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A MULTIDISCIPLINARY APPROACH TO THE MANAGEMENT OF A NON-NATIVE TROUT SPECIES

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A multidisciplinary approach to the management of a non-native trout species

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ABSTRACT

Non-native freshwater fish are considered a significant threat to the survival of native freshwater fish populations. Traditional management strategies for dealing with non-native fish such as chemical or mechanical removal have limitations and can be unsuccessful. A novel method for the removal of non-native fish is a technique where the addition of non-native male fish with a YY genotype (M_{YY}) theoretically results in a shift of the population sex ratio towards all or mostly all males, driving population extirpation. However, many aspects that affect the success of this method have not been thoroughly tested. My doctoral research incorporated multiple disciplines to enhance the management strategies for a non-native trout, the brook trout (*Salvelinus fontinalis*). Currently, in the Boundary Dam reservoir in Washington, an extensive management program of these non-native fish is underway, including suppression and chemical removal as well as introduction of M_{YY}. I first used genetic monitoring to provide managers with information regarding the genetic structure of brook trout populations and provide information about population resilience in the face of management efforts. I found evidence of significant genetic substructure within the system and highlighted three populations that were most likely to be successfully eradicated due to limited gene flow. I also found evidence of isolation by distance within the largest Boundary tributary (Sullivan Creek) suggesting that partial eradication within this system would likely be followed by recolonization. Next, I performed a study of the reproductive performance of M_{YY} brook trout compared to hatchery XY brook trout in a lab-based setting. My results indicate that M_{YY} brook trout perform similarly to hatchery XY males at fertilization and their offspring survive similarly at early development stages suggesting they could be an effective tool in non-native brook trout eradication efforts. Simulation studies testing the effectiveness of M_{YY} have suggested that eradication success and/or minimum population size of the non-native population may be affected by many different factors. However, no studies to date have looked at the possible consequences on the remaining population if M_{YY} management plans result in failure to eradicate. Suppression and M_{YY}

introduction cause reductions in the abundance of the population, essentially forcing these populations through a population bottleneck and increasing the chance of inbreeding depression (ID). I performed a simulation study that looked at the effects of ID on the bottleneck and recovery of the remnant brook trout population after suppression and M_{YY} introduction if it does not result in eradication. I found that during M_{YY} introduction, ID resulted in a decrease in the population abundance compared to models that did not include fitness effects. However, because of increased genetic variation due to hatchery M_{YY} admixture, populations recovered to above pre-treatment levels for most simulations post-suppression and M_{YY} treatment. This result suggests that even if populations are driven to very low abundance, managers should not rely on them going extinct due to the effects of ID. Finally, I conducted a survey of wildlife managers to determine how manager characteristics influence the likelihood that managers will implement two novel management methods (M_{YY} implementation and genetic rescue) to conserve native headwater stream fish populations. Findings suggest that risk tolerance was a good indicator for managers willingness to implement novel strategies. Additionally, we found differences for managers from different states and regions in their willingness to implement novel strategies. These results show that understanding the individual characteristics of managers is important for identifying factors that hinder the implementation of novel methods in the conservation of species. Overall, this research demonstrates that genetic tools can be informative when managing non-native species. M_{YY} may be an effective approach to the management of non-native species, however, caution should be taken as incomplete eradication could result in full recovery of the population. Finally, understanding manager characteristics could be beneficial for determining whether managers are willing to implement novel management strategies.

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CHAPTER 1: Introduction and overview

The spread of non-indigenous species is increasing worldwide due to globalization and an increase in international commerce (Sala et al. 2000; Gozlan 2008). Non-indigenous species (also referred to as non-native species, alien species, exotic species) are species that have been introduced into an area beyond their native range due to human action (Jeschke et al. 2014). The rate with which species are being introduced outside their native range is increasing drastically, with almost 40% of the known exotic species having been introduced in the last 50 years (Seebens et al. 2017).

For the majority of introduced species, the ecological consequences of their introduction are unknown. However, non-native species are considered one of the greatest causes of species becoming endangered or going extinct second only to habitat destruction (Pejchar and Mooney 2009). In the United States, 400 of the 958 species listed as threatened or endangered under the Endangered Species Act are considered to be on that list because of interactions with non-native species (Wilcove et al. 1998; Pimentel et al. 2005). Non-native species can cause global ecosystem damage and biodiversity loss through competition, displacement, hybridization, introgression, or predation within the environments that they invade, ultimately impacting ecosystem function (Mooney and Cleland 2001; Jeschke et al. 2014). Mollot et al. (2017) ran a global meta-analysis and reported that a 16.6% decrease in species richness can be caused by a single introduced species. If the introduced species is a top predator, then they can exhibit "top-down" control of the food web, impacting the abundance and biomass of native lower-level species. Alternatively, if the introduced species is at a lower trophic level, it can impact the amount of total energy available through nutrients, resulting in a "bottom-up" effect through the trophic system. For example, non-native zebra mussels (*Dreissena polymorpha*), predators of phytoplankton, reduce the abundance of phytoplankton considerably within their invasive range. This causes negative consequences for higher trophic levels within those systems, indirectly impacting the species composition of the ecosystem (Ward and Ricciardi 2007).

Unfortunately, it is extremely difficult to remove a non-native species once they have become established due to what is known as the lag effect (Mooney and Cleland 2001). The lag effect is where introduced species exist at low population numbers for years before a sudden

expansion in population size. This can be the result of a normal increase in population size and distribution over time, however, environmental change can also cause a lag effect if the change supports population growth of the non-native species (Crooks et al. 1999; Mooney and Cleland 2001). As a result of the lag effect, managers may not know that a species is present until it is too late, and populations have become established (Crooks 2005).

It can be incredibly expensive to try and eradicate an established non-native population. For example, the United States Department of Interior reported spending $143 million on invasive species management in 2020 (USDOI 2021; Fantle-Lepczyk et al. 2022). Additionally, control and eradication programs are often unsuccessful, resulting in wasted effort and money (Manchester and Bullock 2001). For example, Pluess et al. (2012) ran a meta-analysis of 173 different eradication campaigns of 94 species worldwide and found that only around half (50.9%) were successful at eradicating non-native species.

Non-native Fishes

One of the major causes of the worldwide decline of native aquatic fauna is the introduction of non-native freshwater fishes (Ribiero and Leunda 2012, Cambray 2003; Clavero and García-Berthou 2006; Helfman 2007). According to USGS, there are over 600 introduced fish species in the United States alone (USGS 2023). Unfortunately, despite the measures used to decrease fish introductions, the rates of fish invasions continue to increase and impact almost every major watershed within the United States (Thomas et al. 2008).

Fish are considered non-native if they are introduced to new areas through intentional stocking or unintentional stocking (Rahel and Olden 2008). Intentional stocking is when fish are stocked specifically to increase recreational fishing. Unintentional stocking is the accidental release of non-natives through enclosure escapes or pet releases (Donaldson and Cooke 2016). Once a non-native fish species has been introduced, its successful establishment is dependent upon the reproduction rate, growth, and mortality of the non-native species, as well as the successful competition of resources of the non-native species with native species (Sammarco et al. 2015). Non-native fish species can cause different impacts on the ecosystem, in some cases completely displacing the native species, in others becoming the dominant competitor for resources (Sammarco et al. 2015).

Brook Trout

The brook trout (*Salvelinus fontinalis*) is a non-native species to western North America. Brook trout were intentionally introduced in the late 1800's to early 1900's by the U.S. Fish Commission (MacCrimmon and Campbell 1969; MacCrimmon et al. 1971; Crawford 1979) to promote recreational fishing. They have now established populations ranging from Southeast Alaska to Texas (Fuller and Neilson, 2019). Brook trout can tolerate a wide variety of environmental conditions making them less specialized in terms of habitat demands than other members of the salmonidae family (Karas 1997). These traits have allowed them to invade many different environments. Once established, an introduced brook trout population can easily spread, usually upstream as they have the capacity to travel up steep slopes (>13%) (Dunham et al. 2002). However, if sufficient space is limited, they will disperse downstream as well (Karas 1997). Given enough time, this allows for a single introduced population to spread through an entire river system (Karas 1997; Dunham et al. 2002). They continue to colonize new habitats (Dunham et al. 2002) and are now the most common trout in small (typically headwater) streams in the Western United States (Behnke 1978; Schade and Bonar 2005).

Brook trout are thought to be one of the primary causes of the decline of native cutthroat (*Oncorhyncus clarkii*) and native bull trout (*Salvelinus confluentus*) populations in western North America (USFWS 1999a; USFWS 1999b; Rieman et al. 2006; Warnock and Rasmussen 2013). Bull trout are federally listed as threatened by the U.S. Fish and Wildlife Service (USFWS 1999b). Brook trout are highly phenotypically plastic (Kennedy et al. 2003) and higher size-specific fecundity and earlier maturation results in competitive advantages over and predation of cutthroat and bull trout populations (Kennedy et al. 2003; Kennedy et al. 2018). Additionally, brook trout are able to hybridize with bull trout populations. Hybrid offspring are typically sterile, wasting the reproductive output of bull trout (Allendorf et al. 2001).

Management of brook trout can be complicated and typically there is no comprehensive solution. Common methods utilized by fisheries managers to eradicate non-native brook trout populations include chemical or mechanical removal (Donaldson and Cooke 2016). In the presence of native species, eradication usually includes mechanical removal as this poses the least risk to native species, however, it can often be unsuccessful (Meyer et al. 2006). Chemical removal is faster and usually more effective than manual removal, however, it must be done in

areas where no native species are present as the chemical will also eliminate native populations. Managers can also choose to intentionally isolate native populations through barrier installation to prevent the spread of brook trout. However, this also has issues in that it prevents movement and gene flow into the native population and can lead to extinction (Fausch et al. 2009).

An alternative method, the addition of brook trout with a YY genotype (hereafter M_{YY}), represents an alternative tool for non-native fish eradication (Schill et al. 2016; Schill et al. 2017; Kennedy et al. 2017; Kennedy et al. 2018). In this approach, the artificial propagation and subsequent introduction of M_{YY} into a wild population theoretically results in a shift of the population sex ratio towards all or mostly all males, causing demographic population extirpation due to the elimination or shortage of one sex. Recent studies have indicated the use of M_{YY} male brook trout in conjunction with suppression activities may accelerate the rate in which the brook trout population declines as compared to suppression alone (Kennedy et al. 2018). While the M_{YY} approach appears promising, many factors that will determine the success of this method have not been thoroughly tested (Kennedy et al. 2017; Kennedy et al. 2018; Day et al. 2020; Day et al. 2021).

The aim of my dissertation was to take a multidisciplinary approach to the management of non-native brook trout through four different chapters. Note that due to the highly collaborative nature of my dissertation, I use the term “we” and “our” throughout this document. The first chapter used the discipline of population genetics and focused on an analysis of the genetic structure of brook trout within Washington state to inform adaptive non-native species management. We found significant genetic substructure and determined three populations with limited gene flow that were most likely to be successfully eradicated. We also found evidence of isolation by distance within the largest Boundary tributary (Sullivan Creek), where suppression and M_{YY} introduction are currently being implemented, suggesting that partial eradication within this system would likely be followed by recolonization.

The second chapter involved the discipline of experimental biology and consisted of an analysis of the reproductive performance of M_{YY} brook trout compared to hatchery XY brook trout in a lab-based setting. Our results indicate that M_{YY} brook trout fertilize eggs at a rate comparable to hatchery XY males and their offspring survive similarly at early development stages. These results suggest they have similar reproductive performance to XY males and could be an effective tool in non-native brook trout eradication efforts.

The third chapter included a model simulation of the fitness effects of releasing M_{YY} brook trout into populations of wild non-native brook trout if it does not result in the eradication of brook trout populations. We found that inbreeding depression during suppression and M_{YY} introduction resulted in a decrease in the population abundance of brook trout compared to simulations that did not include a fitness effect. However, because of increased genetic variation due to hatchery M_{YY} admixture, populations recovered to above pre-treatment levels for most simulations post-suppression and M_{YY} treatment. This result suggests that even if populations are driven to very low abundance, managers should not rely on them going extinct due to the fitness effects of the bottleneck.

Finally, the fourth chapter used the discipline of human dimensions to conduct a survey of how the characteristics of managers influence their willingness to use novel techniques such as M_{YY} brook trout to help eradicate non-native fish populations. Characteristics such as risk tolerance were found to be good indicators of managers' willingness to implement novel strategies. Additionally, we found managers from different states and regions differed in their willingness to implement novel strategies. These results show that understanding the individual characteristics of managers is important for identifying factors that prevent the implementation of novel methods in the conservation of species.

Overall, this research demonstrates that multi-disciplinary approaches provide a comprehensive and compelling method for non-native species management. Understanding the genetic structure of non-native populations can help to inform management strategies (Chapter 1). M_{YY} have reproductive success that is similar to that of hatchery XY, which suggests they can successfully reproduce and may be successful at shifting the sex ratio of the population (Chapter 2). However, if the population is not completely eradicated, then brook trout populations may recover after introduction of M_{YY} has ceased (Chapter 3). Finally, understanding managers' characteristics could provide a better understanding of how novel methods are implemented in non-native species management (Chapter 4).

CHAPTER 2: Using population genomic analysis of lower Pend Oreille River Brook Trout (Salvelinus fontinalis) to inform eradication efforts.

Abstract

Examination of the current population genetic structure of non-native populations can provide insight into the potential origin of genetic diversity within non-native populations and provide information about population resilience in the face of management efforts. We examined genetic variation within populations, genetic differentiation among populations, and attempted to determine the source of brook trout (*Salvelinus fontinalis*) populations from select tributaries to the lower Pend Oreille River in the vicinity of Boundary Dam in eastern Washington. We also performed a finer spatial scale analysis to test the null hypothesis of isolation by distance (IBD) within the Sullivan Creek watershed. We were able to narrow the likely hatchery source to a region within the native range, and could not rule out a single source, which would be consistent with historical records. Genetic variation was the highest in naturalized Washington populations, even higher than the most likely hatcheries of origin, and substantially higher than populations from the native range. Three tributaries located above barriers were substantially genetically differentiated from the remainder of sites. We observed significant genetic subdivision within some tributaries. Among physically connected sites within Sullivan Creek, we detected a highly significant pattern of isolation by distance. Eradication of highly differentiated sites would likely not be followed by recolonization from other nearby tributaries. Partial eradication within tributaries would likely be followed by recolonization at different rates, ranging from slowly in tributaries with population subdivision, to more quickly in Sullivan Creek with its pattern of isolation by distance. Additionally, the observed pattern of isolation by distance suggests that for Sullivan Creek, management plans to introduce M_{YY} should involve reintroduction locations that are closer together to increase the likelihood of eradication. This work provides an example of how genetic monitoring can inform adaptive non-native species management.

Introduction

Non-native freshwater fishes are one of the major causes of the decline of native aquatic fauna worldwide (Cambray 2003; Clavero and García-Berthou 2005; Helfman 2007; Ribiero and Leunda 2012). It is estimated that around 624 freshwater fish species have been introduced outside of their native range (Gozlan 2008). Non-native fish species are thought to be one of the main threats to at-risk fish species (Jelks et al. 2008) and are estimated to be the cause of about 68% of fish extinctions in North America (Miller et al. 1989). Unfortunately, despite measures to decrease fish introductions, rates of fish invasions continue to increase (Gozlan et al. 2010) and impact almost every major watershed in the world (Ricciardi and MacIsaac 2011; Pyšek et al. 2020).

After a non-native species has become established, an increasingly common management strategy is to attempt to eradicate them (Gozlan et al. 2010; Britton et al. 2011). Eradication can be attempted by mechanical removal, but this can take time and effort and might not be successful (Meyer et al. 2006). For aquatic species, eradication can also be accomplished through a chemical treatment applied to the water. Chemical removal is usually less time and labor-intensive compared to manual eradication in aquatic systems and has higher rates of success (Rytwinski et al. 2019). However, chemical removal requires that a native species does not co-occur or that the native species is temporarily removed from the habitat (Britton and Brazier 2006; Britton et al. 2011). Otherwise, managers often resort to suppression, where they reduce the abundance of the non-native species with the hope of lessening the negative effects of biotic interactions (Peterson et al. 2008).

A new management strategy meant to aid in eradication efforts is the approach often referred to as M_{YY} male introduction (Schill et al. 2016; Schill et al. 2017; Kennedy et al. 2017; Kennedy et al. 2018). M_{YY} males offer an opportunity for non-native local eradication in cases where managers have otherwise had to settle for suppression. This approach involves the introduction of hatchery-produced M_{YY} males of the non-native species (Schill et al. 2016; Schill et al. 2017; Kennedy et al. 2017; Kennedy et al. 2018). M_{YY} are males that have two Y chromosomes and the introduction of M_{YY} into a wild population can theoretically cause population extirpation through a shift of the population sex ratio towards all or mostly males. Recent empirical (Kennedy et al. 2018) and simulation (Day et al. 2021) studies have indicated

that the use of M_{YY} in conjunction with manual suppression activities may accelerate the rate of non-native population decline compared to suppression alone.

Genetic tools can play an important role in non-native species management by informing the likelihood of recolonization and susceptibility to management efforts. First, for local eradication to be successful, through chemical, mechanical, or M_{YY} approaches, recolonization must not occur (Britton et al. 2011). Surveys of population genetic structure prior to eradication efforts can inform the likelihood of recolonization from nearby populations. For example, by identifying connected subpopulations that, if overlooked, could be a source of immigration into focal management areas.

Second, determining the population genetic structure of a set of potentially connected non-native populations can inform susceptibility to eradication or suppression. In theory, small, inbred populations with low genetic variation are expected to have weaker demographic vital rates (Westemeier 1998) and therefore should be more susceptible to mechanical eradication efforts or M_{YY} introduction. Alternatively, if a set of populations was founded by multiple intentional introductions from either the same or multiple sources, this scenario could create high genetic variation and novel genetic combinations that allow a response to natural selection under novel conditions (Facon et al. 2008; Neville and Bernatchez 2013). Analyzing the potential sources of non-native populations can provide insight into the potential origin of genetic diversity within these systems and provide information about population resilience in the face of management efforts. If historical events have led to highly genetically variable and demographically robust populations, this could make populations more difficult to eradicate.

Third, genetic approaches can help to inform the number of release locations of M_{YY}. Simulations have shown that the number of release locations can impact the overall success of M_{YY} implementation in eradicating non-native populations, however, this is dependent upon the movement and dispersal of the non-native population as well as M_{YY} within the system (Day et al. 2020). By determining the genetic spatial structure of populations, it is possible to determine the movement and dispersal of genes across a landscape (Kanno et al. 2011). This could then be used to inform simulations as to how far non-native fish move within watersheds and to determine where to introduce M_{YY} and how many release locations of M_{YY} are needed.

A brook trout eradication program is underway in tributaries of the lower Pend Oreille River in eastern Washington, USA. The brook trout (*Salvelinus fontinalis*), native to eastern

North America, is a common non-native species to western North American aquatic systems (Dunham et al. 2002). The lower Pend Oreille River runs from Lake Pend Oreille in Idaho through Washington until it meets with the Columbia River in Canada. Historically both native bull trout (*Salvelinus confluentus*) and cutthroat trout (*Oncorhynchus clarkii*) populations occurred in tributaries to the lower Pend Oreille River, but due to the construction of several dams and the introduction of brook trout within some of the tributaries, native trout populations are declining or have been extirpated (USFWS 1999a; USFWS 1999b). The electrical company that owns nearby mainstem Boundary Dam, Seattle City Light (SCL), along with state, tribal, and federal cooperators, began a comprehensive suite of measures to benefit populations of native salmonids within the lower Pend Oreille River and its tributaries near Boundary Dam (hereafter Boundary tributaries; R2 Resource Consultants 2014) in 2015. The eradication and suppression of non-native brook trout is a key component of aquatic habitat measures currently underway for Boundary tributaries. Chemical eradication has or will be used where brook trout are allopatric. Where brook trout are sympatric with native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*), manual electrofishing suppression and M_{YY} introduction are used. In the largest sympatric drainage (Sullivan Creek), brook trout suppression began in 2016 and M_{YY} introduction began in 2018.

Here, we examined the population genetic structure of brook trout from Boundary tributaries to inform these suppression and eradication management actions. We conducted genetic analyses at two different spatial scales. At a larger spatial scale, we performed a population genetic analysis of brook trout collected from 15 Boundary tributaries, eight natural populations from the native range, and 11 hatchery strains. At this larger spatial scale, our goals were to examine genetic variation within and among populations within the Boundary system and provide insight into the potential origin of brook trout populations within these tributaries. At a finer spatial scale, our goal was to test the null hypothesis of isolation by distance (IBD) within brook trout occupied Sullivan Creek along with four Sullivan Creek tributaries lacking physical barriers. Our results inform (1) the likelihood of success of various eradication efforts within Boundary tributaries (suppression, eradication, and M_{YY} introduction) and (2) release strategies for M_{YY} in Sullivan Creek.

Methods

Study Organism

Brook trout were intentionally introduced outside of their native range in the United States in the late 1800s to early 1900s by the U.S. Fish Commission (MacCrimmon et al. 1971; Kennedy et al. 2018) to promote recreational fishing (USDA 2019). These introductions led to the establishment of brook trout populations ranging from Southeast Alaska to Texas (Fuller and Neilson, 2019; USGS 2023), and brook trout are now often the most common trout in small (typically headwater) streams in the western United States (Behnke 1979; Schade and Bonar 2005). Brook trout are thought to be one of the primary causes of the decline of native cutthroat and native bull trout populations in western North America (Rieman et al. 2006; USFWS 1999a; USFWS 1999b; Warnock and Rasmussen 2013). Brook trout are highly phenotypically plastic (Kennedy et al. 2003), and higher size-specific fecundity and earlier maturation result in competitive advantages over, and predation of, cutthroat and bull trout populations (Kennedy et al. 2003; Dunham et al. 2004). Additionally, brook trout have been shown to hybridize with bull trout (Allendorf et al. 2001). Brook trout have been stocked in most tributaries to the Pend Oreille River near the Boundary Dam (R2 Resource Consultants 2014; Figure 2-1).

