) may be needed.
- Genotypes not open access. Non-standardized marker sets also hamper meta-analyses.
- Several participants expressed that the USGS overhead rate is too high.
- Funding, lack of in-house technical expertise, time (this is long-term planning, but there are fires to put out now).

What are the key knowledge gaps that you perceive as important?

- Adaptive genetic variation linked to specific environmental conditions that are expected to change over coming years.
- How much adaptive capacity exists to cope with projected environmental change.
- We put a lot of stock in microsatellites to evaluate genetic "health" of populations- what about all the genetic adaptations found in genes under selection that aren't accounted for?
- More baseline data needed in larger watersheds and priority areas.
- Are localized adaptations important to consider when moving fish around, for either genetic recuse or reintroductions? Or does natural selection supersede?
- Difference in microsatellites vs. adaptive regions of the genome.
- Knowledge of adaptive genetic variation in Brook Trout.
- Identifying barriers to integrating genetic information into management decision-making and best ways to address them.
- Delineating local adaptations from phenotypic plasticity.
- Using genetics to inform management how to determine what are good source stocks for streams?
- Knowing what kinds of questions can be asked with which genetic analyses (and what kind of data I should be collecting).
- Identifying when a populations "needs" genetic rescue or another intervention.
- Should we use unique but low allelic richness populations within the watershed for stock populations or should we choose populations that are outside of the watershed but high allelic richness for stock populations?
- Better understanding of link between genetic variation, population size, and actual population persistence in a non-stationary world.
- Relative cost of getting genetic baseline vs. collection traditional population measures.
- Identifying locations of metapopulations.

- Can we effectively say what happens to a population of Brook Trout with N_E <50 and over 10s, 100s, and 1000s of years? Likelihood of extirpation? Lowered genetic diversity and extinction is linked, but outcomes likely change by species?
- Identifying when it is appropriate to consider genetic rescue.
- Why do genetically "poor" Brook Trout populations persist when they appear doomed to extirpation?

Do you have access to sufficient genetic information to support management in the area where you work?

- Three responded yes, three no.
- We have the data, but lack consensus on how to use it.
- At what point is it important to move beyond applying best genetic principles and genetic knowledge from other locations to having site-specific data at each location prior to initiating management?
- More populations have been genotyped than most other states, but majority of populations still not genotyped. Problem because of population-specific management.
- More baseline data are needed in larger watersheds and priority areas.
- Yes and no. We have enough to know what we don't have, but more comprehensive (all populations statewide) information would better inform management decisions.

What are the biggest challenges you've found for reintroduction?

- What genetic metrics are most important to consider from source populations?
- With genetics information, deciding which populations represent the best ones to use as source populations.
- Deciding how to balance multiple priorities (similarity to original stock, introgression level, and diversity metrics).
- Understanding how many individuals from how many source stocks are needed to establish a new population (in a restored segment)? We have been using 200-300 fish/mile of all age classes.
- Getting the genetics data necessary to prioritize projects and identify donor (source) populations.
- What about knowing the adaptive potential to temperature increases?
- Jurisdictional boundaries. Lack of source populations. Private property-bounded streams are difficult to work on due to public funding.
- Determining appropriate streams for reintroduction and the reasons for extirpation-how to mitigate for the causes of extirpation.
- Disagreement between managers on what source stocks to use and lack of guidance from geneticists on what metrics to uses. We have the genetic baselines, but are lost now.
- Restoring habitats/water quality suitable for Brook Trout.
- It's not a 'genetics' issues per se, but some managers have stated that habitat suitability and temperatures are the limiting factors, so they are not doing reintroductions.

What is preventing us from doing more reintroductions (e.g., finding unoccupied habitat, source populations, logistics, etc.)?

- Finding streams with suitable habitat and water quality/temperature.

- Most streams with suitable habitat have populations of Brook Trout or competition from other salmonids. Should we remove competition from a stream before reintroductions? Would we need to install a barrier to not allow colonization from competition?
- Finding unoccupied and reasonable habitat is tough.
- Understanding what genetic metrics to use from our baseline data to determine how to do reintroductions. We also don't have funding for genetic monitoring afterwards.
- Limited number of suitable streams with barriers to prevent invasion by non-native salmonids from further downstream.

Have you considered using genetic rescue?

- Eight respondents said yes, one said no.

Would more publications of successful genetic rescue implementation help you make a decision to implement this in your management area?

- Six respondents said yes, one said probably, one said no.

What is your most pressing concern about implementing genetic rescue?

- 8 responded with causing loss of local adaptation in the recipient population.
- 0 said disease.
- 6 causing more harm than good.
- 1 causing too much demographic harm to the source populations.

If YY brown or rainbow trout were developed, would you consider attempting YY-based eradication?

- Yes, in select location.
- Yes, for very specific locations.
- In certain isolated systems, yes.
- Yes. We have much interest, but no hatchery space.
- Potentially, but only in certain waters.
- Maybe, most of our Brown Trout and Rainbow Trout thrive in the warmer sections of rivers where eastern Brook Trout don't.
- Yes, but would have a very limited application.
- Yes, in select, high priority locations.
- On a case-by-case situation. If nonnatives are impacting native Brook Trout. If stream is isolated from future immigration. If the nonnative fishery has little value.
- If mechanisms to prevent future invasion are in place first.
- Yes, a primary limiting factor is likely hatchery operations.
- Not likely. We value our wild trout downstream of our Brook Trout. Mechanical removal has proven to be successful in short term.
- Yes. We have several small, isolated populations that seem suited for this application (mechanical or chemical removals are not possible).
- Hatchery space would be a huge roadblock to clear.

Under what scenarios do you envision using YY-based eradication if it becomes an option (open/closed system, in conjunction with manual suppression)?

- Closed system, following suppression, experimental basis.
- Would be useful to restore metapopulation structure in select watersheds as this is generally lacking across the range, especially the southeast. In a system, initial suppression would be necessary prior to YY introduction.
- Closed, medium-sized system in conjunction with manual suppression. Small systems would just use manual (electrofishing); large systems probably not suitable because of large number of fish needed.
- Documented declines attributed to nonnatives, closed system, limited value of nonnative brown/rainbow fishery.
- Closed system, with manual suppression, and limited public exposure.
- Upstream of a barrier, likely in conjunction with previous suppression (chemical or mechanical) and where a priority Brook Trout population would be secured.
- In conjunction with suppression.

What other tools or actions would be helpful to facilities communications regarding Brook Trout genetics?

- Interactive data visualization.
- Face-to-face meetings.
- Centralized information (Eastern Brook Trout Joint Venture [EBTJV]), more workshops like this, engage a wider audience.
- Fact sheet for talking points that can be used to communicate why the public and administrators should care.
- A well-designed resource website to refer people to, pitched at a level for educated, but not formally genetics-trained people.
- Central information portal (EBTJV).
- More informal discussion.
- A brief video that explains why genetics matter to Brook Trout (inbreeding, outbreeding effects, what happens to populations with low genetic diversity) to the public, targeting anglers.
- This group today would be great as an email contact list so that I could periodically ping to ask for those publications or news that could be added at the end of the newsletter. Or, just for general communication within ourselves to continue the discussion.
- Mandate 50-m riparian buffer from Maine to Georgia.
- A very brief informational website/pamphlet to direct stakeholders to for background, so they can know what questions to ask.
- Resources on how to do a genetics study. How much will it cost? What do I need to do to answer management questions? Study design?
- Help with designing study. Costs, how many different populations should be surveyed? One-on-one discussions with experts.
- More EBTJV involvement on social media.
- Maybe an available slide presentation to be presented at a variety of venues.
- Video-based educational tools.
- Something online with basic information and how it applies to Brook Trout conservation to which audiences could be referred.
- Practical guidance.