Boundary tributary brook trout tissue collection

A total of 637 brook trout tissue samples were collected over the summers of 2016, 2017, and 2018, by collaborators from SCL, WDFW, the Kalispel Tribe of Indians, the University of Montana, and Eastern Washington University. Tissue samples from 20-30 mixed-age brook trout (> 70 mm fork length) were collected from sites within Flume, Lime, Slate, Slumber, Styx, Sweet, Sullivan, and Uncas Gulch Creeks (Figure 2-1). In sites where 20-30 fish were not found, fin clips from all fish were collected. In the Sullivan Creek watershed, tissues were collected at eight mainstem locations (established monitoring sampling units) and within five tributaries (Table 2-1). All brook trout were captured by electrofishing and euthanized with MS-222 as part of eradication efforts. Fork length (mm) and wet weight (g) of each individual were recorded. Fin clips were preserved in 95% ethanol or dried in Whatman paper and stored in coin envelopes before extraction of genomic DNA.

Tissue Collection of Hatchery and Wild Brook Trout Populations From Their Native Range

To attempt to determine the original source of brook trout introduced into the Boundary tributaries, we used collections from 19 hatchery strains or wild populations meant to represent a wide range of possible sources. Beginning in the 1910's, brook trout thought to have originated from the Paradise Brook Trout Company, Henryville, Pennsylvania, were planted in the area around Republic, WA (Crawford 1979). Some fish from these plants were then used as a source for Owhi Lake, WA. Eggs obtained from Owhi Lake were eventually used to establish the Ford Hatchery broodstock in 1966 (Crawford 1979). Ford Hatchery is thought to be the hatchery strain that was used to stock brook trout within the Boundary Reservoir (Bill Baker, WDFW, personal communication). Sixty-two fin clips were collected from Ford Hatchery in 2018 for subsequent genotyping. These include 25 age-1 (sex unknown) and 37 age-2 (22 males and 15 females) fish. Additionally, brook trout samples were collected from within their native range including eight eastern US and two midwestern US hatcheries, and eight eastern US wild populations (three from the mid-Atlantic region and five from the northeastern region as defined by Kazyak et al. (2022)) (Table 2-1). Hatchery samples include (state in parentheses): Berlin Hatchery (NH), Burton State Hatchery (GA), Milford Hatchery (NH), Paint Bank Hatchery (VA), Iron River Hatchery (WI), 'MN wild' (MN, mixture of two wild MN populations), Hyde Pond Hatchery (NY), Big Hill Pond Hatchery (NY), Walhalla State Hatchery (SC), and Wytheville State Hatchery (VA). These additional hatchery sources have been chosen to capture extant genetic variation that occurs among hatcheries from the native range of the species (Pregler et al. 2018, Kazyak et al. 2022)(see http://bte.ecosheds.org/ for a visualization of genetic patterns of hatchery and wild populations within the brook trout native range from Kazyak et al. 2022). We included wild eastern populations to make sure that we had a more complete representation of genetic variation within the native range of brook trout. These include the following east coast wild populations (state in parentheses): Upper Cohocton Creek (NY), Cohocton Creek (NY), Wiley Brook (NY), West Brook (MA), and Stanley Brook (ME) and the following mid-Atlantic population Savage Creek (split into two subpopulations Upper Savage River 41 and Upper Savage River 90; MD) and Fridley Gap (VA). The northeastern and mid-Atlantic genetic groups appear to be the source of most brook trout hatchery strains (Kazyak et al. 2022).

DNA Extraction

Total genomic DNA was extracted from 20 to 50 mg of tissue (fin clips) using a modified version of the extraction method found in Ali et al. (2016). Extracted DNA was then sent to the Idaho Fish and Game (IDFG) Eagle Genetics Lab for library preparation and sequencing analysis. Library preparation was done following the GTSeq approach developed by Campbell et al. (2015). GTSeq was performed for each individual using an established 240 SNP panel. A subset of the SNPs on this GTSeq panel have been used to examine genetic structure within other areas of the non-native range of brook trout (Neville and Bernatchez 2013). This SNP panel has also been used for genetic assignment of offspring to parental type in experimental systems into which M_{YY} male brook trout have been released (Kennedy et al. 2018). SNPs on this panel were originally discovered from wild populations in Quebec, Canada, introduced populations in Chile, and from Paradise Hatchery in Pennsylvania (Narum et al. 2017).

Screening of SNPs

We initially conducted a data screening step based on missingness. Loci with less than 80% genotype success were removed from the analysis. We tested for conformance to Hardy-Weinberg (HW) proportions and for linkage disequilibrium. We conducted a locus-specific analysis of conformation to HW proportions by examining raw *P*-values and calculating the number of collections with significant departures from HW expectations ($P < 0.05$). We compared observed HW deviations to binomial expectations. Loci that showed significant deviation from HW proportions across large numbers of collections (≥ 7) were removed (Waples 2014). Deviations from HW expectations and elevated LD can occur when samples contain single cohorts that have multiple siblings from the same family (Waples and Anderson 2017). Therefore, when more than two YOY were included in a collection, we ran COLONY (version 2.0.5.0; Jones and Wang 2010) and implemented the yank-2 procedure of Waples and Anderson (2017), where if a full-sibling family is greater than two individuals, two full-siblings were retained randomly. Next, because loci that deviated widely from HW expectations in few collections could have been retained by our first HW filtering step, we re-tested loci for conformation to HW using collections with sample size of 20 or larger. We used Bonferroni corrected *P*-values from exact tests for conformance to HW proportions performed with the R package *genepop* (Rousset 2008). We corrected for the number of tests performed based on the number of loci genotyped within each collection. We excluded from subsequent analyses loci

that yielded significant tests (after correction for multiple tests) in more than one population sample. In pairs of loci that showed significant LD across large numbers of collection samples (≥ 8), one locus was randomly chosen to be removed. LD was calculated using the *genepop* package in R (Rousset 2008).

Genetic Analyses

Observed (H_O) and expected (H_e) heterozygosity per population and mean number of alleles per collection (A) were estimated with custom R scripts. We did not calculate summary statistics for sites with six or fewer individuals.

Larger Spatial Scale Analyses

Within tributaries, we performed initial tests to determine whether nearby collections should be pooled or analyzed separately. F_{ST}'s were calculated with R package *hierfstat* (Goudet 2005) using the Nei F_{ST} approach. Chi-squared P-values were calculated to test for allele frequency divergence and to assign significance to pairwise F_{ST}'s using the *adegenet* R package (Jombart et al. 2010). Sullivan Creek was more extensively sampled compared to other tributaries. Therefore, to avoid bias in clustering analyses due to uneven sample sizes (Puechmaille 2016), we grouped Sullivan Creek collections into upper (S18.6, n=23 and S20.1, n=47), middle (S17.2, n=39), and lower (S13.1, n=28 and S14.2, 24) sampling units (hereafter referred to as SU's) based on tests of statistical significance of F_{ST} values (Table S2-1). SUs are consecutive 200m sampling reaches and numbers correspond to location within the tributary (lower SU numbers occur closer to the mouth of a tributary). Additionally, the four collections from Lime Creek (1.3, 1.6, 1.7, and 1.9) were grouped into three separate collections (Lower Lime: 1.3, n=10; Middle Lime: 1.6, n=39; and Upper Lime: 1.7 and 1.9, n=10) based on our F_{ST} analysis. The three collections from Flume Creek (Flume 1.3, Flume 1.15, and Flume 1.27) were grouped into two separate collections (Lower Flume: 1.3 and 1.15, n=33; and Upper Flume: 1.27, n=28) based on the F_{ST} analysis. Samples collected from Upper Slate (1.27, n=22) and Lower Slate (1.9, n=8 and 1.12, n=11) were kept separate based on our F_{ST} analysis. Samples collected from Sweet Creek (1.20, n=11; 1.21, n=14; 1.23, n=7; and 1.25, n=4) were grouped together (Upper Sweet Creek) based on the F_{ST} analysis as well as spatial proximity.

To examine initial broad-scale genetic patterns among all collections, an unweighted pair group method with arithmetic mean (UPGMA) dendrogram was built from genetic distances

calculated with the R package *poppr* (Kamvar et al. 2014) using Nei's genetic distance (Nei 1972, Nei 1978). We also conducted DAPC using the R package *adegenet* and conducted a STRUCTURE (Pritchard et al. 2000) analyses using all the collections. DAPC is a multivariate approach that first transforms data using a principal components analysis (PCA) and then clusters genetically related individuals using discriminant analysis (DA). We visually determined the number of PCs corresponding to cumulative variance explained (N = 50 PCs). For STRUCTURE, we used 100,000 replicates and 10,000 burn-in cycles under an admixture model for each run with no location prior. We performed five replicate runs for each of $K = 2$ to 20.

Next, based on initial results, we performed a second DAPC and STRUCTURE analysis using a subset of collections including all the Boundary tributary samples along with only the hatcheries that initially most closely clustered with collections from the Boundary tributaries. We conducted this second set of analyses to test for more subtle genetic differentiation that might have been masked by the inclusion of highly divergent eastern populations and hatchery samples. We also constructed a dendrogram on hatchery populations only to examine more closely the relationships among the hatchery samples.

Finer Spatial Scale Analysis

At a finer spatial scale, we examined patterns of gene flow within the Sullivan Creek watershed (Sullivan Creek Collections, Table 2-1). For analyses at this spatial scale, we used n = 336 of the mixed-aged samples collected from eight Sullivan Creek mainstem reaches and the four Sullivan Creek tributaries lacking physical barriers to the main stem (Leola, Deemer, Gypsy, and Pass Creeks). Chi-squared P-values were calculated to test for allele frequency divergence and to assign significance to pairwise F_{ST}'s using the *adegenet* R package. We tested the relationship between geographic and genetic distance by performing mantel correlograms. Individuals were grouped by SU and pairwise F_{ST} was used as the estimate of genetic distance. Geographic distances (riverine distances between each pair of sampled SUs) were determined from Google Earth. Mantel correlograms were calculated using the *vegan* package in R (Oksanen et al. 2013). Statistical significance was obtained by running 9999 permutations with an α = 0.05. We ran two separate mantel correlograms. For the first, we treated each sample as a separate collection and examined all pairwise genetic distances. For the second, we used the same grouped Sullivan Creek collections (Lower Sullivan, Middle Sullivan, and Upper Sullivan)

used in the larger spatial scale analyses to account for small sample size in some SUs and possible associated bias in F_{ST} values.

Results

Screening of Loci

We examined 1,387 mixed-age individuals at 240 SNPs. Fifteen loci with less than 80% genotype success were removed from the analysis. Significant departures from HW proportions occurred in 226 of the 8,614 tests performed ($P < 0.05$) where 224 were expected by chance ($\boldsymbol{\alpha} = 0.05$). We excluded from subsequent analysis ten loci that yielded significant tests in seven or more populations (Figure S2-1). Next, we performed a Bonferroni correction for multiple tests applied at the population level and removed an additional five loci that were significant in two or more populations. This left a total of 210 loci for subsequent analysis. Next, we performed a COLONY sibship analysis on the collections that included more than two YOY. Collections with full-sibling family sizes of three or more were Upper Sullivan, Slumber, and Leola Creeks. We selected two random YOY from each family and kept those for subsequent analysis. We then re-ran HW and LD based on collections with 20 or more individuals. We did not remove any more loci based on HW. For LD, one locus was removed randomly from locus pairs for which there was evidence of significant LD in eight or more populations. This resulted in a total of 43 loci being removed and 167 loci were retained for subsequent analyses.

Genetic Variation Within Populations

Genetic variation was highest in the naturalized Boundary tributary populations, slightly lower in hatchery collections, and substantially lower in eastern natural populations. Mean ± SD H_e was 0.33 ± 0.009 for the 13 Sullivan Creek collections (n = 13), 0.32 ± 0.02 for the other Boundary tributary collections (n = 13), 0.29 ± 0.02 for midwest hatcheries (n = 2), 0.22 ± 0.02 for northeast coast wild collections (n = 5), 0.16 ± 0.002 for the mid-Atlantic wild collections (n=3) and 0.28 ± 0.05 for east coast hatcheries (n = 8; Figure 2-2). We observed a similar pattern with allelic diversity (*A;* Figure S2-2).

Origin of Brook Trout Within the Lower Pend Oreille River Watershed

The dendrogram using Nei's distance and including all collections revealed allele frequency similarity between all Boundary tributary collections (Figure 2-3). Collections that had the highest dissimilarity from the rest of the Boundary tributary collections were Highline, Upper Sweet, Slumber, Styx, and the Lime Creek collections. This result was similar to the preliminary STRUCTURE analysis and the preliminary DAPC analysis (Figures S2-3 and S2-4). Burton State Hatchery, MN Wild Hatchery, and Ford Hatchery were most similar to Boundary tributary collections with the exception of the Lime Creek collections. Iron River Hatchery and Paint Bank Hatchery were the two hatcheries most similar to the Lime Creek collections (Figure 2-3).

The DAPC analysis excluding the most highly divergent hatcheries and eastern collections revealed close clustering of all the Boundary tributary collections except Upper Sweet, Highline, and all three Lime Creek collections. The closest clustered hatchery to the majority of the Boundary tributary collections was MN Wild Hatchery. Additionally, Ford Hatchery clustered closely to Burton State Hatchery and all three Lime Creek collections clustered closely with Iron River Hatchery. Upper Sweet Creek clustered closely with Paint Bank Hatchery (Figure 2-4). STRUCTURE analysis showed similar results to the DAPC (Figure S2-5).

The dendrogram analysis including only hatchery populations showed similar results to the above analysis. The most closely clustered hatcheries were Ford, Burton State, Iron River, MN wild, and Paint Bank hatcheries (Figure S2-6).

Genetic Differentiation Among Collections Within Boundary Tributaries

Genetic differentiation among Boundary tributary collections ranged from slight and non-significant for sites within Sullivan Creek to large and highly significant for sites in adjacent drainages. Estimates of pairwise F_{ST} ranged from -0.002 to 0.09 (Table S2-2). Comparisons including collections from Lime, Highline, Upper Sweet, and Slumber Creeks tended to have the largest pairwise F_{ST} estimates. F_{ST} estimates revealed significant genetic divergence between all the tributaries within the Slate Creek system, especially between Slumber and Styx and Slumber and Uncas Gulch Creeks. F_{ST} estimates also showed significant genetic divergence between all sections of Flume Creek (Upper and Lower Flume Creek, SF Flume Creek, and MF Flume Creek).

Pattern of Gene Flow Within the Sullivan Creek System

There was statistically significant evidence for isolation by distance within Sullivan Creek and adjacent connected tributaries with genetic similarity decreasing gradually with distance among sampling sites. For the first analysis, where we treated each sample section as a separate collection, mantel correlation coefficients were statistically significant for the first three distance classes, or approximately 5500 m stream distance. Negative Mantel correlation coefficients, though not significant ($P = 0.34$), were first detected at over 7000 m stream distance (Figure 2-5). The second mantel correlogram, where Sullivan Creek collections were grouped (Lower Sullivan, Middle Sullivan, and Upper Sullivan), showed similar results (Figure S2-7).

Discussion

We examined the population genetic structure of naturalized brook trout in a portion of the western US non-native range. We specifically aimed to inform current and future management strategies that attempt to eradicate brook trout in tributaries to the Pend Oreille River in Washington. We conducted genetic analyses at two different spatial scales. At a larger spatial scale, we performed a comprehensive genetic analysis of brook trout collected from 15 Boundary tributaries. Naturalized populations contained substantially more genetic variation than wild eastern populations and likely hatchery sources. We found clear evidence of genetic substructure and high genetic divergence of three tributaries (Lime Creek, Highline Creek, and Sweet Creek) from the remainder of the tributaries. Additionally, we were able to provide insight into the potential origin of brook trout populations in the Boundary tributaries. At a finer spatial scale, a strong signal of isolation by distance (IBD) has developed within Sullivan Creek and four of its tributaries since brook trout introduction. This result has important implications for an ongoing suppression and M_{YY} eradication effort.

Genetic Variation Within and Among Collections

Overall, genetic variation within the Boundary tributary collections was higher than genetic variation observed at this marker set for the most closely related hatchery strains and the northeast coast and mid-Atlantic wild collections. The higher value of both H_e and A within Boundary tributaries, even though these populations were introduced into this range relatively recently, could provide a partial explanation for why brook trout within their introduced range

tend to be so successful at establishing and maintaining populations. For example, Bell et al. (2022) found that brook trout occupied more habitat than any other trout in Montana.

Recent genetic studies have focused on what has been termed the "genetic paradox" of invasive species (Frankham 2005; Schrieber and Lachmuth 2017). Introduced species are often founded with small population sizes, which should lead to reduced genetic variation in general, compared to source populations. Additionally, introduced populations face novel environments and therefore should not be locally adapted. The paradox comes, however, in that we often see successful invaders outcompeting and replacing native species. While non-native brook trout populations in the US have expanded rapidly (e.g. Bell et al. 2021), native brook trout populations in the eastern US are declining due to various anthropogenic stressors (Lovich and Lovich 1996; Robinson et al. 2017; Kazyak et al. 2021). One explanation for this paradox is propagule pressure (defined as the number of individuals released and the number of release events; Lockwood et al. 2005). Propagule pressure is an important factor that can result in higher than expected genetic variation and novel genotypes in non-native populations, which can in turn help them to establish and adapt to a novel environment (Facon et al. 2008; Neville and Bernatchez 2013). Many recent studies have shown subsequent admixture after establishment as an additional reason for increased fitness of non-native populations (Allendorf et al. 2012; Neville and Bernatchez 2013). Higher genetic variation in the Boundary brook trout populations than native wild eastern populations could be a result of multiple introductions of brook trout into the Boundary system over many years as well as subsequent admixture after establishment. Though we cannot specifically link the higher genetic diversity seen here in the non-native populations with higher fitness, numerous studies have shown advantages of higher genetic variation (Vandewoestijne et al. 2008; Facon et al. 2008; Kardos et al. 2021).

Origin of Brook Trout Within the Pend Oreille River Watershed

Our results were inconclusive about the precise hatchery of origin of Boundary tributary brook trout. Ford Hatchery was expected to be the hatchery most likely to have stocked brook trout within the Boundary tributaries based on historical records. Records indicate that the source of the Ford Hatchery broodstock originated from Pennsylvania (Crawford 1979) indicating that Ford and other East Coast hatcheries should cluster closely with Boundary tributaries. In our analysis, Ford Hatchery did cluster closely with the Boundary tributaries, but so did mid-west hatcheries as well as Burton State and Paint Bank hatcheries. A previous genetic analysis of

Paint Bank Hatchery showed similarity to both north-eastern populations as well as mid-Atlantic populations (Kazyak et al. 2022). MN Wild is a hatchery that was stocked by two wild Minnesota populations, which, due to presumed extirpation of wild brook trout in the state, were most likely stocked by eastern strain populations in the late 1800s (Hoxmeier et al. 2015). Thus, historical records suggest the stocking populations for these hatcheries all originated on the East Coast. That, along with our dendrogram analysis of hatchery-only populations, shows that these collections are all genetically similar (Figure S2-6). Therefore, given the resolution of the current marker panel and the lack of historical samples, it is difficult to conclusively determine which hatchery stocked the Boundary tributaries. We cannot rule out the possibility that Ford Hatchery founded the Boundary tributary populations. The slightly higher observed H_e found within Boundary reservoir populations compared to Ford Hatchery is consistent with Ford Hatchery as the sole source of the Boundary reservoir populations if the Ford Hatchery broodstock has lost genetic variation faster than the naturalized populations since the time of introduction. Small N_e is often found in hatchery strains, which could have led to the subsequent loss of H_e over time within the extant Ford Hatchery strain. Large population size and metapopulation structure within Boundary tributaries could also have led to greater relative maintenance of H_e in the Boundary tributary collections compared to the Ford Hatchery strain.

An alternative hypothesis is that multiple sources were used to stock the Boundary tributary populations and subsequent admixture between source populations occurred to create higher overall genetic variation within the Boundary tributary populations compared to other collections. This could include different source hatcheries besides Ford Hatchery, or possibly that Ford Hatchery contained multiple (unsampled) sub-strains of brook trout at one point in time.

Ascertainment Bias

One caveat to our study is the possibility of ascertainment bias. Ascertainment bias can occur due to the populations that are used as discovery panels, which have differences in genetic diversity to the populations that are being tested. For this study, the SNP panel used arose from wild populations in Quebec Canada, Chile, and from Paradise Hatchery in Pennsylvania (Narum et al. 2017). Therefore, we would expect that any bias occurring would likely bias estimates of genetic variation within the eastern populations high since those

populations are likely very similar to some of the populations used to develop the SNP panel. However, we saw higher genetic diversity within the Sullivan and Boundary tributary collections than the East Coast wild collections, suggesting that if anything, we might have underestimated relative differences in genetic variation in our comparison of western and eastern populations.

Number of Subpopulations Within the LPO Watershed

Overall, we found evidence of genetic subdivision within the Boundary watershed. The highest genetic differentiation within the larger Boundary watershed was seen from collections from Lime Creek, Slumber Creek, Highline Creek, and Sweet Creek. We saw genetic differentiation within the Slate and Flume Creek watersheds as well. This suggests that there is limited gene flow among these collections and they should be treated as separate subpopulations. This would suggest that if future eradication or suppression treatments are unsuccessful (i.e. if a few brook trout remain in areas difficult to eradicate), recolonization could be expected to unfold relatively slowly at the among-tributary geographic scale. Further, because subdivision occurred within tributaries, spatially incomplete eradication would be expected to be followed by recolonization, albeit possibly slowly, from within tributaries.

There was no evidence of genetic subdivision at the tributary scale within the Sullivan Creek drainage, with the exception of the above dam and now extirpated Highline Creek. Suppression actions targeting brook trout within Sullivan Creek should include these connected tributaries to prevent recolonization of adjacent sections of Sullivan Creek and tributaries that had been recently suppressed. On the other hand, Highline Creek, which is separated from Sullivan Creek by an impassable crib dam, was highly genetically differentiated from the Sullivan Creek mainstem. This indicates there is a very low likelihood that Highline Creek will experience a reinvasion of brook trout from within Sullivan Creek in the future as long as the crib dam remains intact.