- Seminars and webinars for both technical and nontechnical audiences. Fact sheets. Recommendations for management from EBTJV Genetics Team or similar technical group of scientist and managers. Model Explainer.
- A tool using specific metrics for determining source stocks.
- Repetition is important to retain information.
- Periodic seminars on specific aspects such as genetic tools and their capabilities and limitations, update on conservation genetics terms/definitions, costs, case studies, interpretation of results, etc.
- Decision workflow tool for management applications like introductions or translocations of Brook Trout.
- Knowing what other managers are doing and their successes. What is being done in different states?
- Frequent opportunities for all of us to continue interactions like this. There is a value to the repetition of these discissions- we can all keep learning from each other.
- Training for those who are not able to travel or pay for training due to organizational constraints. Short webinars.

Rate on a scale of 1 (strongly disagree) to 5 (strongly agree) how useful each of these platforms would be. Average across all responses is shown.

- Bibliography of key resources and studies: 3.9
- Announcement of publications on listserv (e.g., EBTJV): 4
- Glossary of genetics terminology: 3.9
- Links to online training resources: 4.3
- Contact list of geneticists who are available to help: 3.9

What obstacles make it challenging to communicate about Brook Trout genetics? Numbers represent total number of responses for each option.

- It is not necessary: 0
- There is no clear audience: 0
- The information is too technical: 17
- Lack of institutional support to engage in this manner: 7
- Lack of available materials to support this communication: 5

What topics are most important to communicate? Each respondent ranked each topic on a scale of 1-10, number shown is average number across responses.

- Genetic methods: 3.4
- Genetic concepts and principles as they apply to Brook Trout: 7.8
- Application of data management: 8.8

Who do you communicate with most frequently? Numbers represent total number of responses for each option.

- Peers within your agency: 21
- General public: 2
- Policy makers: 2
- Anglers: 2
- Environmental interest groups: 1

Please rate on a scale of 1 (lowest priority) to 10 (highest priority)

- Do you personally have enough information on Brook Trout genetics to guide your work? 4.9
- How important do you feel genetics is to the conservation of Brook Trout? 8.7

How are you currently using eDNA within your conservation program?

- Surveyed 32 streams where Brook Trout were not found since 1990. Eight streams were absent with eDNA, targeting those for reintroductions. Is eDNA more efficient (effort and cost) vs. traditional electrofishing.
- Worked with Trout Unlimited to sample 8-10 streams from previously undocumented streams. Some success in detecting Brook Trout. Putting those streams on list for possible reintroductions.
- Currently conducting SWG project with University of Maryland Eastern Shore. Appalachian Laboratory. Will have to redo the field work portion in 2022 because samples from 31 did not yield DNA hits.
- Advantages: don't need access. Take samples at road crossings.
- Spot checking locations from locations where Brook Trout were not detected via electrofishing. Taking samples above and below locations.
- Looking into detecting American Shad reproduction possibilities.
- We are not using it.
- Monitoring species presence: Brook Trout, American eel, other species.
- Metabarcoding for species assemblages.
- Tracking invasive species distribution.
- Species presence/absence for streams with limited access (private land, etc.).
- No active eDNA projects.
- To see if culvert replacement is successful.
- Have used to determine fish locations in streams using longitudinal surveys; early detection of invasives.
- Video monitoring as potential alternative approach.
- Citizen science has concerns with contamination.
- Look for Brook Trout extirpation, possibly metabarcoding to look at community changes, but more likely species-specific.
- Monitoring sites don't capture full range of stream sizes, limiting resources for electrofishing. High sample size is important for broad spatial and temporal coverage.

What additional opportunities are there to apply eDNA to support your conservation program?

- Cost/effort advantages over traditional electrofishing.
- Confirm presence/absence of streams thought to be extirpated.
- Work with regulatory agencies to accept eDNA detections.
- Field eDNA test kits.
- Quickly confirm presence in unknown tributaries when reviewing construction projects.
- Post restoration immigration assessment.
- Post mechanical removal of invasive species.
- Sample difficult habitat.
- Individual population screening.

- Continued resolution of species ranges and presence.
- Might be helpful for detected Brook Trout in lower probability settings, instead of sending crews out to those locations.
- Detections of species with similar habitat requirements to support identification of candidate streams for reintroduction.
- Verify eradication and continued blocking of non-natives from stream reach.

What areas of uncertainty do you still see? Roadblocks to implementation? Any negative indications that would cause you to either not need it or not use it?

- Cost to process samples vs. electrofishing.
- Longitudinal distribution to ensure we're capturing comprehensive sample from stream/reach.
- Impacts of flow, temperature, and biomass/density on eDNA detections.
- False positives and false negatives, in low density populations. If sampling error I greater than electrofishing, then undesirable.
- Lack of funding.
- Hesitancy to adopt new methodologies.
- Potential cost.
- Identification of unexpected species in results-eDNA detection may be from species further upstream.
- Have other reasons for continuing to use electrofishing and haven't gotten to the point in priority list for using eDNA (lower probability streams). Haven't exhausted lower-hanging fruit yet.
- Regulatory ties to documenting Brook Trout with electrofishing.
- How close do you have to be to the source?
- Depends on biomass, hydrology.
- Spatial specificity.
- Complex hydrology- gaining and losing streams and potential complication.
- eDNA flows downstream, could still get positives in downstream areas where trout are most likely to blink out first.
- Lots of questions related to abundance where do they actually occur?
- Uncertainty in abundance estimates- makes it not that useful, better to just use electrofishing.
- Doesn't replace traditional surveys.
- Need to use ground truthing to confirm positive results.

How do you we use eDNA to track Brook Trout restoration progression?

- Genetic metrics supersede eDNA, electrofishing is still needed.
- Confirm presence of Brook Trout in restored reaches of currently occupied streams.
- Can be used for Aquatic Organism Passage (AOP) retrofits, before and after removing barriers to tributaries.
- Confirm if a barrier is preventing upstream migration of nonnative salmonids.
- Removal of nonnative salmonids (brown and rainbow). Did we get them all?
- Post mechanical removal monitoring of competitor species.
- Screening populations for which populations are lacking Brook Trout.
- Monitoring generalized "densities" or correlated abundance.

36

To ask if barriers are limiting passage and if individuals are moving upstream after passage has been restored.

Key Findings

Finding: Many wild Brook Trout populations in the eastern U.S., including those in the Chesapeake Bay watershed, are small and isolated. Under these conditions, genetic drift is expected to erode genetic diversity and limit future adaptive potential.

Finding: Where they occur, larger interconnected habitats that support wild Brook Trout are most likely to be resilient to genetic drift and other perturbations.

Finding: Many studies have reported that stocking hatchery lineages of Brook Trout has not consistently resulted in genetic introgression and populations can only be assessed using genetic tools. However, many populations within the Chesapeake Bay watershed have not been genetically assessed.

Finding: Environmental DNA is now established as a tool to assess Brook Trout occupancy. Environmental DNA can sometimes be used to assess abundance, but many factors can obfuscate the relationships between DNA counts and abundance.

Finding: Rapid, unprecedented environmental changes are underway which will challenge wild Brook Trout populations. Standing genetic diversity is essential for rapid adaptation to change. However, it is uncertain how much adaptive potential wild Brook Trout populations have, or how that potential is distributed across the landscape.

Finding: Genetic rescue is a candidate tool for augmenting gene flow among recently (<500 years) isolated populations. This tool is a low-cost, scalable tool to help maintain genetic diversity, but has not been rigorously evaluated in wild Brook Trout populations.

Finding: Many Brook Trout populations have been restored across the eastern United States. However, genetic evaluations have been very limited, and meaningful questions remain as to what approaches are most likely to be successful.