Pattern of Gene Flow Within the Sullivan Creek System

There was a clear pattern of isolation by distance (IBD) within Sullivan Creek, suggesting that gene flow tends to be spatially restricted, but that Sullivan Creek and its tributaries (with the exception of the now eradicated Highline Creek) represent a single large population. While IBD patterns have been observed for brook trout populations in their native range (Kanno et al. 2011), this is the first demonstration of isolation by distance for brook trout

in a system outside their native range. This result has important implications for future suppression efforts. Brook trout in their native range have been observed to have a spatially limited dispersal distribution with a large tail of longer distance movements (Kanno et al. 2014). For example, Kanno et al. (2014) observed a range of movements of 0 to 820 meters, with 62% of movements within 20 meters. This dispersal pattern would be expected to create an IBD pattern of gene flow. This pattern of gene flow and dispersal creates the expectation for recolonization of vacant patches on an ecological time scale. Even though gene flow tends to be spatially constrained within the Sullivan Creek watershed, it is likely high enough that recolonization would occur should one portion of the population be eradicated. The rate of recolonization would depend on how many individuals are traveling longer distances and the distance they travel. It also indicates that "patchy" suppression of brook trout within Sullivan Creek would be unsuccessful as brook trout from unsuppressed adjacent sections would likely move into the more open and less dense suppressed areas.

Our results also have implications for M_{YY} introduction. Options for M_{YY} introduction include having few more spatially spread-out release locations or having more release sites, closer together. Day et al. (2020) found that more release locations distributed closer to known brook trout density hotspots resulted in lower population sizes and lower proportion of patches occupied after M_{YY} introduction, however, this was dependent on the distance of brook trout movement. Our results showed most movement of brook trout was restricted to nearby sampling units. Therefore, we suggest more M_{YY} reintroduction locations that are closer together may be required throughout the basin to increase the likelihood of eradication.

Implications for Larger-scale Non-native Management

Determining the genetic population structure of non-native brook trout within Boundary tributaries provides important information to inform effective management actions and future research. High genetic variation suggests that naturalized brook trout populations in this portion of Washington will have high adaptive potential and could be generally challenging to eradicate. Our results help to inform management efforts by determining populations that should be most easily eradicated due to lack of gene flow from other populations. Additionally, our results have highlighted strategies that are most likely to be successful when implementing M_{YY} within the Sullivan Creek system, namely a larger number of release locations that are closer together. This

study can be used as a guide for using genetic analysis for future management strategies not only for brook trout in the Boundary tributary but for management of non-native fishes in many other systems.

Table 2-1. Brook trout samples examined, sample sizes (*N*), mean expected heterozygosity's (H_e), mean allelic diversity (*A*), and state (Location) where samples were collected. Sample numbers reported from Boundary tributary collections represent what we refer to as 'mixed-age brook trout', that is those that are age-1 or older from a mixture of age classes and do not include YOY. SLVN = Sullivan Creek. The first number following SLVN indicates the SU that the individual was collected from. Summary statistics were not calculated for sites with six or fewer individuals.

Watershed/Hatchery	*Collection*	*N*	H_e	*A*	**Location**
Sullivan Creek Collections					
Sullivan Creek	SLVN12.2	32	0.37	1.98	WA
Sullivan Creek	SLVN13.1	28	0.37	1.98	WA
Sullivan Creek	SLVN14.2	24	0.37	1.98	WA
Sullivan Creek	SLVN17.13	11	0.37	1.96	WA
Sullivan Creek	SLVN17.2	39	0.37	1.99	WA
Sullivan Creek	SLVN17.3	14	0.37	1.94	WA
Sullivan Creek	SLVN18.6	23	0.37	1.97	WA
Sullivan Creek	SLVN20.1	47	0.36	1.97	WA
Sullivan Creek	Gypsy	19	0.36	1.97	WA
Sullivan Creek	Highline	30	0.34	1.95	WA
Sullivan Creek	Leola	28	0.35	1.95	WA
Sullivan Creek	Deemer	8	0.35	1.89	WA
Sullivan Creek	Pass	10	0.36	1.93	WA
Other Boundary Tributary Collections					
Flume Creek	Lower Flume	33	0.38	1.99	WA
Flume Creek	Upper Flume	28	0.33	1.97	WA
Flume Creek	MF Flume	20	0.36	1.98	WA
Flume Creek	SF Flume	20	0.35	1.97	WA
Lime Creek	Lower Lime	10	0.31	1.89	WA
Lime Creek	Middle Lime	39	0.33	1.94	WA
Lime Creek	Upper Lime	10	0.32	1.91	WA
Sweet Creek	Upper Sweet	36	0.33	1.94	WA
Slate Creek	Lower Slate	19	0.37	1.98	WA
Slate Creek	Upper Slate	22	0.37	1.99	WA
Slate Creek	Slumber	29	0.31	1.89	WA
Slate Creek	Styx	11	0.33	1.88	WA
Slate Creek	Uncas Gulch	22	0.35	1.96	WA

Watershed/Hatchery	*Collection*	*N*	H_e	*A*	**Location**
Washington State Hatchery					
Ford Hatchery	-	61	0.32	1.92	WA
Midwest Hatcheries					
Iron River Hatchery	-	41	0.31	1.98	WI
MN Wild Hatchery	-	13	0.33	1.86	MN
Northeast Coast Wild Populations					
Stanley Brook	Stanley Brook	44	0.23	1.77	ME
Cohocton Creek	Upper Cohocton	97	0.22	1.95	NY
Cohocton Creek	Cohocton Brook	43	0.22	1.90	NY
West Brook	West Brook	49	0.16	1.91	MA
Wiley Brook	Wiley Brook	45	0.26	1.86	MA
Mid-Atlantic Wild Populations					
Savage Creek	Upper Savage River 41	50	0.18	1.68	MD
Savage Creek	Upper Savage River 90	43	0.17	1.57	MD
Fridley Gap	Fridley Gap	76	0.18	1.81	VA
East Coast Hatcheries					
Burton State Hatchery	-	20	0.33	1.95	GA
Hyde Pond Hatchery	-	20	0.29	1.87	NY
Milford Hatchery	-	20	0.28	1.86	NH
Paint Bank Hatchery	-	20	0.34	1.90	VA
Walhalla State Hatchery	-	19	0.30	1.90	SC
Wytheville State Hatchery	-	20	0.25	1.71	VA
Berlin Hatchery	-	20	0.28	1.81	NH
Big Hill Pond Hatchery	-	19	0.29	1.85	NY

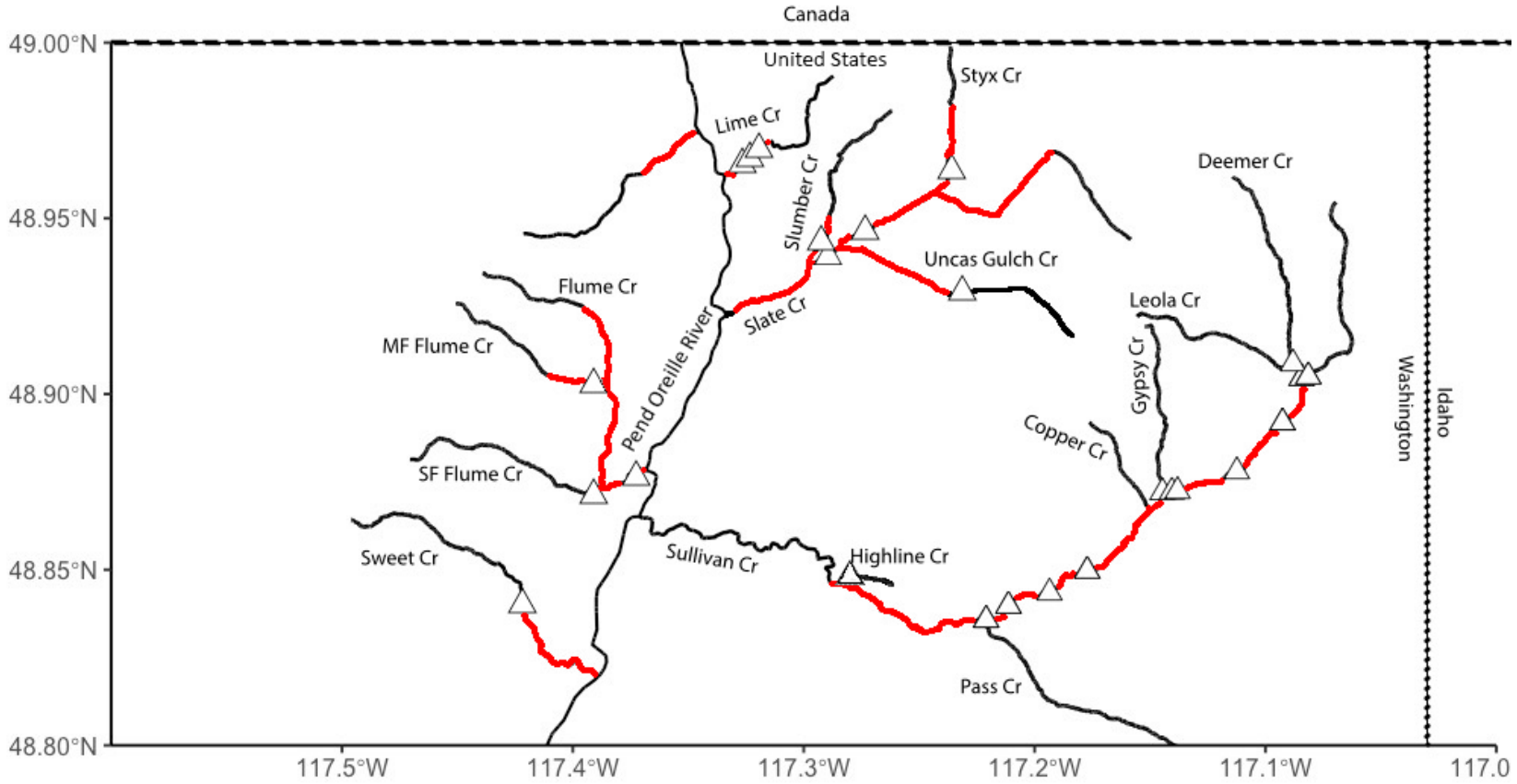


Figure 2-1. Map of the Boundary Tributaries. Triangles indicate Sampling Units from which tissue collections were used for this study. Red lines indicate distribution of brook trout within the watershed.

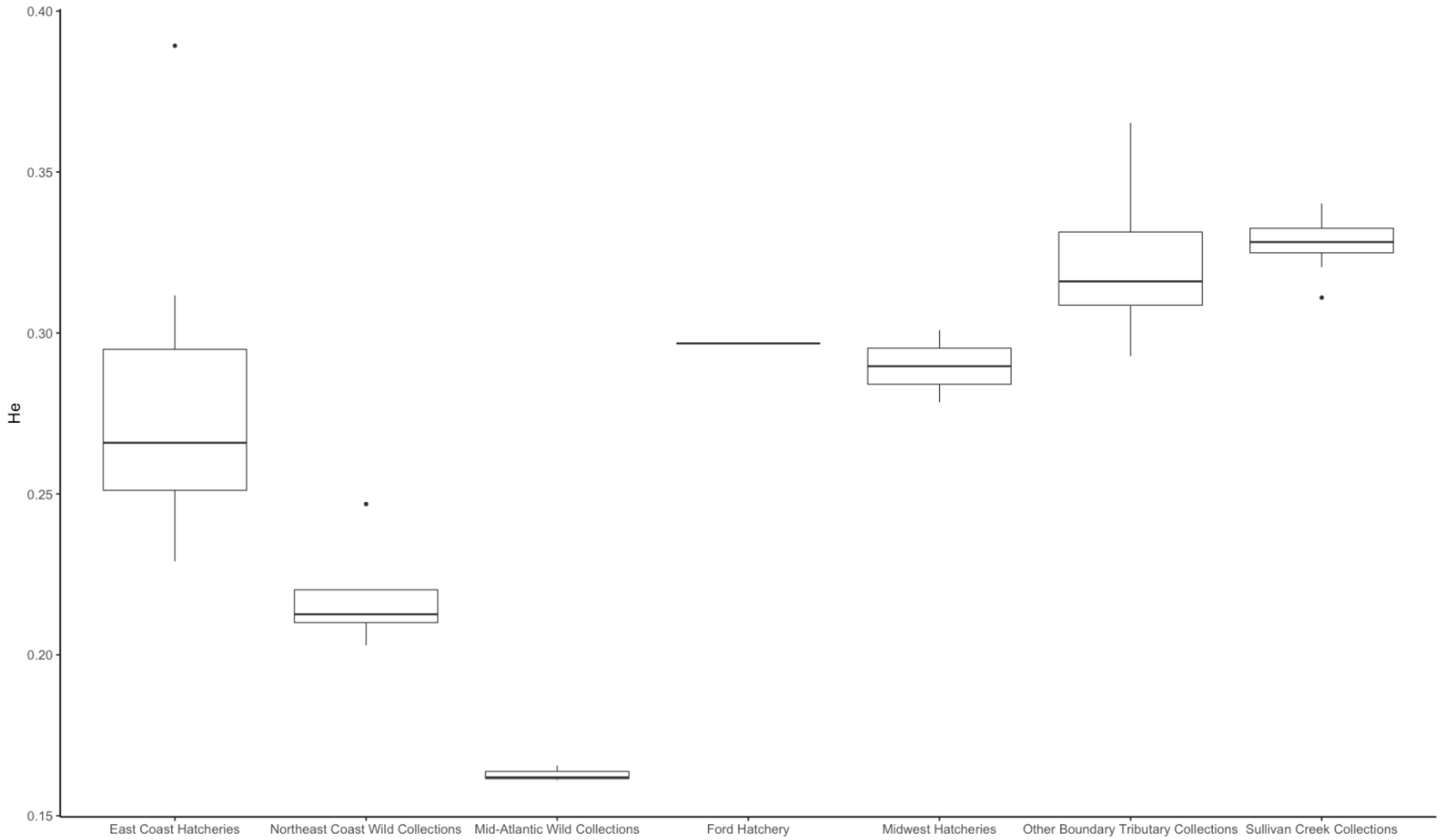


Figure 2-2. Boxplot of expected heterozygosity (H_e) per population. Other Boundary tributaries = Other Boundary Tributary Collections according to Table 1. Within each box, horizontal black lines denote median values; lower (Q1) and upper (Q3) quantiles represent the 25th to the 75th percentile of each group's distribution of values; data falling outside the Q1 –Q3 range are plotted but are considered outliers of the data.

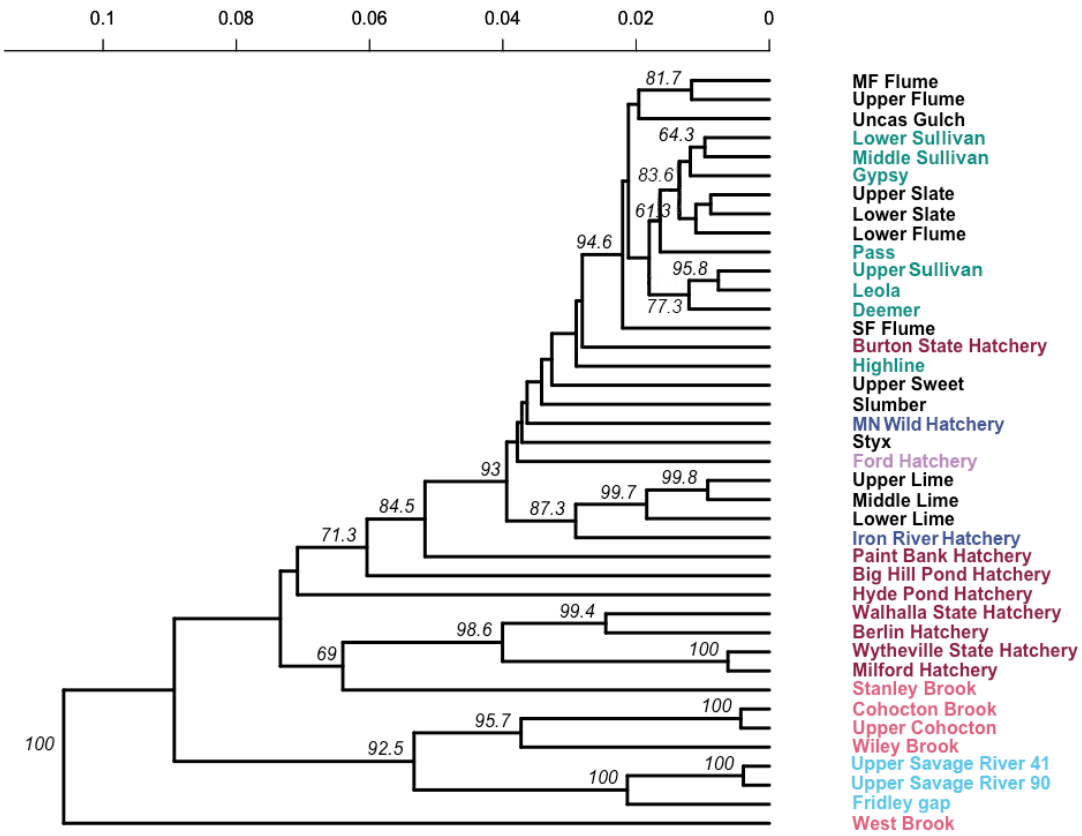


Figure 2-3. UPGMA dendrogram analysis of all populations using Nei's distance. Colors represent watershed/hatchery groups according to Table 2-1. The horizontal axis is representative of the allele frequency distance between clusters. Bootstrap values > 50 (percent out of 1000 iterations) are shown.

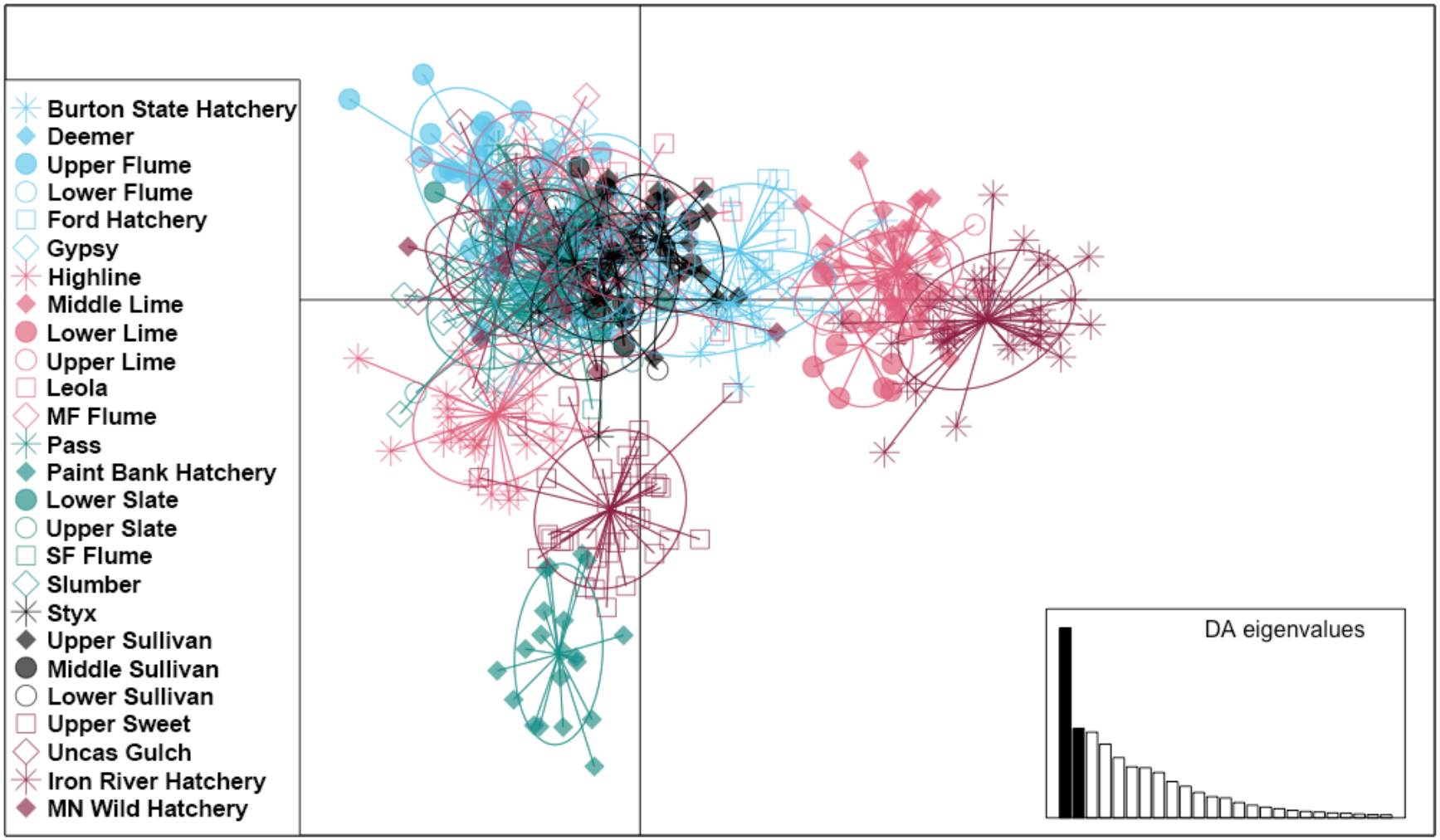


Figure 2-4. DAPC analysis including all Boundary tributary populations and Ford Hatchery, Burton State Hatchery, Paint Bank Hatchery, MN Wild Hatchery, and Iron River Hatchery.