Recommendations

Through the exchange of ideas facilitated by the STAC Workshop, the committee identified a series of recommendations which could advance the objectives of the Chesapeake Bay Program with respect to Brook Trout conservation:

Programmatic recommendations

- 1. Encourage managers and conservation practitioners to further use genetics as a set of tools to support the conservation of wild Brook Trout.
- 2. Increase consideration of the role that genetics plays in the conservation of wild Brook Trout, particularly in isolated populations facing rapid environmental change.

Research recommendations

- 1. Genetic characterization of additional populations in the Chesapeake Bay watershed.
- 2. Implementation of eDNA as a tool to track restoration progress.
 - a. Evaluation of the potential for eDNA to correlate with Brook Trout abundance.
- 3. Encourage regional collaboration for genetic evaluation of reintroductions.
- 4. Encourage regional collaboration to evaluate genetic rescue as a tool to support isolated populations against a backdrop of rapid environmental change.
 - a. Review current science on genetic rescue as it relates to wild Brook Trout.
 - b. Consider experimental tests of genetic rescue to evaluate efficacy and guide future efforts.

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Appendix A: Workshop Agenda



Chesapeake Bay Program's (CBP) Scientific and Technical Advisory Committee (STAC) Workshop

Understanding Genetics for Successful Conservation and Restoration of Resilient Chesapeake Bay Brook Trout Populations

Fall 2021 Workshop Webpage

10:30 am Introduction | Presentation Slides

– Dave Kazyak (USGS), Eric Hallerman (VT), Lori Maloney (Eastern Brook Trout Joint Venture), Steve Faulkner (USGS)

11:00 am Major Processes that shape Brook Trout Genetic Structure | Presentation Slides

- Dave Kazyak (USGS), Eric Hallerman (VT)

Overview of major processes impacting Brook Trout genetics. Discussions on the following topics: Glaciation and recolonization, Connectivity, Isolation and drift, Natural selection, and Hatchery introgression.

11:50 am 10-minute break

12:00 pm Review of What We Know – Dave Kazyak (USGS), Eric Hallerman (VT) | Presentation Slides

An examination of Brook Trout range-wide patterns and a discussion of how the Chesapeake Bay watershed fits into the broader context. Major considerations to consider within in the Chesapeake watershed are connectivity, small population size, hatchery introgression, local adaptation, and changing landscapes and climate.

12:45 pm Group Discussion

1:15 pm Lunch

2:15 pm Tools for Studying Genetics – Amy Welsh (WVU) | Presentation Slides

With a focus on how information has changed over time, Welsh will provide a short background on various techniques and major metrics for studying genetics.

3:00 pm Group Discussion

3:15 pm Using Genetics to Identify Robust & At-Risk Populations – Jason Coombs (USFWS)

Presentation Slides

Identifying and conserving strongholds: 'what constitutes a stronghold?', Key considerations, Protection strategies

3:45 pm Genetic Rescue – Andrew Whiteley (University of Montana) | Presentation Slides

Whiteley will provide an overview of genetic rescue, associated risks and rewards, and a review of the current State of the Science.

4:15 pm Group Discussion

4:45 pm Synthesize and Overview of Day 1

5:00 pm Recess

Wednesday, September 29

9:00 am Synthesize and Overview of Day 1

9:15 am Role of Genetics in Supporting Reintroductions

- Dave Kazyak (USGS), Jake Rash (North Carolina Wildlife Resources Commission), Shannon White (Contractor to the U.S. Geological Survey)

- Selection of source stocks
- Strategies (pros and cons)
- Evaluation of success
- Case studies (LeConte, Purlear, others)
- Need for further evaluation

10:00 am 10-minute break

10:10 am Group Discussion

10:40 am Environmental DNA: Utility and Application – Meredith Bartron (USFWS) | Presentation Slides

- Overview of approaches and applications
- Limitations
- Key considerations for successful implementation

11:20 am Group Discussion

12:20 pm Lunch

1:20 pm Communicating to the Public and Decision Makers | Presentation Slides

- Dave Kazyak (USGS) and Jake Rash (North Carolina Wildlife Resources Commission)

1:30 pm Panel discussion | Video Recording

- Jake Rash (North Carolina Wildlife Resources Commission), Matt Kulp (Great Smoky Mountains National Park), Shannon White (PSU), Shawn Rummel (Trout Unlimited)

- How to translate genetics to the general public?
- What resonates?

2:10 pm Lightning Talks: Where are we and where are we going? | Video Recording

- Introduction/format Dave Kazyak (USGS)
- Local adaptation Eric Hallerman (Virginia Tech |) Presentation Slides
- Adaptation to warming temperature Mariah Meek (Michigan State University)
 Presentation Slides
- YY Brown or Rainbow Trout for suppression of non-native trout *Andrew Whiteley (University of Montana)* | Presentation Slides

3:10 pm Workshop Adjourns

Appendix B: Workshop Participants

Participant	Affiliation	Email
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Appendix C: List of Figures

Figure 1. Theoretical loss of genetic diversity (as measured by heterozygosity) over generations
in isolated populations. A range of effective population sizes are presented
Figure 2. Relationship between one measure of genetic diversity (allelic richness) and effective
population size in wild Brook Trout populations from Maryland. Isolated populations are shown
with dark gray points. Populations from the interconnected stream network of the Upper Savage
River watershed are shown in yellow. Data from Morgan et al. (2021)
Figure 3. Relationship between one measure of genetic diversity (allelic richness) and the extent
of differentiation (mean-population specific F'ST) in wild Brook Trout populations from
Maryland. Isolated populations are shown with dark grey points. Populations from the
interconnected stream network of the Upper Savage River watershed are shown in yellow. Data
from Morgan et al. (2021)
Figure 4. Latitudinal patterns of effective population size in wild Brook Trout populations.
Populations from within the Chesapeake Bay watershed are shown in green, except those
populations from the interconnected Upper Savage River watershed which are shown in yellow.
All other populations are shown in dark grey. Data from Kazyak et al. (2022)
Figure 5. Latitudinal patterns of genetic diversity (as measured by allelic richness) in wild Brook
Trout populations. Populations from within the Chesapeake Bay watershed are shown in green,
except those populations from the interconnected Upper Savage River watershed which are show
in yellow. All other populations are shown in dark grey. Data from Kazyak et al. (2022) 14
Figure 6. Screenshot of the Brook Trout Explorer tool with HUC 4 Watershed overlay
Figure 7. An example of a multi-step filtering process which highlights how demographic and
genetic data were used in the design of a Brook Trout reintroduction effort in North Carolina 17
Figure 8. Conceptual diagram showing common pathways of information related to Brook Trout
genetics
Figure 9. Venn diagram showing similarities and differences of gene expression patterns among
four lake-dwelling populations of wild Brook Trout in Adirondack Park, NY. Although 45 genes
were up- or down-regulated in response to heat stress in all four populations, the majority of up-
or down-regulated genes were unique to a particular population. Adapted from "Adaptation to
Warming Temperatures" [Presentation] by M. Meek (2021, September)
Figure 10. Sample relationships between average temperatures and allele frequencies of genes
associated with thermal response in Brook Trout. Many relationships were statistically
significant and showed reasonable strong correlation coefficients between genotypes and
temperature. Adapted from "Adaptation to Warming Temperatures" [Presentation] by M. Meek
(2021, September)
Suppression of the population in conjunction with the introduction of YY males is represented by
a red, dotted line. Figure from Day et al. (2020)
Figure 12. Comparison of the length of time required to achieve extirpation with varying
treatment strategies and electrofishing mortality rates. Figure from Day et al. (2021)
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Appendix D: List of Tables

Table 1. Management	applications and a	ssociated	questions r	elated to I	Brook Trout	genetics	
which were identified	prior to the STAC	workshop)				2