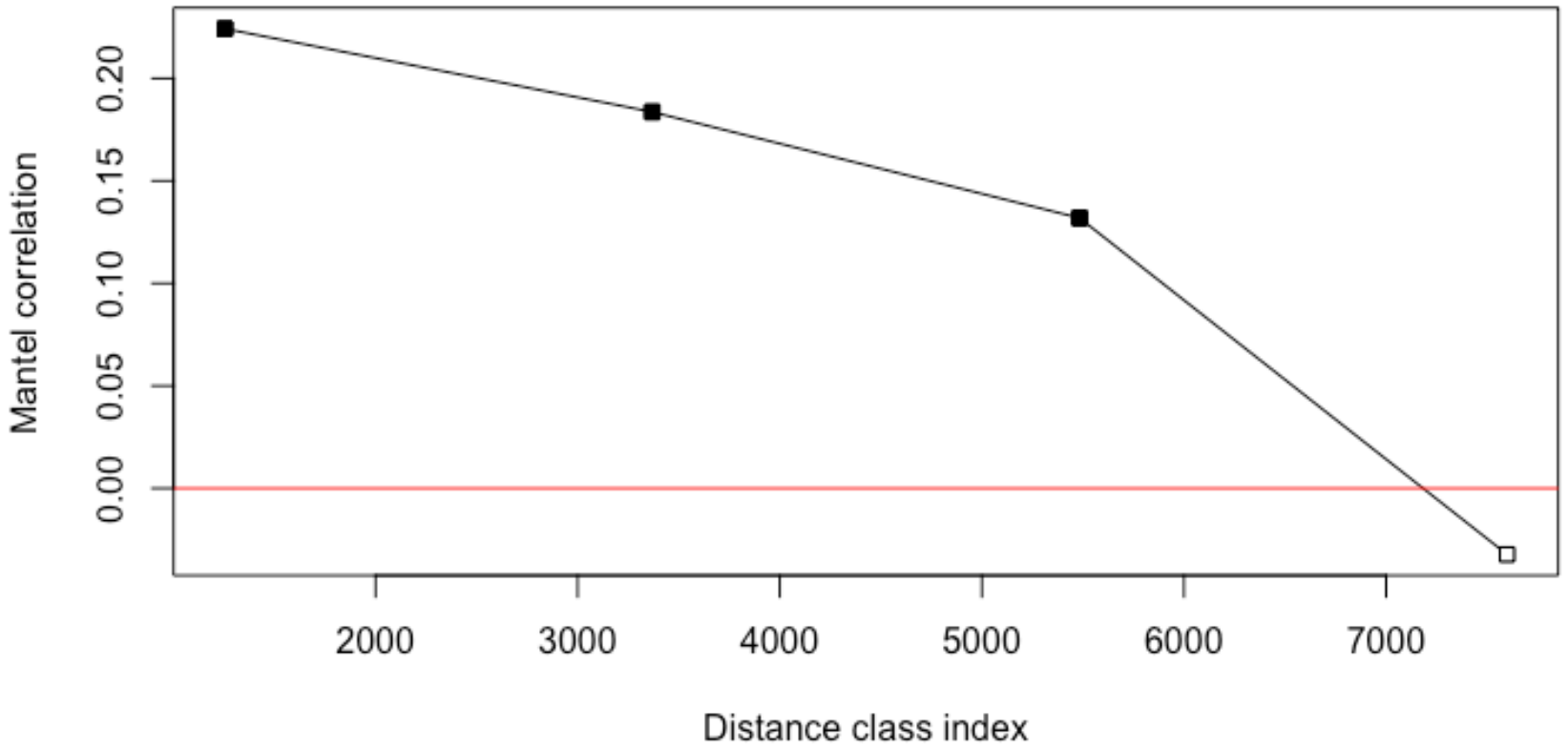


Figure 2-5. Mantel correlogram of isolation by distance within Sullivan Creek where Sullivan Creek sampling units were treated as separate populations. Brook trout were sampled from Leola, Deemer, Pass, and Gypsy Creeks along with adjacent mainstem Sullivan Creek locations. Geographic distances are riverine distances measured between each pair of collections.

CHAPTER 3: Lab-Based Evaluation of the Reproductive Performance of Trojan (M_{YY}) Brook Trout (*Salvelinus fontinalis*).

Abstract

The use of trojan males with two Y chromosomes (M_{YY}) appears promising for the eradication of non-native fish species when combined with manual suppression. However, due to the novel nature of this method, many aspects remain to be tested. The goal of this study was to evaluate the reproductive performance of hatchery age-0 and age-1 M_{YY} brook trout compared to hatchery XY males using laboratory crosses. Offspring of XY males had significantly higher survival than offspring from both age classes of M_{YY} one day post-fertilization (P <0.001). We found no differences in survival from eyed-egg to swim-up, but survival was significantly higher for offspring of M_{YY} from one-month post swim-up to the fry stage (P = 0.01). Offspring of XY males were larger at the fry stage in both length (P < 0.001) and weight (P < 0.001) than those of M_{YY}. We also determined survival to the eyed-egg stage for milt mixture crosses fertilized by both male type (M_{YY} and XY) using a parentage analysis. A significantly higher proportion of offspring within families that survived to the eyed-egg stage were sired by M_{YY} rather than XY males (P < 0.001). These results indicate that M_{YY} brook trout perform similarly to XY males at fertilization and their offspring survive similarly at early development stages suggesting they could be an effective tool in non-native brook trout eradication efforts.

Introduction

Non-native fishes are a major source of the decline of native fish species (Miller et al. 1989; Buckwalter et al. 2018). Common methods available to fisheries managers to eradicate non-native fish populations include the targeted application of a piscicide or by mechanical removal (Donaldson and Cooke 2016). The success of piscicides is dependent on environmental conditions such as water temperature and pH (Finlayson et al. 2000), and due to conservation concerns, can be problematic to use when the target species co-occurs with native aquatic fauna. Alternatively, managers may use mechanical methods of removal (e.g., electrofishing), which have the advantage of selecting specifically for non-native species, but this often results in suppression and not eradication (Meyer et al. 2006; Shepard et al. 2014; Day et al. 2018).

The introduction of artificially propagated males with two Y chromosomes (hereafter M_{YY}) represents an alternative tool for non-native fish eradication. This approach theoretically results in a shift in the sex ratio of the target population toward all males, causing demographic population extirpation due to the elimination or shortage of females (Gutierez and Teem 2006; Gutierez et al. 2012; Wang et al. 2016). The development of M_{YY} has been pursued in several fish species to date, including common carp (*Cyprinus carpio*; Bongers et al. 1999*),* Nile tilapia *(Oreochromis niloticus;* Mair et al. 1997*),* yellow catfish *(Pelteobagrus fulvidraco*; Liu et al. 2013), crucian carp (*Carassius carassius*; Zhou et al. 2015), and brook trout *(Salvelinus fontinalis*; Kennedy et al. 2018*).* However, many aspects pertaining to the performance of M_{YY} compared to their wild counterparts remain poorly understood and warrant further investigation (Cotton and Wedekind 2007).

The brook trout, which is native to eastern North America, was introduced to western North America in the late 1800s (MacCrimmon and Campbell 1969; MacCrimmon et al. 1971; Crawford 1979). Non-native populations have been established from Alaska to Texas (Fuller and Neilson 2019; USGS 2023), and competition with native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and bull trout (*Salvelinus confluentus*) populations in western North America is thought to be one of the primary causes of decline for those species (Rieman et al. 2006; Dunham et al. 2002; USFWS 1999a; USFWS 1999b; Warnock and Rasmussen 2013). Brook trout are highly phenotypically plastic and earlier maturation and faster generation time

result in a demographic advantage over cutthroat and bull trout (Gunckel et al. 2002; Kennedy et al. 2003; Rieman et al. 2006; Peterson et al. 2008). Additionally, brook trout can hybridize with bull trout and though hybridization appears to not often proceed beyond the F2 generation (Kanda et al. 2002), it causes wasted reproductive effort for bull trout (Allendorf et al. 2001). Where native species are present, attempts at brook trout eradication via mechanical removal have had mixed results (Meyer et al. 2006; Buktenica et al. 2013; Shepard et al. 2014; Kennedy et al. 2018), identifying a clear need for alternative methods for their removal.

The use of M_{YY} male brook trout in conjunction with mechanical suppression has been recently evaluated via simulation modeling (Schill et al. 2017; Day et al. 2018; Day et al. 2021) and through M_{YY} release programs in natural stream systems (Schill et al. 2016; Kennedy et al. 2017; Kennedy et al. 2018; Armstrong et al. 2022). While the M_{YY} approach shows promise, many factors mediating the success of this method have not been empirically tested. This includes the number of M_{YY} that should be introduced relative to the number of wild fish, behavior and dispersal of M_{YY}, and fitness (survival and reproductive success) of M_{YY} (Kennedy et al. 2018; Day et al. 2021). If M_{YY} are less fit than wild conspecifics, assumptions of equal fitness would overestimate the effectiveness of the M_{YY} strategy (Day et al. 2020). Recent studies of M_{YY} brook trout fitness in natural stream systems have shown mixed results, ranging from indirectly inferred fitness equal to or lower than wild conspecifics (Kennedy et al. 2018; Armstrong et al. 2022). Therefore, studying M_{YY} reproductive performance (fertilization success as well as survival and growth of offspring) relative to XY brook trout is warranted to inform future implementation of M_{YY} programs.

Fitness effects related to the size and age of M_{YY} brook trout at time of release could also influence the success of M_{YY} programs. Most commonly, managers release M_{YY} brook trout as either mature fingerlings (age-0) or overwinter them in the hatchery and, depending on feeding and growth strategy in the hatchery, release them at a larger size (commonly referred to as catchables) the following year as age-1 fish. Larger size could give the catchables a size advantage over wild trout and therefore could increase their spawning success and post-release survival (Williamson et al. 2010; Kennedy et al. 2018). However, the rearing of M_{YY} to age-1 requires considerable resources such as time in the hatchery and hatchery space. Alternatively, release of mature age-0 M_{YY} fingerlings is much less expensive from a hatchery rearing perspective (Kennedy et al. 2018) and therefore, it might be preferable to stock at this age to

avoid these costs. There are still questions however, regarding the fitness of age-1 vs age-0 M_{YY}. For example, it is unknown how the reproductive performance of mature age-0 M_{YY} compares to the reproductive performance of age-1 M_{YY}. Demonstration of a fitness advantage during reproduction by age-1 M_{YY} could justify the additional expense of overwintering these males in a hatchery and releasing them at a later point in time. Laboratory crosses could provide an initial examination of several aspects of fitness such as fertilization success and survival and growth post-fertilization.

In this study, we evaluated the reproductive performance, defined here as relative fertilization rates and offspring survival and growth under controlled conditions, of age-0 and age-1 M_{YY} brook trout compared to hatchery (chromosomally XY) brook trout using controlled laboratory crosses. Our goals were to 1) estimate the difference in fertilization rates (measured as proportion survival one-day post-fertilization) between age-0 M_{YY}, age-1 M_{YY}, and XY males; 2) compare the proportion of offspring survival at specific developmental stages between age-0 M_{YY}, age-1 M_{YY}, and a single age-class (age-2) of XY males; 3) determine the proportion of offspring sired by either age-0 M_{YY} or XY males that survived to the eyed-egg stage from crosses fertilized by an equal volume mixture of milt from both male types; and 4) compare the growth to fry stage of offspring sired by M_{YY} versus XY males. Comparison of survival and growth of offspring produced under controlled conditions by M_{YY} and XY males will inform the use of M_{YY} brook trout as a supplement to manual suppression to eradicate nuisance populations of wild non-native brook trout.

Material and Methods

Crosses

Approval of this study was received from the University of Montana Institutional Animal Care and Use Committee (under University of Montana IACUC protocol 061-21). All spawning was performed in fall 2021 at Abernathy Fish Technology Center in Longview, WA. M_{YY} brook trout were obtained as eyed-eggs from Hayspur Hatchery (Idaho) and reared at Abernathy Fish Technology Center. Age-0 M_{YY} were mature fingerlings that hatched in spring 2021 and age-1 M_{YY} were catchables that hatched in spring 2020. Age-2 hatchery-origin females and age-2 hatchery-origin XY males (Ford Hatchery, WA) were utilized in this study because of known

high female fecundity, availability of known-sex mature fish, and spawn timing. Additionally, we used hatchery XY males to account for the potential for a reproductive fitness advantage of hatchery fish over wild fish brought into a hatchery environment. Females were assessed for ripeness weekly to determine the timing of fertilization. Females that were considered ripe (eggs readily released when light pressure was placed on the abdomen) were then placed into a separate holding area to prepare for spawning (See Table S3-1 for total number of females spawned per day).

Before spawning, all four fish (female, XY male, age-0 M_{YY}, and age-1 M_{YY}) to be used for each cross were anesthetized using MS-222 and weighed (g) and measured for fork length (mm). Each female was wiped dry and eggs were expelled using hand-stripping into a colander to remove excess fluid. Overall weight (g) of the eggs was determined and divided into four evenly weighted groups and placed into four large Petri dishes. Each group was then fertilized using milt from one male type: XY male (cross-type 1), age-0 M_{YY} male (cross-type 2), age-1 M_{YY} male (cross-type 3), or a mixture of milt from XY and age-0 M_{YY} (cross-type 4). For each female, we randomized the order of fertilization across each cross-type. Collection of milt and fertilization of eggs for each cross was as follows: Milt from one randomly chosen anesthetized male was expressed into an individual Petri dish. It was then collected using a 200 to 1000µL pipette and deposited directly into the Petri dish containing the weighted group eggs for that cross. For each female, milt volume for each cross-type was standardized by determining the male with the lowest total volume of milt and halving it. For example, if the male with the lowest amount of milt for all males used to fertilize one female had 100 µL of milt, then 50 µL would be used to fertilize each egg group for that female. For cross-type 4, the same volume of milt from the XY and age-0 M_{YY} used for each of the other crosses for that female was combined within a separate 0.5 mL microcentrifuge tube and gently mixed using the pipette. Half of that mixture was then used to fertilize the eggs so that the same volume of milt was used to fertilize each egg group for each female. We chose to combine milt between the two male types before fertilization to increase the effects of sperm competition (Campton 2004). Milt volumes used to fertilize each egg group for each female ranged from 50 to 150 µL. Once milt was added to all groups, water was poured onto each cross-type, then gently mixed and allowed to fertilize for 10 minutes. After fertilization, all adults were euthanized and tissue samples were collected and stored in 95% ethanol for subsequent parentage analysis. Fertilized eggs were placed in four

circular screened rearing vessels (one for each cross-type) within Heath trays, such that each tray contained the four crosses for a single female.

This procedure was replicated across all 43 females that were spawned. Each Heath stack was supplied with ~12° C well water at a flow rate of 3 gallons per minute. Placement of individual rearing vessels within each Heath tray was randomized by cross-type to account for potential variability in flow from front to back within each tray. Twenty-four hours after fertilization, unfertilized eggs were removed and enumerated.

Early Rearing

Developing embryos were examined daily, and mortalities were removed and enumerated. At the eyed-egg stage, eggs were physically shocked by dropping them into a 600 mL glass beaker, and non-viable eggs were removed twenty-four hours later. Surviving eggs from cross-type 4 were incubated until the eyed-egg stage (Figure 3-1), whereupon eggs were removed and preserved in 95% ethanol for subsequent dissection and parentage analysis. Surviving eggs from cross-type 3 (age-1 M_{YY} sire) were incubated until hatch (Figure 3-1) and were then removed and enumerated. Eggs from cross-types 1 (XY) and 2 (age-0 M_{YY} sire) were incubated until swim-up fry stage with continued daily checks for non-viable eggs. Once the swim-up fry stage was reached (after yolk-sac depletion), individuals from crosses 1 and 2 were transferred into 15-gallon fiberglass tanks supplied with 12°C well water at 0.5 gallons per minute. Fry were then fed a daily ration of 3.5% of body weight (biomass)/day. Tanks were monitored and cleaned daily, and all mortalities were removed and enumerated. After approximately one month post swim-up, (post swim-up fry stage) we standardized fish density by selecting a random sample of 80 individuals from each cross (i.e., tank) for continued rearing. The remaining fish within each cross (in excess of 80) were removed, euthanized (using an overdose of MS-222), and enumerated. The 80 individuals remaining in each tank were reared to the juvenile-fry stage (Figure 3-1) with continued daily cleaning and mortality checks. At juvenile-fry stage, all fish were removed from tanks and euthanized with an overdose of MS-222. All crosses were photographed, and fry lengths were measured with ImageJ (Schneider et al. 2012). We collected weight (g) data for each individual offspring of a subset of six different crosses (one of each cross-types 1 and 2 from three randomly chosen females).

Survival and Growth Analysis

Survival was calculated for six stages of development: 1-day post-fertilization (1 dpf; measure of fertilization success), eyed-egg stage (30 dpf), hatch (40 dpf), swim-up fry (65 dpf), post swim-up fry (105 dpf), and juvenile-fry (150 dpf). For cross-types 1 and 2, survival to each stage was calculated. For cross-type three, only survival to 1-day post-fertilization, the eyed-egg stage, and hatch stage was calculated. Survival to the first five stages of development was calculated by determining the number of individuals per cross at fertilization, then subtracting the number of mortalities observed to the designated developmental stage (Figure 3-1). Survival to juvenile-fry stage for cross-types 1 and 2 was determined by subtracting the number of individuals that survived to 150 days post-fertilization from the number of individuals at post swim-up (determined upon subsampling to approximately 80 fish per cross).

Differences in proportion of offspring survival to each developmental stage for cross-types 1-3 were analyzed using a beta-binomial generalized linear mixed model (glmm; Gelman and Hill 2007) with cross-type as a fixed effect and female as a random intercept. Growth differences at the juvenile-fry stage were examined by comparing length (mm) between all offspring of XY and all offspring of age-0 M_{YY} males and comparing weight (g) for the subset of six crosses using a glmm in R. For the growth analyses, total number of individual fry within each cross was used as a random intercept to account for density of tanks (either length or weight was the dependent variable, and cross-type was a covariate). All glmm's were fit using the R package *glmmTMB* (Brooks et al. 2017; R v 4.1.1).

Parentage of Milt-mixture Crosses (Cross-Type 4)

Parentage of cross-type 4 offspring was determined through a genetic analysis of eyed-eggs. DNA was extracted from 100 randomly chosen eggs from each cross using a modified version of the extraction method found in Ali et al. (2016). Extracted DNA was then sent to the Idaho Fish and Game (IDFG) Eagle Fish Genetics Lab for library preparation and sequencing analysis. Library preparation was completed following the GTSeq approach developed by Campbell et al. (2015). GTSeq was performed for each individual using an established 240 GTSeq SNP panel. This SNP panel has been used to determine sire type (either XY or M_{YY}) of offspring sampled from experimental systems into which M_{YY} brook trout have been released (Kennedy et al. 2018).

Screening of SNPs

We conducted a locus-specific analysis of conformation to Hardy–Weinberg proportions in a companion brook trout population genomic analysis (see Chapter 2). Loci that showed significant deviation ($P < 0.05$) from HW proportions across large numbers of population samples (≥ 7) in this analysis were removed (Waples 2015). We did not screen for linkage disequilibrium among loci as that is not an assumption of exclusion-based parentage assignments.

Hiphop Analysis for Milt-mixture Crosses

Parentage of cross-type 4 offspring at the eyed-egg stage was estimated from genotypes based on the exclusion approach implemented by the R package *Hiphop* (Cockburn et al. 2021). Adults were excluded from possible parentage if the offspring possessed two identical copies of alleles at that locus and adults possessed two identical copies of the alternate allele at that same locus (i.e were alternate homozygotes). Additionally, adults were excluded from parentage if both possible parents were homozygous for the same allele while the putative offspring was heterozygous at that locus. Each cross was examined separately and the putative parent with the fewest number of mismatches was the assigned parent. We expected high accuracy of parent assignments for each cross because the female parent was known and there were only two possible male parents (the XY and M_{YY} used for that cross). Differences in the proportion of offspring that were assigned to an XY compared to an M_{YY} was tested using a beta-binomial glmm (Gelman and Hill 2007) in R with sire type as the fixed effect and female as a random intercept.

Results

Survival Analysis

The number of offspring per cross per female at spawning ranged from 205 to 765 across the 43 females spawned. Crosses from three females were removed from the survival and growth analysis since no offspring from those females survived to the final developmental stage regardless of cross-type. Mean ± SD survival of offspring across all cross-types from spawning to each of the following developmental stages was as follows: 1-day post-fertilization 84% ± 16%; eyed-egg, 61% ± 23%; hatching, 55% ± 23%; swim-up fry, 50% ± 23%; and post swim-up

fry, 39% ± 21%. Survival from post swim-up fry to juvenile-fry stage was 99% ± 3% (Figure 3-2).

A significantly higher proportion of offspring of XY survived 1-day post-fertilization than offspring of age- 0 M_{YY} ($P < 0.001$) and age-1 M_{YY} ($P < 0.001$). No significant difference occurred in the proportion of surviving offspring of M_{YY} and XY males to the eyed egg, hatch, swim-up, or post swim-up stages, but a significantly higher proportion of offspring of age- 0 M_{YY} survived from the post swim-up to fry stage ($P < 0.001$; Figure 3-3 and Table S3-2). Offspring of age-1 M_{YY} had significantly higher proportionate survival 1-day post-fertilization than offspring of age- 0 M_{YY} ($P < 0.001$) but there were no significant differences between proportionate survival of age-0 and age-1 M_{YY} offspring at any of the other survival stages examined for those two crosses (eyed-egg, and hatch; Figure 3-3 and Table S3-2).

Growth Analysis

There were significant differences in growth between offspring of XY males and age-0 M_{YY}. XY offspring were larger in both length ($p < 0.001$) and weight ($p < 0.001$) at the juvenile-fry stage. Mean body length for offspring of age- 0 M_{YY} at the juvenile-fry stage was 3.6% smaller compared to offspring of XY males (Mean ± SD 66.00 ± 7.06 mm vs 68.43 ± 7.67 mm respectively). Mean body weight for offspring of the subset of three crosses of age-0 M_{YY} at the juvenile-fry stage was 25.2% smaller compared to offspring of the subset of three crosses of XY males (Mean ± SD 4.23 ± 1.28 g vs 5.45 ± 1.47 g respectively).