Appendix E: Participant Responses to Mentimeter Introduction Prompt

Your name, affiliation, and one thing you would like to get out of this workshop:

Amy Welsh, WVU, managers' research needs Jerrod Parker, Maine Inland Fisheries and Wildlife Stephen Faulkner, USGS - genetics science needs of brook trout managers Jason Coombs, USFWS: What data/tool needs managers Nat Gillespie, US Forest Service and EBTJV, need to incorporate genetic information into management recommendations for brook trout conservation Nadya Mamoozadeh Michiaan State University How brook trout management, and conversely, how geneticists can better learn about the needs of manager Dan Goetz Maryland Department of Natural Resources Best approaches for reintroductions and genetic rescue for brook trout populations. Nat. Res. Barb Lubinski, USGS EESCMeet some folks (at least Mariah Meek, Michigan State University. excited to meet Sally PetreTennessee Wildlife Resources AgencyInformation virtually!) that we work with everyone and see how our genetic tools can help brook on how to choose stocks to restore populations Brad Fink, Virainia Department of Wildlife Resource - Using Steve Reeser, Virginia Department of Wildlife Resources BKT genetics to determine population health. A better How to use genetics to make the best management understanding of tools and resources available regarding & Sumter NFs in South Carolina - Best way to account for decisions genetics in our brook trout re-introduction projects Jacob Rash - to learn how others are integrating these data Barry Baldiao, USGS-NYWSC, Interested in learning Poss Shramko New Jersey Division of Fish and Wildlife Gain into decision making processes specifics about other projects/programs intended to knowledge on collection of genetic information for protect and restore brook trout populations in different management of our coldwater resources and restoration of Corey Pelletier- Rhode Island Division of Fish and Wildlife-Matt Kulp, National Park Service, Tools to discern genetic Brandon Simcox, Tennessee Wildlife Resources Agency Better understand how genetics are being used to guide health of source stocks in order to inform translocation of utilizing genetics information in restoration decision making progressive management. Brook Trout into newly restored stream sections. Emily Shosh - PA Native Fish Coalition, Potter County Scott Collenburg, NJDFW, understanding the importance of Colin Krause, Forest Service, Southern Research Center Conservation District Educator - Hoping to find out more genetics information and how to apply it to make Center for Aquatic Technology Center. Learn how genetics about how to prioritize restoration projects and how to management decisions for brook trout data is for management. communicate their effectiveness and importance to the general public. David Thorne, West Virginia Division of Natural Resources, Eric Smith - Virginia Tech - Can we combine information on Coldwater Fisheries Biologist - interested in using genetic genetics with information on landscape and other Brandon Keplinger, District 2 Fisheries Biologist, WV characteristics to model and better inform decisions about information to better inform management and restoration DNRAdequate genetic diversity metric thresholds that may indicate long-term population stability. Theoretical or realized consequences of suppressed genetic diversity over Lori Maloney EBTJV meeting needs of the managers across Dave Kazyak, USGS Eastern Ecological Science Guidance on use of genetics information in the re CenterEngage with managers, conservation practitioners, establishment of populations from hatchery propagation or and other researchers to exchange ideas and advance brook trout conservation Philip Light, State Chair Native Fish Coalition - Pennsylvania Chapter. Better understand brook trout genetics and how Ben Letcher, USGS, development of a common SNP panel to best help advocate for brook trout Meredith Bartron, USFWS, I'd like to hear the science needs for brook trout are from the biologists and managers at the field level help them manage the resource Eric Hallerman Virginia TEch, broader familiarity and Matt Lawrence, Maryland Department of Natural appreciation of the importance of genetics, and meet more Resources, A better understanding of the use of genetic people interested in regional brook trout management Shannon White USGS- determine what's needed to techniques for brook trout managment

Mentimeter results from a full-group exercise on Day 1. Participants were asked to input their name, affiliation, and "one thing [they] would like to get out of [the] workshop." Answers live populated as participants responded to the posed question.

translate science into practice