Cross-type 4 Parentage Analysis

We removed from the analysis any individuals where fewer than 90% of loci were successfully genotyped. Crosses with three females were removed from analyses because either a parent was below the genotyping threshold or because no offspring survived to eyed-egg stage. This left a total of 40 crosses for analysis. Age-0 M_{YY} sired a significantly higher proportion of offspring across the 40 families ($P < 0.001$; Figure 3-4). On average, the assigned male ((mean ± SD), 0.15 ± 0.41 mismatches) had fewer mismatches than the unassigned male ((mean ± SD), 18.51 ± 5.8 mismatches) (Figure S3-1). For 43 out of the total 3649 individual offspring, there were fewer than five mismatches separating the assigned and unassigned males, which could have biased results. Following removal of these 43 offspring, disproportionate contribution by

the age-0 M_{YY} compared to the paired XY male remained highly statistically significant ($P<$ 0.001).

Discussion

Determining the reproductive performance of M_{YY} brook trout is a key component to evaluating their efficacy and potential as a tool to extirpate non-native brook trout populations. Using lab-based crosses, we demonstrated that hatchery M_{YY} brook trout can produce viable offspring with similar survival to those of hatchery XY males in early developmental stages. XY males produced slightly larger offspring while M_{YY} males had a larger proportion of offspring of most families survive to eyed-egg stage in our milt-mixture trials. Together, these results are promising for M_{YY} programs and provide a baseline for which to compare future results within wild environments.

Fertilization Success

We observed higher fertilization success (measured as proportionate survival 1-day post-fertilization) of XY males than either age class of M_{YY} in cross-types 1-3. These results likely arose due to pre-fertilization dynamics such as sperm concentration, sperm motility (sperm swimming speed), or the duration of the viability of sperm, though tests of fertilization dynamics were not performed for this study.

Milt-mixture Crosses

In the milt mixture cross (cross-type 4), a significantly higher proportion of families were offspring of M_{YY} compared to offspring of XY males. These results could arise similarly to the individual crosses from factors that occurred pre-fertilization. We saw evidence for higher fertilization rates for XY males than M_{YY} males in crosses 1-3. However, studies have shown that when in direct competition with sperm from other males, some males fertilize fewer eggs due to lower sperm motility (Beirão et al. 2019) or the duration of the viability of sperm (Casselman et al. 2006). Salirrosas et al. (2017) found better mobility, motility, and viability of the sperm of chromosomally M_{YY} tilapia (*O. niloticus)* than hatchery XY male tilapia. It is possible that the XY hatchery males in this experiment also had lower sperm motility or viability of the sperm that did not impact overall fertilization rates in the individual experiment, yet

effects became more apparent under the competitive conditions created by our mixture experiment.

Alternatively, post-fertilization dynamics could also have played a part in our results. It is possible that offspring of XY males had lower survival between fertilization and shortly after eye-up compared to offspring of M_{YY} males in our milt-mixture experiment. We observed no evidence of differential survival of offspring in cross types 1-3 at eyed-egg stage. However, the higher fertilization rates for XY males compared to either age class of M_{YY} in crosses 1-3 suggest that post-fertilization, offspring of XY males had higher mortality than offspring of M_{YY} males. This could be due to possible negative inbreeding effects within the Ford Hatchery strain. Ford Hatchery has high relative genetic variation compared to native east coast populations (see Chapter 2) but high genetic variation does not necessarily rule out high genetic load. However, survival within the Ford Hatchery strain of brook trout is generally high under normal spawning operations suggesting negative inbreeding effects are unlikely. Alternatively, positive fitness effects of outcrossing between two hatchery strains (M_{YY} arose from a different hatchery strain than Ford (Schill et al. 2016)) could be an explanation. The substantially higher proportion of M_{YY} offspring surviving to the eyed-egg stage in our milt-mixture experiment compared to the individual crosses (which showed no difference in survival) and the higher fertilization rates of XY males compared to the age-0 M_{YY} in the individual crosses, suggests this result is likely a combination of both pre-and post-fertilization dynamics.

Survival

Generally, we observed little to no difference in survival of offspring through the earliest life stages among any of the crosses. We did observe greater survival of age-0 offspring of M_{YY} than offspring of XY males at the final developmental stage (post swim-up to juvenile-fry stage) though the effect size was small. Since we saw higher fertilization success of XY offspring than M_{YY} offspring, this suggests that subsequent mortality post-fertilization was higher in XY compared to M_{YY} offspring. Overall, this result suggests that, under controlled conditions, M_{YY} brook trout can produce viable offspring that can survive equally or better than offspring of hatchery XY males to the fry stage.

Growth

We found offspring of XY males grew significantly larger as fry in both length and weight than those of M_{YY}, hinting at a possible growth advantage for the offspring of XY males. We did not control for density of fry for a period of approximately 30 days after fish were transferred to larger tanks, but we did control for density for the last 42 days. We assumed that during early swim-up and the days of first feeding, density would have less of an impact on growth, given the smaller size of fry and the substantial amount of food that we supplied. It is possible that density-dependent growth differences influenced our results beyond what the statistical model could account for, however, densities would have needed to be consistently lower among tanks containing offspring of XY males to have led to our results. We saw no differences in overall survival of offspring of M_{YY} compared to offspring of XY between eyed-egg and the post swim-up stage (Table S3-2). Therefore, it is unlikely that density-dependent growth is the explanation. Larger size at age-0 can have a positive relationship with fitness in some fish species (Wiegmann et al. 1997; Zabel and Achord 2004; Xu et al. 2010), possibly through increased overwinter survival following the first year of life (Kallis and Marschall 2014; Geissinger et al. 2021). Additional experimental and simulation work are warranted to explore the demographic consequences of the differences observed here to test how they might influence future use of M_{YY} brook trout.

Previous brook trout studies have shown mixed results for the indirectly inferred reproductive success of M_{YY} males compared to wild XY males in stream experiments. Kennedy et al. (2018) found the overall average proportion of offspring of M_{YY} was roughly equal to that of the proportion of M_{YY} stocked within their experimental streams, suggesting similar relative fitness of XY males and M_{YY}. Armstrong et al. (2022) found lower reproductive success of M_{YY}, where an average of 29.5% of progeny were offspring of M_{YY} though ~50% of milt producing brook trout within streams were stocked M_{YY}. Our results suggest that XY males may have higher fertilization rates than M_{YY} males, however, in direct competition M_{YY} may have an advantage. Our results also suggest that early (first year) survival differences between offspring of M_{YY} and XY males are small and therefore do not entirely explain why M_{YY} may have lower relative fitness in some cases. Possible explanations could include behavioral differences associated with mating and sexual selection (e.g. female preference) (Fleming and Gross 1993; Dickerson et al. 2005) or outbreeding depression observed in the wild (Miller et al. 2004; Araki

et al. 2008) but not under our hatchery conditions (if anything, we observed an outcross advantage in our mixture experiment).

Our results address some of the uncertainties associated with the tradeoffs with releasing M_{YY} males at different ages. We found significant differences in fertilization success between offspring of age-0 and age-1 M_{YY} though the effect size was small. We also saw no differences in survival between offspring of age-0 and age-1 M_{YY}. Costs associated with hatchery rearing time could lead managers to favor use of age-0 M_{YY} in eradication programs, especially if age-0 M_{YY} are mature. Model results from Day et al. (2021) found release of age-0 M_{YY} was more effective at achieving eradication than release of age-1 M_{YY}, likely due to size and density-dependent mortality. Our results further support the release of age-0 M_{YY} by showing relatively equal fertilization rates and survival of age-0 M_{YY} offspring compared to offspring of age-1 M_{YY}. Understanding other aspects of fitness, such as size-assortative mating in the wild (Xu et al. 2010; Kennedy et al. 2018), would require further testing.

Overall, we saw lower survival for all crosses at all life stages than we expected. Cumulative survival for all crosses was 84% one-day post-fertilization, 63% to eyed-egg stage, 57% to hatching, 49% to swim-up, and 35% to ponding. Expected survival for Ford Hatchery stock brook trout is typically 85% to eyed-egg, 80% to hatch, and 75% to swim-up (Kevin Flowers; WDFW Spokane Hatchery Manager; *personal communication*). However, during the Spokane Hatchery 2021 broodstock spawn, survival rates were also lower than expected (Kevin Flowers, personal communication). Replication would be required to test for an effect of year of spawning on our results. Our study design controlled for female egg quality by comparing male-type reproductive performance between egg groups for each female. Therefore, if an effect of year of spawning were to impact our results, it would have been through some mechanism of XY male sperm quality that translates to lower post-fertilization survival compared to M_{YY}. We saw higher fertilization rates of XY compared to M_{YY} and we are not aware of any reasons why we would observe disproportionate survival among offspring of male sire types due to our experimental conditions. Therefore, we do not expect the overall survival rates to influence our conclusions.

We also observed substantial differences in fertilization of eggs and survival rates of offspring among females. This could be due to female egg quality, e.g. stage of egg development, environmental impacts, or other aspects of a female's eggs that could lead to

higher fertilization rates (Bobe 2014). Our experimental design controlled for some of this variation by spawning each female with each of the four cross-types. Additionally, differences in fertilization and survival rates of females could be due to variation among Heath rack trays. Female and Heath rack tray were confounded in our experimental design because all of one female's eggs (all four cross-types) went into a single rack. While we cannot tease apart these two sources of among-female variation, we included female as a random effect in our statistical models to account for the differences of both female egg quality and possible environmental differences among Heath tray racks. Thus, variation among females should not influence our conclusions.

Simulation models of the application of M_{YY} for the eradication of non-native brook trout provide an effective way to explore relative effects of various factors that influence application success. Modelling work by Day et al. (2020) found fitness (survival and reproduction) of M_{YY} had a significant effect on the success of M_{YY} implementation and the timeframe for eradication of non-native brook trout populations (Day et. al. 2018). However, these authors state that empirical estimates of fitness of M_{YY} would be informative to improve model reliability. Our results suggest that fitness of M_{YY} may be higher than currently expected within these model simulations and suggest that implementation of M_{YY} may be more effective than model estimates predict.

In summary, our results provide insight into the reproductive performance of M_{YY} hatchery brook trout and inform further implementation of M_{YY} as a management tool for the extirpation of nuisance brook trout populations in their non-native range. To date, ours is the first study that has formally evaluated fertilization rates and survival to several early development stages of M_{YY} and XY males, along with growth and sperm competition. Overall, our results indicate that M_{YY} produce milt that is at least as viable as their XY male counterparts, and even more viable when in direct competition and their offspring survive at an equal, if not higher rate than XY males. However, M_{YY} progeny did exhibit somewhat slower growth than progeny of XY males. Future studies could include exploring pre-fertilization processes (e.g. mate choice) as well as examination of reproductive performance of M_{YY} compared to wild male brook trout under wild or semi-natural conditions.

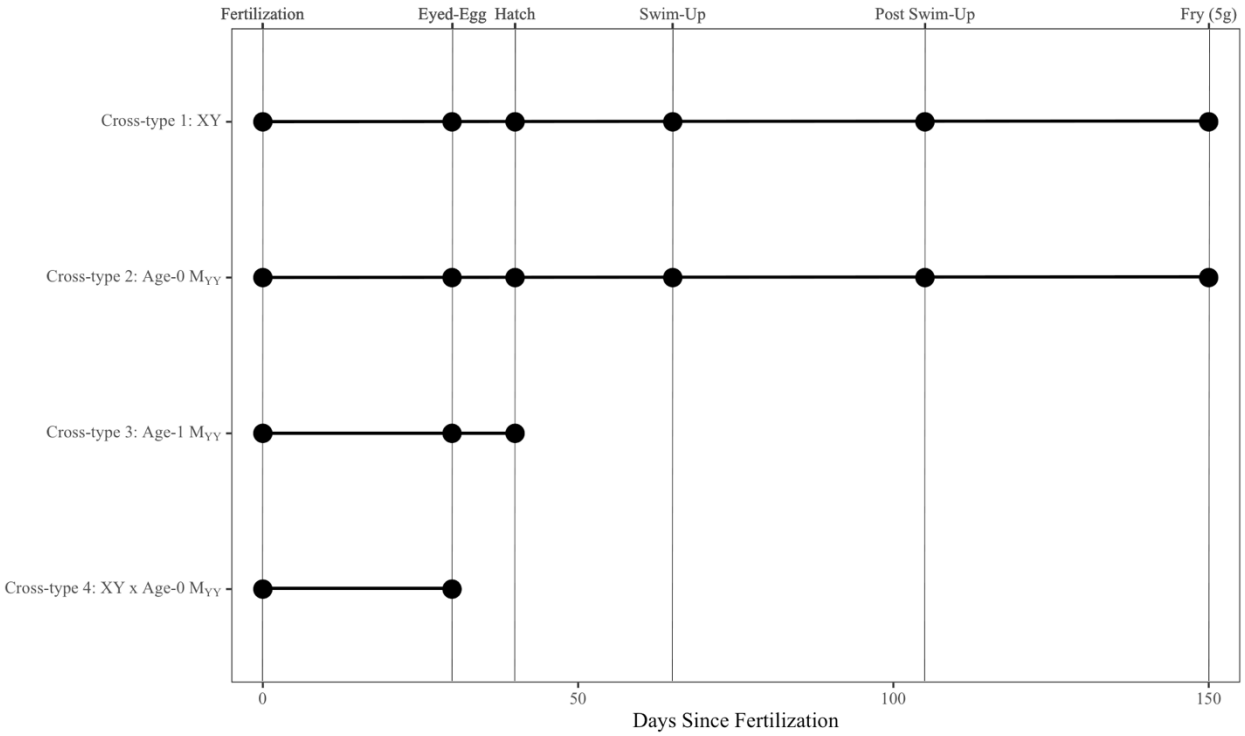


Figure 3-1. Timeline of offspring rearing by developmental stage. Line length along the x-axis represents rearing duration; black circles indicate the developmental stages in which survival was measured.

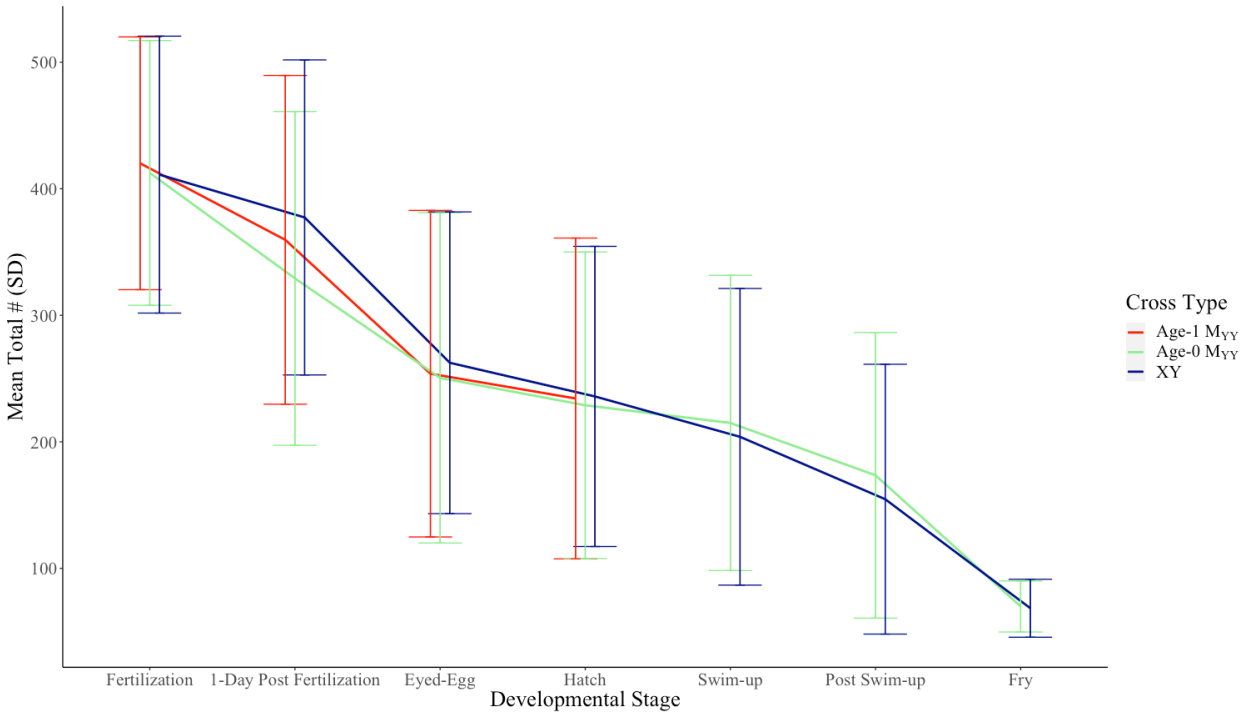


Figure 3-2. Mean total number of offspring survived at each developmental stage for different cross types. Bars represent standard deviation.

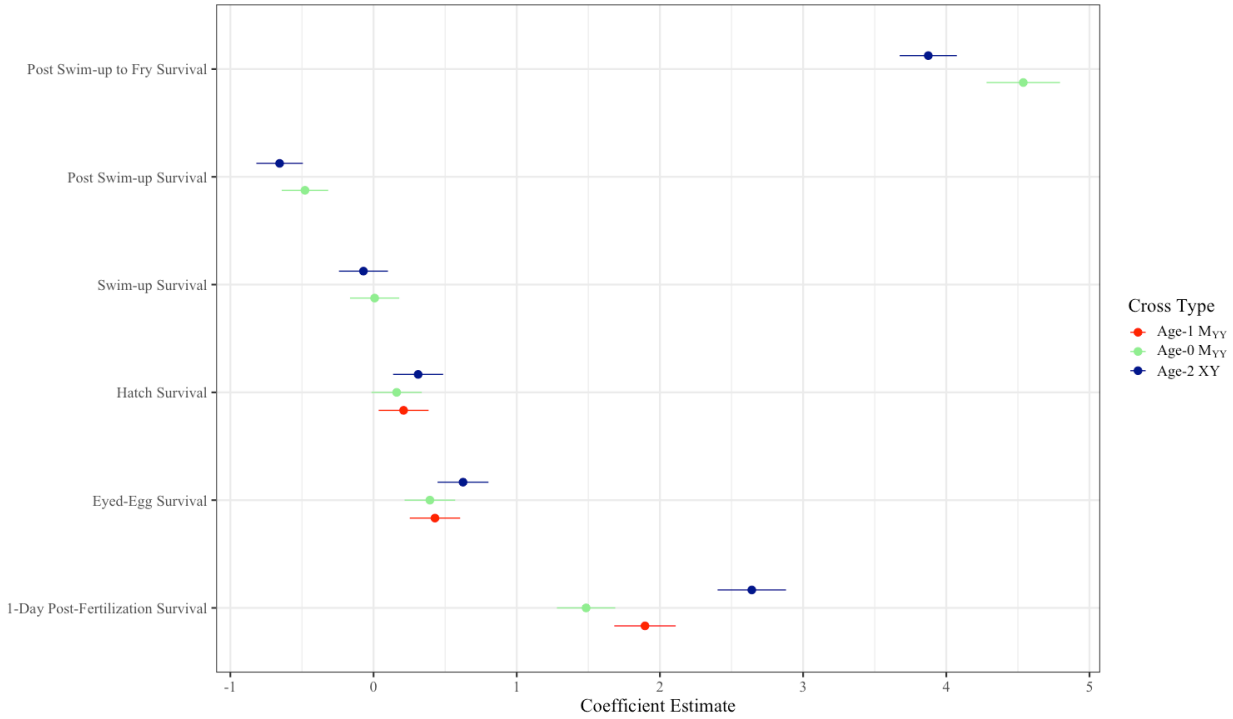


Figure 3-3. Plot of coefficient estimates (+/-) of the beta-binomial models run for each offspring survival stage measured. Note that survival at the final stage (post swim-up to fry) was based on survival values after post-swim-up stage when crosses were counted down to 80 individuals per tank and therefore overall survival was higher. Bars represent standard error.

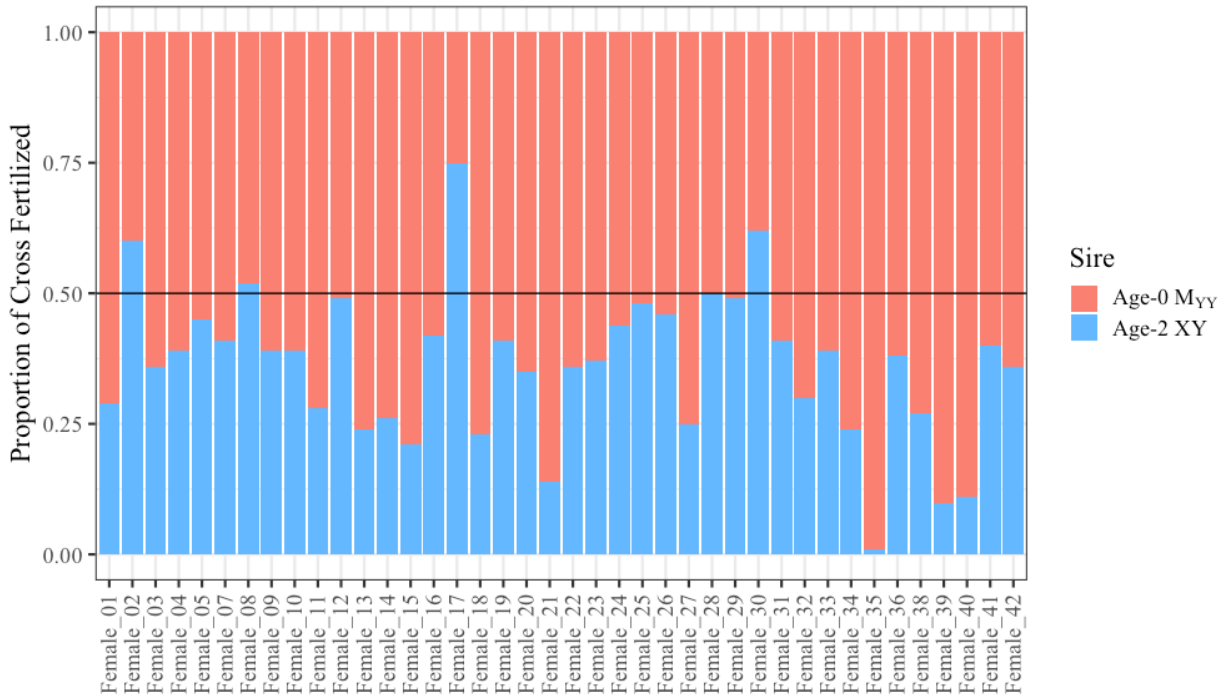


Figure 3-4. Proportion of mixture crosses fertilized at the eyed-egg stage by each different male type (Age-0 M_{YY} in red, XY males in blue) for each female. Male parentage was determined using *Hiphop* mismatch values where the possible parent with the lowest number of mismatches was the assigned parent. The black line represents an even proportion fertilized by each male type.

CHAPTER 4: Population viability when eradication falls short: simulation-based analysis of brook trout recovery following an M_{YY} program

Abstract

The introduction of chromosomally YY male fish (M_{YY}), is a novel management method that, when used with other suppression techniques (i.e. mechanical suppression) can theoretically be used to eradicate non-native fish populations by shifting the sex ratio of the population until only males remain. Though this method appears promising, there are many aspects affecting the success of this tool that have yet to be tested. Simulation models can be useful for helping to make predictions of novel management actions. Previous simulation studies testing the effectiveness of M_{YY} suggest that it may be affected by many different factors such as the fitness of M_{YY} and the number of M_{YY} released. No studies to date have looked at the possible consequences on the remaining population if M_{YY} management plans result in failure to eradicate. Suppression and M_{YY} introduction cause reductions in the abundance of the population, increasing the chance of inbreeding depression (ID). Alternatively, heterosis, due to admixed crosses of M_{YY} and wild fish, may dampen the negative fitness effects of the bottleneck. Our goal was to use a demogenetic, spatially explicit model (CDMetaPOP) to examine the effect of inbreeding depression and the impact of admixture of M_{YY} alleles on the mean minimum number of individuals (bottleneck size), number of individuals per patch over time, percent observed homozygosity over time, number of fitness deaths over time, and the recovery of populations. ID had a clear negative impact on the bottleneck size of populations, however, at the final year of the simulation, populations recovered to above pre-treatment levels for most simulations. Additionally, admixture from genetically divergent M_{YY} alleles resulted in an increase in the observed homozygosity during M_{YY} implementation which possibly worked to counteract the effect of the population bottleneck and result in the recovery of populations after suppression and M_{YY} introduction ended. These results demonstrate how fitness aspects of suppression and M_{YY} implementation and subsequent bottleneck size may impact overall implementation success and suggest that even if populations are driven to very low abundance, managers should not rely on them going extinct due to the effects of fitness.

Introduction

Non-native freshwater fishes are a significant threat to biodiversity, causing severe challenges for native species conservation and management (Jelks et al. 2008; Crystal-Ornelas and Lockwood 2020). Traditional options for managing a non-native species after they have become established include eradication or control (Donaldson and Cooke 2016). Eradication can occur through mechanical or chemical removal; however mechanical removal oftentimes is unsuccessful (Meyer et al. 2006) and chemical eradication is challenging when a native species is present (Britton et al. 2011). Otherwise, managers must resort to control, where they suppress the abundance of the non-native species to try to reduce their impacts on the ecosystem (Peterson et al. 2008).

Alternative management options include biocontrol, one of which is the introduction of M_{YY}. M_{YY} are hatchery-produced males of the non-native species with two Y chromosomes that are introduced into the wild population, potentially causing eradication through manipulation of the sex ratio of the non-native species (Guiterrez and Teem 2006; Schill et al. 2017). Used in conjunction with suppression, M_{YY} introduction is an option to accelerate the rate of the decline of the non-native population compared to suppression alone (Kennedy et al. 2018; Day et al. 2021). The M_{YY} approach has been gaining momentum in the United States. Though this method appears promising, many aspects affecting the success of M_{YY} introduction have yet to be tested including the fitness of M_{YY}.

In the absence of empirical data sets, exploring the factors that affect the success of an M_{YY} eradication strategy can be achieved through simulation models. Day et al. (2020 and 2021) simulated how eradication success and/or minimum population size of the non-native population may be affected by the relative fitness (survival and reproduction) of M_{YY} males, the number of M_{YY} released relative to the number of wild fish, the number of years for which M_{YY} are released, and the number of release locations. These simulations and others (Teem et al. 2014; Schill et al. 2017) predict that anywhere between 2 and 25 years of introduction of M_{YY} is needed for eradication to occur in the most optimistic simulations. Further, these simulations found that lower fitness of M_{YY} compared to wild-type males increased time to eradication and, when M_{YY} fitness was much lower, resulted in failure to eradicate the population entirely.

No studies to date have looked at the possible consequences if M_{YY} management plans fail to eradicate non-native populations. How the remnant population of the non-native species responds is an important element to consider for an M_{YY} management plan. Incomplete eradication is likely to have negative consequences for the genetic variation of the remnant non-native population. Suppression and M_{YY} introduction cause reductions in the abundance of the non-native population, essentially forcing these populations through a population bottleneck (Allendorf et al. 2012). Population bottlenecks can increase the chance of inbreeding among related individuals as the population decreases and can lead to reduction in fitness, termed inbreeding depression (ID; Emlen 1991; Kardos et al. 2015). In the case of M_{YY} implementation where eradication is not achieved, a reduction in fitness due to ID could limit the ability of the non-native population to recover and recolonize after treatment has ended. Directly conflicting ID is an unusual aspect of M_{YY} introduction: a sustained pulse of admixture from a genetically divergent source precedes the population bottleneck. This could lead to heterosis, where admixed crosses have higher fitness than their parents, dampening the negative fitness effects of the bottleneck. Determining the balance of possible fitness effects due to suppression and M_{YY} introduction lends itself well to exploration through simulations, which are critical tools for reducing uncertainty around the effects of parameters that are unknown.

In this study, we explored through simulations the effects of fitness on the rate and magnitude of recolonization of a non-native population after suppression and M_{YY} introduction have ceased and complete eradication failed to occur. Building on the framework used by Day et al. 2020 and 2021, we modeled suppression and the release of M_{YY} male brook trout (*Salvelinus fontinalis*) into an established population of non-native wild brook trout using spatially explicit, individual-based simulations. Non-native brook trout are well-suited model as they have previously been studied for simulations that assess how management factors affect the success of M_{YY} implementation. Specifically, we examined how fitness effects of inbreeding depression (ID; measured as mortality as a function of the change in genetic variation (homozygosity)) influences overall population bottleneck size and subsequent patterns and rates of recovery once suppression efforts and M_{YY} introductions have ceased. Our goal was to examine the effect of the fitness penalty of ID on several factors including the mean minimum number of individuals (bottleneck size), number of individuals per patch over time, percent observed homozygosity over time, the number of fitness deaths over time, and the recovery index of populations. Our

results can help to reduce the uncertainty associated with the unknown fitness impacts of suppression and M_{YY} implementation on the non-native population should eradication not occur.

Methods

Study Site

The spatial extent of this study is based within the occupied range of brook trout in the Sullivan Creek Watershed in Washington State, USA. Sullivan Creek (roughly 25 km of habitat) is a large tributary to the Pend Oreille River in northeastern Washington State. Historically Sullivan Creek was occupied by native bull trout (*Salvelinus confluentus*) and westslope cutthroat trout (*Oncorhynchus clarkii lewesi*), but due to the construction of several dams and the introduction of brook trout, bull trout were extirpated and westslope cutthroat trout populations are dwindling (USFWS 1999a; USFWS 1999b). Brook trout were extensively stocked into the system between 1933 and 1981 (Kloempken, 1996) and they now occur throughout most of the mainstem and tributaries of Sullivan Creek. A collaboration between Seattle City Light (SCL), the Washington Department of Fish and Wildlife (WDFW), and the Kalispel Tribe of Indians has resulted in habitat restoration as well as brook trout eradication and suppression work within Sullivan Creek and some of its tributaries. Recent management efforts have included the removal of one of the dams (Mill Pond in 2018) and mechanical (electrofishing) suppression of brook trout since 2016. Additionally, in November 2018, an M_{YY} management project began with M_{YY} being released annually. Modeling work was previously performed to inform this ongoing large-scale implementation of M_{YY} releases into Sullivan Creek, including simulation of the effects of dispersal distances and rates, mortality, growth, fitness, and survival of M_{YY} brook trout on overall brook trout abundance (Day et al. 2020; Day et al. 2021).

Model Description

The simulation of population dynamics, movement, suppression, and introduction of M_{YY} was done using CDMetaPOP version 2.51 (Landguth et al. 2017; Day et al. *in press*). CDMetaPOP is a demogenetic model that simulates the movement and exchange of genetic material of individuals that occupy discrete patches (subpopulations) arranged across a heterogeneous landscape (Landguth et al. 2017). Within the model, every time step (i.e. year) individuals can grow (as a function of temperature), mature, mate and reproduce (as a function of

size), and move (as a function of riverine distance). Additionally, size-based density-dependent mortality and age-based density-independent mortality occur annually. Day et al. (2018) developed a model within CDMetaPOP to simulate mechanical suppression of brook trout within the Sullivan Creek system through electrofishing. Day et al. (2020) built upon that model to incorporate the use of M_{YY} brook trout within the system. Our study uses many of the parameterizations from these previous analyses (See Table 4-1) but expands on the model to incorporate the fitness effects of ID on the remnant brook trout population.

Introduction of M_{YY}

To simulate the use of M_{YY}, we used a previously developed module within CDMetaPOP that allows for the introduction of fish at the beginning of any time step during the simulation. Individual fish can be assigned sex chromosomes (XX, XY, and YY). Individuals with YY chromosomes were introduced into the upper sampling units of Sullivan Creek consistent with management practices currently being used and following Day et al. (2020 and 2021). Introduced M_{YY} were assigned their own allele frequencies (as described below). M_{YY} were also assigned an independent set of parameters for mortality, maturation, fecundity, and movement based on Day et al. (2020) (See Table 4-1).

Allele Frequency Parameterization

Patch genetic data for the wild brook trout populations were initiated with allele frequency files generated from empirical adult brook trout genotypes (240 locus SNP panel) from seven locations within the Sullivan Creek system. Locations were selected if they had at least 20 individuals with genetic data per location. Allele frequencies for M_{YY} brook trout were generated using M_{YY} brook trout genotypes (using the same SNP panel) from Hayspur Hatchery in Idaho (N=386). Hayspur Hatchery is the hatchery that provides M_{YY} brook trout currently being introduced into the Sullivan Creek system. Loci that had missing data for at least 10 individuals per population were removed, which left a total of 219 loci. Chi-squared P-values were calculated to test for allele frequency divergence and to assign significance to pairwise F_{ST}'s using the *adegenet* R package (Jombart et al. 2010). No significant difference was found between male and female allele frequencies (chi-squared Goodness-Of-Fit Test: $P > 0.05$) and therefore allele frequencies were not separated based on sex. Summaries of genetic variation and differentiation of these two simulated gene pools are as follows: Mean heterozygosity for the

Sullivan Creek populations was 0.35 ± 0.05 SD and for Hayspur Hatchery was 0.47 ± 0.04 SD. Pairwise F_{ST} (calculated with R package *hierfstat* (Goudet 2005) using the Nei F_{ST} approach) between the two populations was 0.125.

Since estimates of individual inbreeding coefficients are sensitive to number of loci (Kardos et al. 2015, Nietlisbach et al. 2017), we conducted a preliminary analysis to determine the number of loci to simulate that would balance both precision and total run time. We calculated R^2 for the correlation between pedigree and observed homozygosity (hom_O) for 500 loci, 1000 loci, 5000 loci, and 10000 loci. All loci were unlinked and therefore independently inherited. We increased the number of simulated loci until we reached a value where R^2 was not sensitive to further increases. We selected 5000 loci for the final simulations as it created a good balance between total run time for the simulation and R^2 value (0.918; Figure S4-1). Additionally, using 10000 loci vs 5000 loci did not substantially affect the R^2 value (10000 loci R^2=0.945, 5000 loci R^2= 0.918).

Study Design

The study area was divided into 152 discrete patches approximately 200 m in length, representing brook trout distribution in Sullivan Creek (West Fork Environmental 2012). Carrying capacity, habitat suitability, temperature, capture probability, and resistance to movement between patches were parameterized at the patch level following Day et al. (2020; Table 4-1). Patch genetic data were initiated for all patches with wild brook trout allele frequencies (see Allele Frequency Parameterization) that were closest geographically to that specific location.

Baseline Scenario

We created a baseline scenario for comparison of alternative scenarios. Within this baseline scenario, no ID fitness penalty was implemented. The baseline simulation began with a burn-in phase of 10 years to stabilize population abundance before genetic exchange between individuals occurred. At year 25, manual suppression within patches began on a schedule of three years on, two years off following current management efforts within Sullivan Creek (Bearlin and Simmons 2015). Beginning at year 28, annual releases of 9,110 fingerling size M_{YY} brook trout occurred within all patches. This number is based on previous simulations performed by Day et al. (2020) and on the carrying capacity of each patch. M_{YY} brook trout are assumed to

distribute themselves evenly across the watershed in proportion to brook trout density. Since we lack empirical data to parameterize fitness (survival and reproduction) of M_{YY} brook trout compared to wild-type male brook trout, fitness was parameterized as equal to that of wild brook trout. Mating between individuals occurred as a function of distance, with no female selection towards male-type. Treatments continued until year 38 (10 years of M_{YY} introduction and 13 years of manual suppression (three years on, two years off)) or until the female portion of the population was eradicated. Once suppression and M_{YY} introduction were halted, brook trout populations were allowed to recover (in the subset of simulations that do not lead to extirpation prior to recovery) for an additional 32 years (70 years total). We ran 10 replicates of this baseline scenario.

Alternative Scenarios

To demonstrate the effects of ID on the recovery of the wild brook trout population after suppression and M_{YY} introduction (hereafter treatment) ceased, we executed two alternative scenarios: (1) no treatment and no fitness penalty (Null model), (2) treatment with ID fitness penalty. All other parameters were held the same as in the baseline scenario.

ID Fitness Penalty Parameterization

To simulate the impact of ID on the population bottleneck size and subsequent recovery of brook trout once suppression and M_{YY} introduction ceased, a new module was developed within CDMetaPOP that allows for the implementation of fitness (measured as mortality) based on the observed homozygosity (hom_O) value of individuals. We opted to use hom_O as a measure of inbreeding depression after running preliminary analyses to determine how this measure is related to overall pedigree relatedness (determined using R package *pedigree* (Coster and Coster 2010)). One of the criticisms of using pedigree-based estimates of individual inbreeding coefficients is that it does not account for inbreeding caused by distant ancestors not included in the pedigree (Kardos et al. 2015). We were confident that we were accounting for any inbreeding from distant ancestors, since through CDMetaPOP it is possible to determine parents of individuals and track the pedigree through the entirety of the simulation. Therefore, we determined individual pedigrees were a good measure to track inbreeding. Additionally, we selected hom_O as a measure of inbreeding after running preliminary simulations to determine

how closely hom_O followed what we expected to occur after M_{YY} admixture: hom_O decreased following M_{YY} admixture.

To parameterize the relationship between homozygosity and fitness, we drew values from the literature. Few studies have attempted to determine this relationship within salmonids (Thrower and Hard 2009); therefore, we used empirical data from five different studies estimating the effect of inbreeding on early life survival of mammals (red deer (Walling et al. 2011; Huisman et al. 2016); soay sheep (Bérénos et al. 2016), black and white ruffed lemurs and north island robins (Armstrong and Cassey 2007)). We determined the slope of the regression that predicted the level of mortality as a function of the inbreeding coefficient for each study. We calculated the mean slope across all five studies (mean = 9.57) and then varied the parameter space around the mean to understand the sensitivity of model outcomes to parameter uncertainty by calculating several additional penalty slopes: +/- 1 SD of the mean slope, +/- ¼ SD of the mean slope, and +/- ⅛ SD slope of the mean.
The logit relationship between probability of mortality of individuals from hom_O was calculated as:

$$ProbMortality\_ID = \exp(bint + m * hom_O / (1 + \exp(bint + m * hom_O))$$

Where bint is the intercept and m is the slope value. Using the logit function is standard for modeling survival (or mortality) as it constrains the survival probability between 0 and 1 (Armstrong and Cassey 2007).

We ran seven simulation scenarios that varied the slope of mortality based on the hom_O of individual brook trout. ID fitness penalties were based on the mean slope value that we determined from the empirical data (mean = 9.57). Slopes ranged from 12.44 for the + 1 SD simulations, where 60% homozygosity resulted in a 61% chance of mortality, to 6.71 for the -1 SD simulations, where 60% homozygosity resulted in a 22% chance of mortality. Intercept was calculated as -7.03 to obtain ~ 50% probability of mortality when ~50% of the genome is homozygous for the highest penalty slope (+1 SD above the mean) (Figure S4-2). Each simulation scenario was replicated 10 times. The slope penalty of +1 SD above the mean resulted in extirpation of the population for all runs before M_{YY} introduction and suppression could begin

(mean number of years for simulation 22 ± 1 SD) and therefore it was removed from subsequent analysis.

Analysis

To compare the fitness effects of ID on population bottleneck size and subsequent recovery we calculated 95% confidence intervals (mean ± SD) for all output metrics; the mean minimum number of individuals (bottleneck size), number of individuals per patch over time, percent observed homozygosity over time, number of fitness deaths over time, and recovery index (total N at the final year of the simulation/ total N pre-treatment). Pre-treatment year was calculated as year 20 as that gave the population enough time to stabilize after burn-in ended at year 10. Final year was calculated as the final year of the simulation. For populations that were eradicated before year 70, final year was equal to the year before eradication occurred, and for all other simulations was year 70.

Results

For all simulations where M_{YY} were introduced, mean N per patch over time was stable until suppression and M_{YY} introduction occurred and then declined in an inverse sigmoidal fashion up to when M_{YY} introduction and suppression ceased (Figure 4-1).

Baseline and Null Scenario

For the baseline model scenario, where there was no fitness penalty, mean minimum number of individuals at the bottleneck was 8578 ± 293 SD (Figure 4-1; blue line). Once suppression and M_{YY} introduction ceased, the population recovered to pre-treatment levels. Mean recovery index was 1.00 ± 0.02 (Figure 4-2). Mean proportion hom_O pre-suppression and M_{YY} introduction was 0.68 ± 2e-04 SD. Hom_O decreased by 8.8% during the bottleneck (mean = 0.62 ± 2e-03 SD) and then increased again by 4.8% in the final year of the simulations, however, it did not return to pre-suppression levels (mean = 0.65 ± 1e-03; Figure 4-4).

For the null model, mean N per patch over time remained stable for the full length of the simulation (~ 55 individuals per patch; Figure 4-1; pink line). Additionally, mean proportion hom_O over time remained stable for the full length of the simulation (Figure 4-4). Mean recovery index was 1.00 ± 0.02 (Figure 4-2).

ID

There was a clear effect of ID on the bottleneck size of populations during treatment with higher penalty ID slopes resulting in a larger bottleneck (smaller minimum N). Mean minimum number of individuals at the bottleneck varied across slope penalties and increased with decreasing slope penalty. The lowest mean minimum number of individuals per replicate occurred in the +¼ SD penalty slope simulation (mean = 334 ± 144 SD) and the highest mean minimum number of individuals occurred in the -1 SD penalty slope simulation (mean = 7262 ± 374 SD) (Figure 4-1; dark green line and red line respectively). Once suppression and M_{YY} introduction ceased, the populations recovered to at least pre-treatment levels for all simulations except the highest penalty slope simulations. Recovery of all simulations (except the two highest penalty slopes) occurred within 13 years of treatment ending and for the second highest penalty slope recovery occurred within 20 years of treatment ending. Mean recovery index ranged from 0.62 ± 0.22 SD for the highest penalty slope to 1.25 ± 0.02 SD for the mean slope (Figure 4-2). For all simulations, the mean number of fitness deaths per patch decreased during treatment. All simulations resulted in a lower number of fitness deaths per patch in the final year of the simulation compared to pre-treatment. However, for the two highest penalty slopes the slope of the line was increasing in the final year of the simulation whereas all the other simulations had plateaued (Figure 4-3). Mean hom_O decreased during the population bottleneck for all simulations. For the highest penalty slope runs mean hom_O decreased by 12.59% during the bottleneck and by 9.24% for the lowest penalty slope. By the final year, mean hom_O had increased again for all simulations, but not up to pre-treatment levels, and was highest for the highest penalty slope simulations (Figure 4-4).

Discussion

Examination of how the surviving brook trout population responds to incomplete eradication is an important element to consider for an M_{YY} management plan. Using a demogenetic, spatially explicit model, we demonstrated the effects of ID and divergent hatchery admixture from M_{YY} on the bottleneck size and population recovery of non-native brook trout populations, should eradication not occur. Overall, ID had large effects on population bottleneck size. However, the admixture from divergent hatchery M_{YY} alleles resulted in an increase in the genetic variation of the population (decrease in hom_O) which allowed for population recovery above pre-treatment levels for most runs.

ID had a large influence on bottleneck size and subsequent recovery of populations in these simulations. Mean minimum number of individuals at the population bottleneck and total number of individuals at the end of the simulation decreased with increasing fitness penalty. Interestingly, for all simulations except the simulation with the highest (+¼ SD above mean) penalty slope, the recovery index was above 1 and complete recovery occurred within 20 years of treatment ending. However, for the highest penalty slope, at the end of treatment the slope of the line was increasing so it is possible it would have recovered above initial pre-treatment levels if left to recover long-term. The result of a recovery index above 1 is likely due to the differences seen between the number of fitness deaths over time, where fitness deaths decreased post-treatment compared to pre-treatment. There are two possible explanations for this result and both likely interacted to produce the higher recovery index. The first explanation is that increased admixture from the genetically distinct hatchery M_{YY} resulted in a decrease in the number of inbred individuals (decreased mean hom_O) during M_{YY} introduction. This is similar to a heterosis effect, when "hybrid" progeny have higher fitness than either parental type. We attribute this to admixture (and subsequent decrease in mean hom_O) from M_{YY} causing an elevation in fitness since we simulated survival as a function of hom_O, not because of an effect of overdominance. Other studies have shown immigration into an inbred population can increase genetic variation and counter the genetic effects of a bottleneck (McEachern et al. 2011; Bell et al. 2019). In our simulations, M_{YY} introduction acted similarly to immigration and led to increased genetic variation (decreased hom_O) which resulted in fewer fitness deaths based on our parameterization. The second possible explanation is that, for the higher slope penalty simulations, the greater number of deaths of individuals with greater homozygosity led to higher mean population fitness. This also could explain why the lowest penalty slope did not result in a recovery index that was much higher than one (1.01 ± 0.02 SD) since not enough fitness deaths occurred in these simulations to cause this shift in mean fitness.

Management implications

These results highlight the importance of fitness effects and how they can impact the overall effectiveness of an M_{YY} management plan if eradication does not occur. Recovery index for all simulations except the highest fitness penalty simulations was 1 or higher, suggesting complete recovery of populations. Current simulations of M_{YY} implementation suggest time to eradication can range from ~2 years to over 100 years depending on factors such as the relative

fitness (survival and reproduction) of M_{YY} males, the number of M_{YY} released relative to the number of wild fish, the number of years M_{YY} are released for, and the number of release locations (Day et al. 2020 and 2021). Our results suggest the impact of admixture of hatchery divergent M_{YY} alleles may result in complete recovery of populations within a short time-period (20 years after treatment ends for six of the seven simulations) if complete eradication does not occur. This would result in a wasted effort of possibly many years of M_{YY} introduction.

The predicted lower abundance of brook trout populations during the population bottleneck could be a valuable outcome. Lower abundance of brook trout is beneficial for cutthroat trout populations currently dwindling due to competition with brook trout and brook trout removal has been shown alleviate competition issues for cutthroat trout (Peterson et al. 2004), possibly giving them enough of a boost to improve population outcomes. However, the continued persistence of brook trout post-treatment in most simulations suggests that this relief may be temporary and that managers should not rely on the fitness effects of inbreeding to result in eventual brook trout population extirpation.

One caveat to our study is that our model only incorporated the positive fitness effects of M_{YY} admixture in that it increased genetic diversity. However, admixture between hatchery M_{YY} and wild fish could have both benefits and limitations toward the success of a non-native eradication program. Outbreeding depression (OD), caused by admixture between genetically divergent individuals (Lynch 1991), can decrease fitness through genomic incompatibilities or maladaptation between genes and the environment (McGinnity et al. 2003). Many studies have demonstrated outbreeding depression of offspring with hatchery introgression in the wild (Gharrett et al. 1999; Miller et al. 2004; Christie et al. 2014). In the case of M_{YY} introduction, the pulse of admixture from the M_{YY} source could induce OD in addition to heterosis. Production of low-fitness offspring could reduce the ability of the remaining population to recover following treatment. Alternatively, this decrease in fitness could result in a decrease in the rate of admixture of M_{YY} alleles. Decreased M_{YY} admixture within the population would likely affect the overall ability of the management plan to skew the sex ratio, ultimately resulting in a decrease in the rate of decline of the non-native populations during M_{YY} introduction and possibly increase the rate of recolonization after suppression and M_{YY} introduction have ceased. Future research within this model framework includes exploring how bottleneck size and

recovery post-suppression and M_{YY} treatment may be impacted by the fitness impacts of the combination of ID and OD.

Additionally, there was likely an impact on the results of both the bottleneck size and recovery of populations due to the differences in the number of individuals before suppression and M_{YY} introduction began for all simulations. For example, the lower number of individuals at year 20 in the highest fitness penalty slope likely impacted the result of a lower minimum N at the bottleneck than other simulations. Parameterizing the populations so that all simulations start out with the same number of individuals before treatment begins would likely result in a different outcome. However, the objective of this study was not to be prescriptive but to explore a range of parameters to understand the sensitivity of model outcomes to parameter uncertainty. Additionally, regardless of the number of individuals in the population prior to treatment and regardless of the size of the bottleneck, the result of all the simulations was the same, a recovery index that was either above one or increasing and could possibly have gone above one eventually. Therefore, our results still stand in that admixture of M_{YY} alleles into the population results in recovery of the populations. Next steps can be to account for the number of individuals in the population pre-treatment to determine how that may impact bottleneck size between simulations.

Model Considerations

In simulations that are applied to real-world systems, simplifying assumptions must be made about model processes where data are lacking. In our case, we lacked specific data on the impacts of ID on survival of brook trout. To characterize the consequences of this uncertainty, we simulated a range of fitness penalties for ID based on empirical data for mammals. Our goal was to explore the fitness parameter space and its influence on recovery rates of brook trout should eradication not occur, without attempting to be predictive.

We also made the assumption in our model that fitness of M_{YY} and wild brook trout was equal. Other studies have shown negative fitness effects of hatchery individuals in the wild (Araki et al. 2008), however, the relative fitness of M_{YY} are unknown (but see Chapter 3). We chose to parameterize our model in the same way as Day et al. (2021) where the fitness of wild vs M_{YY} brook trout were equal. Decreasing the fitness of M_{YY} compared to wild brook trout has been shown to increase the likelihood of eradication (Day et al. 2020) and therefore could

decrease the impact of M_{YY} admixture on the higher recovery rates in our results, however, this would need to be tested further.

When selecting models for landscape genetic studies, most available software is restricted either in terms of genomic or spatial complexity. An advantage of CDMetaPOP is that it incorporates complex demography with spatially heterogeneous landscapes and dispersal behavior. The tradeoff is that it is limited in genomic complexity. The spatially explicit complexity of CDMetaPOP and the fact that it can be calibrated based on empirical results from ongoing fieldwork lends itself well to studies that can be used to inform management decisions. Future work could include greater genomic complexity (e.g. placing loci on chromosomes with simulated recombination rates) to test for influences on ID in the context of M_{YY} eradication programs.

Evolutionary aspects of non-native species are rarely accounted for when managing them. Simulation models provide a useful method for making predictions of the implications of management actions on population fitness, especially when empirical data are lacking. The implementation of M_{YY} as well as suppression efforts creates a complex situation involving evolutionary processes that will require further empirical and simulated investigation. Our results demonstrate how fitness aspects of suppression and M_{YY} implementation may impact the overall success of the management strategy and suggest that even if populations are driven to very low abundances, managers should not rely on them being extirpated due to fitness effects of bottleneck size if eradication does not occur.

Table 4-1. Key parameters and references used in the simulations.

Input Parameter	**Description**	**Values**	**Reference**
Number of patches		152 patches divided into ~200 m reaches	Day et al. 2020
K	Carrying capacity	Variable: based on multi-pass depletion surveys from 2013 – 2017 and set so that N from the simulation model = estimated abundance.	Walston 2018
Monte Carlo replication runs	Number of replicates per simulation	10	
Runtime (years)	Total Years	70	
Burn-in time (years)		10	
Year when suppression starts		25	
Year when M_{YY} introduction starts		28	
Number of Years of Suppression		13; schedule of 3 years on 2 years off	Bearlin and Simmons 2015
Number of Years of M_{YY} introduction		10	
Number of M_{YY} introduced annually		9,110 age-0	Day et al. 2020
Number of release locations		18	Day et al. 2020
Years of recovery		32	

Input Parameter	Description	Values	Reference
ID Fitness Penalty	Mortality as a function of hom_O per offspring individual	ProbMortality_Inbreeding = exp(-7.025 + m * hom_O) / (1. + exp(-7.025 + m * hom_O)); added slopes for +/- 1 SD of mean, +/- 1/4 SD of mean, and +/- 1/8 of mean; Intercept calculated to obtain ~ 50% probability of mortality when 56% of the genome is homozygous for high penalty slope (+1 SD above the mean). Slopes ranged from 12.44 (+ 1 SD simulations) to 6.71 (-1 SD simulations)	Mean slope calculated based on values from emperical data (Huisman et al. 2016, Berenos et al. 2016, Armstrong and Cassey 2007, Walling et al. 2011.
Number of alleles		5000	Based on preliminary analysis determining the relationship between pedigree and hom_O
Initial number of allele frequency files		**Wild**: generated from genotypes from seven Sullivan Creek populations file; $\mathbf{M_{YY}}$: generated from genotypes from Hayspur Hatchery	
Year at when genetic exchange begins		10	
Fecundity	Number of eggs per female	Number of eggs = 3.78 * Length (mm) -455	SCL data; modeled after Downs et al. 1997

Input Parameter	Description	Values	Reference
Maturation		**Wild**: P(mature) = exp(-6.313 + 0.0539 * Length) / (1 + exp(-6.313 + 0.0539 * Length)); **M_{YY}**: Forced to mature at age 1, 1 year following release	SCL data; modeled after Downs et al. 1997
Body Size (mm)	Mean length (mm) of each age class at initialization	**Wild**: 60, 110, 140, 180, 200, 230, 240, 250**; M_{YY}:** All 137 mm (±14)	Day et al. 2020
Growth	Modified Von Bertalanffy	Newsize (mm) = 400 * (1 – exp(-0.47 * (age + 1-0.075))); maxtemp = 10.5; tempcv = 0.33 (see CDMetaPOP user's manual)	Landguth et al. 2017; Von Bertalanffy 1938; Seattle City Light unpublished data
Class-specific mortality		As an age check on old fish, 50% mortality applied on all individuals age 7+	SCL data
Local dispersal distribution	Distance moved each year	Based on Pareto distribution	Letcher et al. 2015
Migration, mating, and straying movement	Resistance to movement surface	Isolation-by-distance + physical barriers (i.e., dams, culverts). Max mating movement = 1 km. Maximum straying and migration distance is determined by Pareto distribution (i.e., very rare long-distance movements)	UNICOR (Landguth et al. 2012; (Landguth and Cushman 2010)

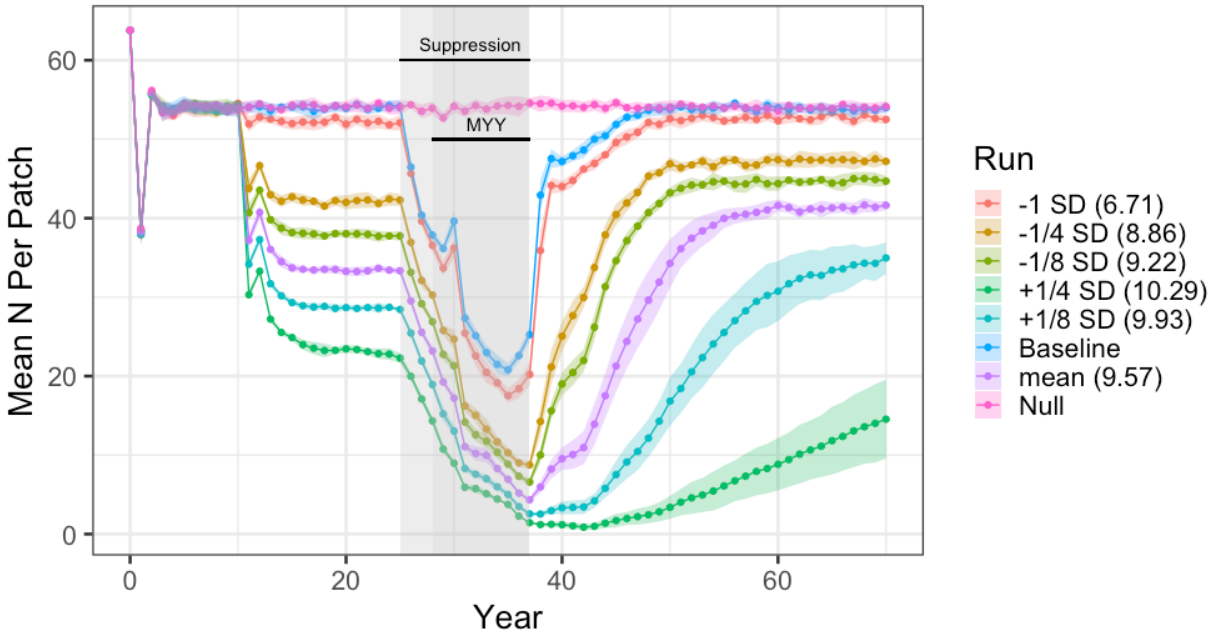


Figure 4-1. Mean number of individuals across patches over time and including the baseline and null simulations (error bars = ± SD). Baseline simulations include treatment but no fitness penalty, null simulations have no treatment and no fitness penalty.

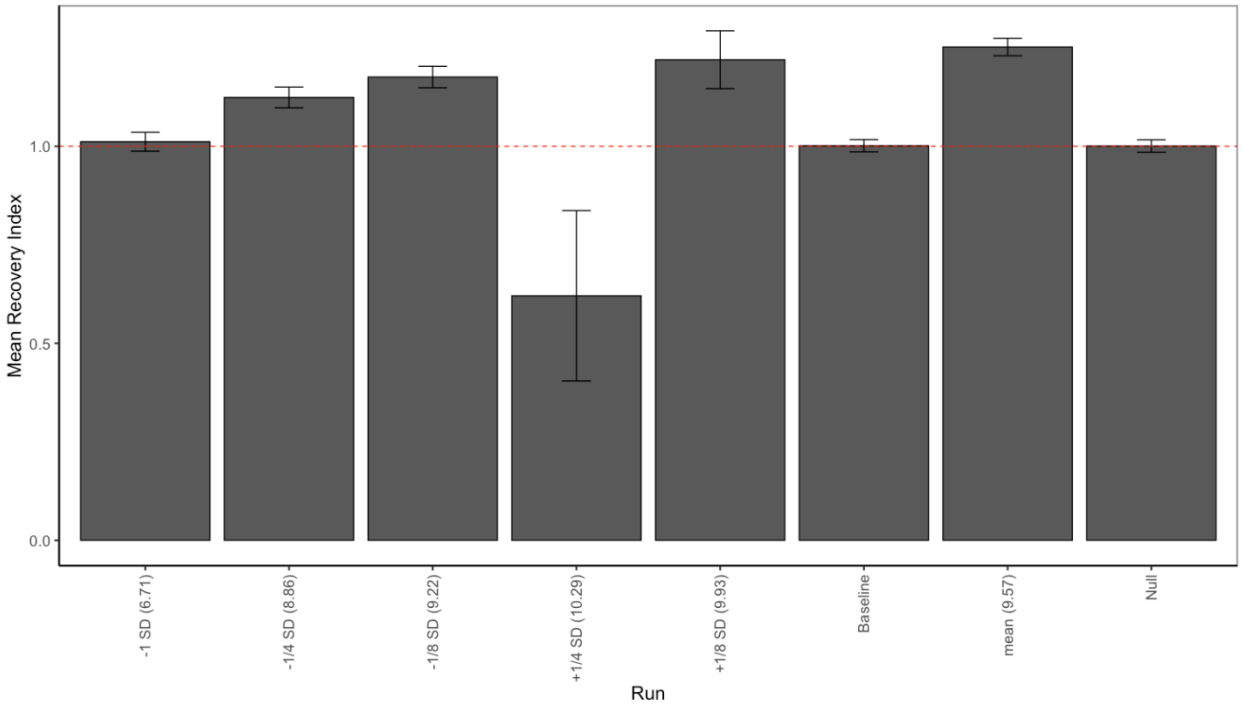


Figure 4-2. Mean recovery index and including the baseline and null simulations (error bars = ± SD). Recovery index is defined as total N at the final year of the simulation/ total N pre-treatment. Baseline simulations include treatment but no fitness penalty, null simulations have no treatment and no fitness penalty. Red dotted line indicates a recovery index of 1 which equates to full recovery.

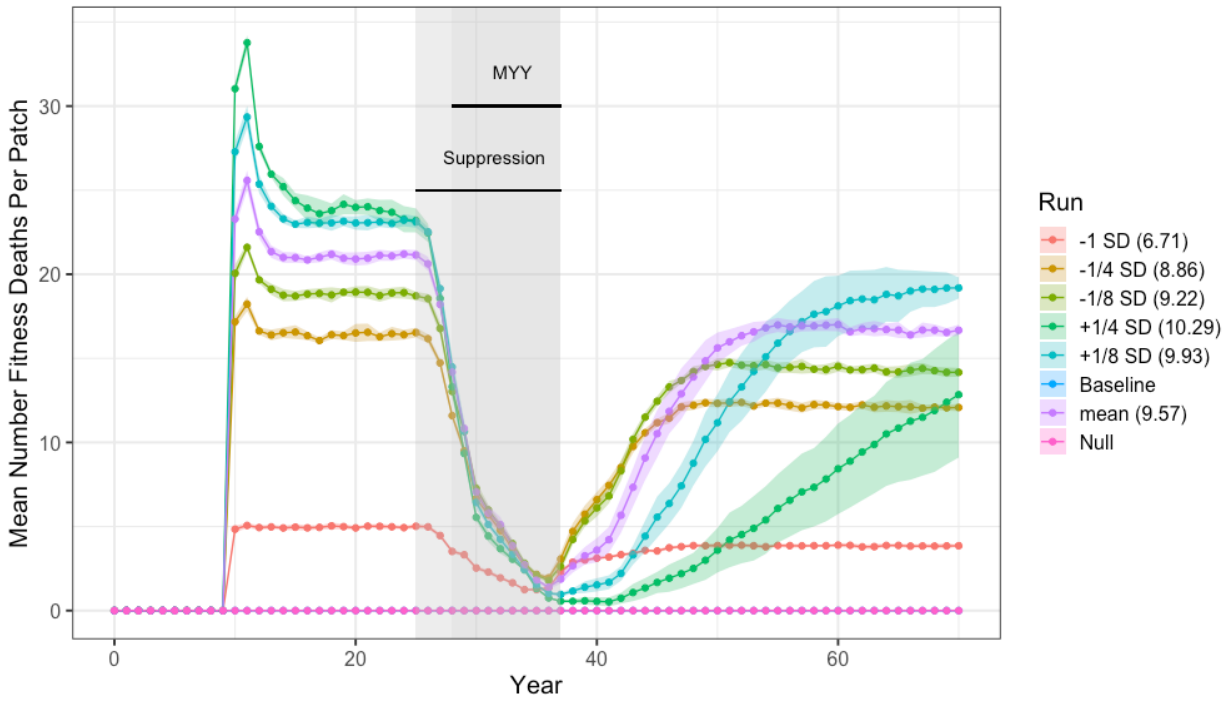


Figure 4-3. Mean number of fitness deaths per patch over time and including the baseline and null simulations (error bars = ± SD). Baseline simulations include treatment but no fitness penalty, null simulations have no treatment and no fitness penalty.

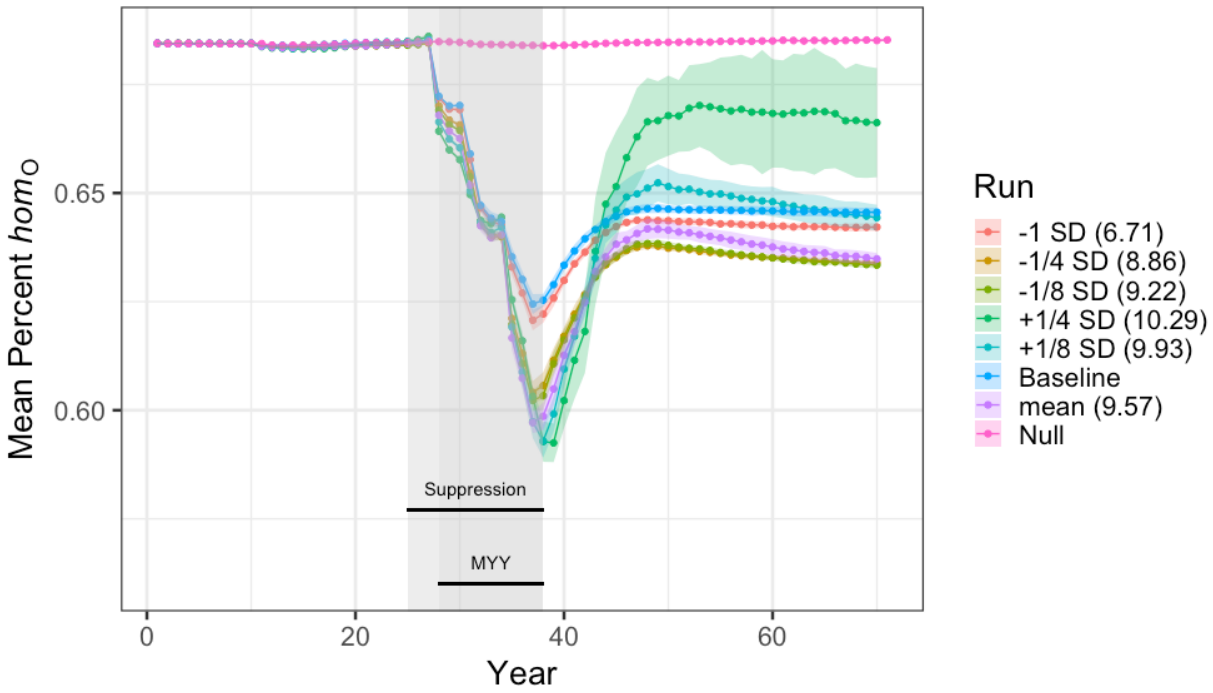


Figure 4-4. Mean percent hom_O over time based on 5000 loci and including the baseline and null simulations (error bars = ± SD). Baseline simulations include treatment but no fitness penalty, null simulations have no treatment and no fitness penalty.

CHAPTER 5: Understanding how characteristics of fisheries managers impact their willingness to use novel approaches to conservation

Abstract

In fisheries and wildlife management, the use of novel methods to conserve native species provides managers with high uncertainty as they have often received limited testing. However, they could provide better alternatives to ineffective traditional methods if successful. The goal of this study was to determine how manager characteristics such as agency affiliation, state affiliation, and risk-tolerance influence the likelihood that managers will implement two novel management methods (M_{YY} implementation and genetic rescue) to conserve native headwater stream fish populations. We presented managers from five different types of agencies across six western US states with surveys that included a modified risk propensity scale to assess their tendency to take or avoid risks. We then presented them with two real-life management scenarios: 1) managing non-native species where suppression or eradication has failed (M_{YY} scenario) and 2) managing a native population of fish that is isolated (genetic rescue scenario) to assess their willingness to use these methods as a management strategy. Our findings suggest that although managers showed possible interest in implementing novel methods, they indicated they were more unlikely than certain to implement novel strategies to conserve native populations. Managers also indicated a need for more research and real-world examples of the use of M_{YY} and genetic rescue in general. Managers from different states and agencies showed differences in willingness to implement these novel strategies. Managers categorized as risk-takers indicated a higher willingness to implement both an M_{YY} eradication strategy and a genetic rescue strategy in all scenarios than managers categorized as risk-averse. Our results show that understanding the individual characteristics of managers is important for identifying factors that hinder the implementation of novel methods in the conservation of species.

Introduction

Native fish species within headwater streams are vulnerable to many threats such as introduced non-native species and habitat fragmentation. Threats from non-native species include hybridization and competition, which can ultimately result in the extirpation of native species (Miller et al. 1989). Habitat fragmentation can result in the elimination of gene flow which can lead to a loss of genetic diversity and inbreeding (Fausch et al. 2009). Traditional management strategies for dealing with these issues include suppression or extirpation (through mechanical removal or the use of chemical piscicides) in the case of non-natives and increased habitat connectivity in the case of habitat fragmentation (Gozlan et al. 2010; Britton et al. 2011). However, there are limitations to both techniques. Suppression or extirpation of non-native fish species can be time-consuming, expensive, and often does not work. Attempts to restore habitat connectivity also have these same issues in that they can be expensive, time-consuming, and may not be successful. Increasing habitat connectivity can also lead to an increase in the spread of non-native species.

Two new approaches to these issues have been suggested in recent years. The first approach, if an undesirable non-native fish species is present, is the addition of YY males of the target non-native species. YY males are hatchery-produced fish with two Y chromosomes, instead of the typical XY chromosomal arrangement. We refer to YY males as M_{YY} following Kennedy et al. (2018). Since M_{YY} only pass on Y chromosomes to offspring, all offspring of M_{YY} will be XY males. Introduction of these fish within a non-native population theoretically results in a shift of the population sex ratio towards all males, causing population extirpation due to the elimination of one sex. The second approach is called genetic rescue, which involves the human-assisted movement of a small number of individuals into an isolated population to provide the fitness benefits of gene flow (Frankham 2015). This option can be implemented if habitat connectivity within a population is low, or a population is isolated and managers have concerns about negative effects of inbreeding on demographic rates. The primary goal is often to increase abundance and genetic variation in the focal population (Whiteley et al. 2015).

In fisheries and wildlife management, decision-making is a critical process involving the application of knowledge, such as data or experience, as well as evaluations of alternative goals, objectives, and actions (Enck and Decker, 1997; Fuller et al. 2020). Perfect solutions rarely exist and often the outcome is unknown so the decision-maker must consider all the consequences of

the management action (Gore et al. 2009; Fuller et al. 2020). The use of novel management methods is one way in which this decision-making process is tested. Novel methods provide managers with high uncertainty as they have often received limited testing, however, if successful, could provide better alternatives to traditional methods that have been ineffective or too costly (Lahoz-Monfort and Magrath 2021).

Understanding managers' decision-making when it comes to novel methods is important for helping to integrate science and policy. A common problem with conservation is that there often is a disconnect between what researchers provide and what managers need (Buckley et al. 1998). Helping scientists to understand the decision-making process of managers and whether they would consider implementing the novel methods that are being studied could help to incorporate more science into management decisions (Cook and Sgrò 2019). Despite the importance of understanding decision-making in conservation management, this topic has rarely been explored for novel wildlife management methods, including the conservation of native fish species.

Both the introduction of M_{YY} and the use of genetic rescue present managers with uncertainty because both are at the early stages of scientific testing in natural populations. One area where there is uncertainty is whether managers are willing to implement novel methods such as these as native fish species continue to decline. For a manager, determining the best method of conservation can be difficult because they are not only accountable to their management entities but also to the public. Therefore, using a method that has little scientific testing may not be something that they are willing to implement. In the case of M_{YY} introduction, success is dependent on aspects of the focal habitat (connectivity, habitat complexity) and how M_{YY} are implemented (e.g. size and age of M_{YY} at release, release strategy) as well as biological considerations (abundance of the focal population, fitness of M_{YY} males; Kennedy et al. 2017; Day et al. 2020). In the case of genetic rescue, managers could cause more harm than benefit if poorly understood outbreeding depression or unintentional disease introduction increases the risk of population extirpation (Tallmon et al. 2004; Hedrick et al. 2014). Managers currently face a large degree of uncertainty as they weigh the costs and benefits of each of these approaches. One component of the decision-making process could be individual manager characteristics, which could help managers make decisions in the face of this uncertainty.

The idea that characteristics of managers could lead to specific management decisions is relatively unstudied. Most research focuses on evaluating the impacts of different management approaches while ignoring the traits and characteristics of the people that make these decisions. Recent studies show the importance of understanding how manager characteristics, including agency type (state, federal, private, or tribal), education level, and experience lead to the success or failure of different management projects (Sher et al. 2020; Clark et al. 2020; Primack et al. 2021). In the case of the use of novel fisheries management approaches, decisions could be influenced by characteristics such as region, agency affiliation, and a manager's willingness to take risks. These first two factors, region and agency affiliation, are affected by external elements such as differences in perceived attitudes of stakeholders, sociopolitical realities, ecological attributes of the managed region, funding, as well as cultural attributes (Kurtz 2003; Fuller et al. 2020). For example, at the state level, whether a state focuses on recreational angling over conservation of native populations will depend partly on the attitudes of stakeholders within that region. Species that occur, along with variations in their life histories, will also affect how a state manages its fisheries. Additionally, agencies all have different missions, which likely impact the prioritization of goals and how projects are managed (Sher et al. 2020). For example, a state agency will work within a smaller spatial scale and will focus more on specific state issues than a federal agency. Underlying agency mission is the culture within an organization, and it can be important to understanding decision-making processes of the managers within that organization (Kurtz 2003). Culture describes the beliefs and practices common within an organization and encompasses values, visions, beliefs, and habits (Kurtz 2003; Friggens et al. 2015). As problems are repeatedly encountered by organizations, a set of decisions regarding those problems will become the norm. Continued success of those decisions will validate and reinforce them for veteran employees, who will then teach them to new employees, establishing a culture of decision-making (Kurtz 2003).

Another characteristic of managers that can influence decision-making is their willingness to take a risk. Risk tolerance is defined as an individual's capacity to accept a certain amount of risk (Hürlimann 2019) and characterizes whether a decision-maker is willing to accept an outcome that may be uncertain (Van Harlow and Brown 1990). Risk tolerance is often studied in business or financial decision-making, but the capacity of natural resource managers to accept a risk can also play an important role in conservation management (Gore et al. 2009; Little et al.

2014; Tulloch et al. 2015). In order to conserve species, managers can implement projects that have low risk but also low reward. Alternatively, they can implement projects that have the potential to be high reward but are high risk. Failure of a risky decision results in wasted resources, a decrease in trust in the management organization, and failure to relieve threats to the conservation species of priority (Tulloch et al. 2015). Despite the importance of understanding risk-taking in conservation management, it has not been explored for novel management methods of species conservation.

The goal of this study was to determine how agency affiliation, state affiliation, and personality (risk tolerance) influences the likelihood that managers will implement an M_{YY} non-native fish eradication strategy or a genetic rescue strategy to conserve native headwater stream fish populations. We presented managers from five different types of agencies across six western US states with surveys that included a modified risk propensity scale, originally developed to determine risk-taking tendencies in a business setting (Sitkin and Weingart 1995; Hung and Tangpong 2010), to assess their tendency to take or avoid risks. We then presented them with real-life management scenarios to assess their willingness to use M_{YY} or genetic rescue as a management strategy. We presented them with two scenarios: 1) managing non-native species where suppression or eradication has failed (M_{YY} scenario) and 2) managing a native population of fish that is isolated (genetic rescue scenario). Within the M_{YY} scenarios, the ecological setting (closed, open) and conservation status (low or high) of the native population varied. Within the genetic rescue scenarios, the risk of outbreeding depression due to the translocation and conservation status of the native population varied. Our research questions include: 1) Does manager region or agency affiliation affect their willingness to implement novel strategies? And 2) Are managers who are more likely to accept risks more likely to implement novel strategies when it comes to the conservation of native fish?

Methods

To assess how the characteristics of management practitioners influence their willingness to implement novel strategies for the conservation of native fishes, we developed a survey targeting fisheries managers across six different states in the western United States. For each state, the specific management scenarios presented involve issues currently under consideration. To identify fisheries managers, we obtained email contact information from the American

Fisheries Society (AFS) membership directory and department databases for Montana Fish, Wildlife & Parks (Montana FWP), Washington Department of Fish & Wildlife (WDFW), Idaho Fish & Game (IDFG), Oregon Department of Fish & Wildlife (ODFW), New Mexico Department of Game & Fish (NMDGF), and Colorado Parks & Wildlife (CPW). Individuals were identified by determining the organization for which they worked and their position. We collected data using a web-based survey program (Qualtrics) where unique survey links were sent to each person. After duplicate removal, the mailing list contains 1308 names. Of those emails, 199 were no longer active, making a total sample of 1109.

Questionnaire Development

Four hypothetical management scenarios were developed to assess the willingness of respondents to implement novel strategies. Scenario 1 and 2 (M_{YY}) involved the management of a native cutthroat trout (*Oncorhynchus clarkii spp*.) population within a manager's region that is being outcompeted by brook trout (*Salvelinus fontinalis*). In these scenarios, respondents were told that managers have already attempted to mechanically removed brook trout, but it was not successful. Bull trout (*Salvelinus confluentus*), a species listed as threatened under the Endangered Species Act (USFWS 1999b), were also not present in this system. Scenario 1 involved a closed population (no movement of individuals into or out of the system) and Scenario 2 involved an open population (free movement of trout into and out of the population). Respondents were then asked the likelihood that they would implement an M_{YY} eradication strategy (on a scale of unlikely to certain) for both a population of high and low conservation priority. Scenarios 3 and 4 involved genetic rescue. These scenarios included the management of a closed native cutthroat trout population with low genetic variation that is likely experiencing negative fitness effects of inbreeding. Respondents were told to consider implementing a genetic rescue strategy where they would translocate a small (<10) number of individuals into the population from a nearby source. For Scenario 3, respondents were told the risks of negative fitness consequences of the translocation were minimal and disease testing revealed no known disease presence within the source population. For Scenario 4, respondents were told the risks of negative fitness consequences of the translocation were possible and disease testing revealed no known disease presence within the source population. Respondents were then asked the likelihood that they would implement a genetic rescue strategy (on a scale of unlikely to certain) for both a population of high and low conservation priority. Respondents were also asked on a

scale of not at all (1) to extremely (5) how familiar they are with M_{YY} and genetic rescue implementation and whether they had any additional concerns about using these novel strategies. We chose not to use generic species in the questionnaire because we assumed that managers would know what species are being considered based on the scenario. After each scenario question, respondents were given the opportunity to explain their answers through an open-ended response.

We asked individuals what state they worked within and whether they worked for a state, federal, non-profit, private, or tribal agency. Additional questions included a modified risk propensity scale, originally developed to determine risk-taking tendencies in a business setting (Sitkin and Weingart 1995; Hung and Tangpong 2010). Respondents were asked to answer on a 5-point Likert scale (Likert 1932) (from strongly disagree (1) to strongly agree (5)) questions relating to their tendency to take or avoid risks. For example, "I believe that higher risks are worth taking for higher rewards". The survey also included attitude questions on a 5-point Likert scale (from strongly disagree to strongly agree) to determine respondents' management approaches to different issues. For example, "Non-native fish are a cause of extinctions of native fish populations".

The questionnaire was piloted with 5 individuals (practitioners, scientists, and University of Montana Wildlife Biology Program graduate students) who provided feedback on the survey. Based on their feedback, minor adjustments were made to the questions. The final survey was deployed in March 2022. We followed a modified Dillman approach by contacting respondents four separate times (respondents were only contacted multiple times if they had not completed the survey) via email with information about the survey and inviting their voluntary participation (Dillman 2011).

Data Analysis

Mean, standard deviation, frequency, and other summary statistics were examined for each response variable separately. Managers were categorically labeled as "risky" or "not risky" based on their responses to the risk propensity scale. Mean risk value was determined and individuals that scored above that value were considered a "risk-taker" and those that scored below were considered "risk-averse". Analysis of willingness to implement novel strategies was conducted using the Potential for Conflict Index (PCI) (Manfredo et al. 2003). PCI is a geographical formula that describes variables in terms of central tendency (mean, median, and

mode), dispersion (standard deviation), and form (modality, skewness) (Vaske et al. 2006). Computed values for PCI ranged from 0 to 1, where 0 indicates no conflict and 1 indicates maximum conflict. Comparisons were made between PCI for state where managers worked, a manager's agency type, and a manager's risk propensity. A total of six PCI analyses were performed. The first three were based on respondents' answers to the M_{YY} scenarios. The final three were based on respondents' answers to the genetic rescue scenarios.

Results

Respondent Characteristics

Four hundred and fourteen managers responded, yielding a response rate of 37.3% (414/1109). We assessed the scenarios for differences in managers of different gender and education level. More males responded than females (n = 217 vs n = 41 respectively). No differences were seen between gender or education level for either scenario (M_{YY} or genetic rescue) (see Chapter 5 supplemental figures 2 - 5). Respondents worked in either MT, WA, ID, OR, CO, or NM). States that had fewer than 30 respondents were removed from the analysis. Therefore, we examined responses from MT (n=35), WA (n=108), ID (n=30), OR (n=63), and a category termed 'multiple states' (any combination of the above states but the respondent works in at least two states; n=77). Respondents worked for federal (n= 136), state (n=127), non-profit (n=28), private (n=57), or tribal (n=45) agencies.

Respondents varied along the risk propensity scale provided. Mean risk score for all respondents was 3.5 ± 0.46 (Figure S5-1). The total number of individuals in the risk-taker group was 183 and the total number of respondents in the risk-averse group was 174.

M_{YY} Scenario

Respondents from each state tended to indicate greater willingness to implement an M_{YY} eradication strategy in closed compared to open populations of cutthroat trout. There was a slight tendency toward greater willingness to implement an M_{YY} eradication strategy for low conservation value populations that were either closed or open. Responses across all states (including the multiple-state category) were similar except for Montana. Respondents from Montana appeared to be less likely to implement an M_{YY} eradication strategy than other states for both closed and open populations. Within open populations (both low and high conservation

values) Montana respondents also had the lowest PCI values (0.19 and 0.20), suggesting closer agreement among managers (Figure 5-1).

Respondents from each agency tended to indicate a greater willingness to implement an M_{YY} eradication strategy in closed compared to open populations of cutthroat trout. Tribal and private agencies indicated a greater willingness to implement an M_{YY} eradication strategy than state or federal managers in both scenarios (open and closed) where cutthroat trout populations were considered low conservation value (Figure 5-2).

Managers that were categorized as risk-takers indicated a greater willingness to implement an M_{YY} eradication strategy across all categories compared to risk-averse managers. Respondents from both groups tended to be more willing to implement an M_{YY} eradication strategy in closed populations than in open populations. Risk takers were slightly more willing to implement an M_{YY} eradication strategy in a low conservation population of cutthroat trout than in a high conservation population. Managers that were categorized as risk-averse showed no difference in willingness to implement an M_{YY} eradication strategy between high conservation populations and low conservation populations (Figure 5-3).

Genetic Rescue Scenario

Managers from different states tended to be more willing to implement a genetic rescue strategy if negative fitness consequences were minimal. Managers from MT, WA, ID, and OR were also more likely to implement a genetic rescue strategy in high conservation priority populations than low. Conversely, managers that worked for multiple states indicated they were more willing to implement a genetic rescue strategy in a low conservation priority population than a high conservation priority population if fitness consequences were possible (Figure 5-4).

Respondents from different agencies also tended to be more willing to implement a genetic rescue strategy if negative fitness consequences were minimal. Managers from federal, state, and tribal agencies indicated they were more willing to implement a genetic rescue strategy for high conservation priority populations than low conservation priority populations if negative fitness consequences were minimal (Figure 5-5).

Managers that were categorized as risk-takers tended to be more willing to implement a genetic rescue strategy across all categories than managers categorized as risk-averse, with the exception of the scenario where negative fitness consequences were possible in a high conservation population. Respondents from both categories showed similar (low) willingness to

implement a genetic rescue strategy when negative fitness consequences were possible in a high conservation population. Respondents from both categories were more likely to implement genetic rescue for populations if the risk of negative fitness consequences was minimal rather than possible (Figure 5-6).

Attitude Questions

On a scale of 1 (strongly disagree) to 5 (strongly agree), managers agreed that brook trout and rainbow trout are major threats to native cutthroat trout (mean ± SD =4.01 ± 0.81; 4.10 ± 0.83, respectively). Managers disagreed that it is never ok to use M_{YY} or genetic rescue as a method for conserving native populations of cutthroat trout (1.69 ± 0.63; 1.68 ± 0.66, respectively). Additionally, managers indicated a need for more information regarding the use of both M_{YY} and genetic rescue as conservation tools (3.48 ± 1.07; 3.48 ± 1.03 respectively). They also indicated that public opinion did not influence their willingness to accept genetic rescue or M_{YY} as a conservation tool, however, it was more likely to influence their willingness for M_{YY} (2.22 ± 0.96) than genetic rescue (2.13 ± 0.91). On a scale of not at all familiar to extremely familiar, managers indicated they were more familiar than unfamiliar with both techniques. Managers also indicated they were more familiar with genetic rescue (3.08 ± 0.83) than M_{YY} implementation (2.98 ± 0.89), though the difference in means was small (Table 5-1).

Discussion

Overall, managers showed a higher willingness to implement a genetic rescue strategy than an M_{YY} strategy as long as the negative fitness consequences of the genetic rescue translocation were minimal. These results could be affected by the fact that managers stated they were more familiar with genetic rescue implementation than M_{YY} implementation, though the overall difference in the mean for this result was small. Managers also indicated a need for more research and real-world examples of the use of M_{YY} and genetic rescue in general. Our findings suggest that although managers showed possible interest in implementing novel methods, overall managers indicated they were more unlikely than certain to implement novel strategies to conserve native populations.

For the M_{YY} scenario's, managers for every characteristic indicated they were more likely to implement an M_{YY} eradication strategy within a closed cutthroat trout population than an open

one. Most managers agreed that a closed population where there is no migration into the population would allow for more control over the M_{YY} introduction area. Specific examples include decreasing the likelihood of recolonization and the possible issues of escaped brook trout, where if density dependence is high in the area where M_{YY} are being stocked, brook trout may move out of the area into previously uninvaded habitats. Managers also indicated this was a concern for bull trout populations that may be in close proximity to the brook trout populations. Most managers also indicated a hesitation to implement a risky novel strategy where populations of cutthroat trout are high priority and at greater risk, with one of the major concerns being the possible increased competition and predation that introducing large amounts of brook trout could incur on the cutthroat trout population. Many managers suggested using this strategy first within a low conservation priority population and if that was successful then expanding this method to also include high conservation priority populations.

For the genetic rescue strategy, regardless of scenario, managers showed a higher likelihood of implementation than for the M_{YY} scenarios if negative fitness consequences were minimal. This result was especially prevalent in the scenario where fitness consequences were minimal, and the population of cutthroat trout was of high conservation value. This is different from the results from the M_{YY} scenario where managers were more likely to implement this method in a low conservation cutthroat trout population than a high one. Some managers stated that they were more comfortable with a genetic rescue approach than with an M_{YY} approach which could explain why managers were more comfortable implementing a genetic rescue strategy in a high conservation value cutthroat trout population. Many managers stated that the populations would become extinct anyway if nothing was done, therefore, implementing a genetic rescue scenario was better than the alternative. Managers still indicated a concern for outbreeding depression, where gene flow from the introduced individuals negatively affects the fitness of the locally adapted population (Whiteley et al. 2015; Robinson et al. 2017), though many said that this "risk" was worth the reward if the population could be restored.

Our results show the importance of the characteristics of management practitioners and how they can influence their management decisions. At the regional level, managers from different states showed differences in willingness to implement these novel strategies. Managers from Montana specifically showed less willingness to implement an M_{YY} strategy than other states but more willingness to implement a genetic rescue strategy as long as the fitness

consequences to the cutthroat trout population were minimal. This suggests a difference in culture between different states, with some managers stating they were waiting to see the results from other states before considering implementation. Other managers indicated their state was already implementing these strategies and therefore they don't have any hesitation in continuing to implement them. Cultural influence could also be seen in some of the open-ended comments from managers. Some managers indicated that they were more likely to use established, proven methods first before resorting to these novel methods. This could be seen particularly with the M_{YY} scenarios where many managers indicated they were more likely to use rotenone to remove brook trout than implement an M_{YY} eradication strategy. Culture within an organization can be difficult and slow to change, which can lead to resistance to new ideas (Kurtz 2003). Broader implementation of these management strategies in some of these organizations may take a long time if a change in culture is necessary.

Socio-political elements were also an important indicator of whether a manager would be willing to implement either of these strategies for native conservation management. Many managers indicated a hesitation to use these novel methods, not because of concern about the actual method, but because of external elements that also impact management decisions, including funding, socio-political aspects, stakeholders, permits, time, and other co-managers or partner agencies. This result is consistent with the agency PCI, where managers from different agency types showed differences in terms of their willingness to implement novel strategies. The biggest differences were found between government (state and federal) agencies and private, non-profit, and tribal agencies. Managers from government agencies are beholden to different entities and have different sources of funding than managers from private, tribal, or non-profit agencies. Some managers indicated that with the proper funding, they would use these methods for native species management. Others indicated that these hurdles of project implementation would likely inhibit any ability to incorporate genetic rescue or M_{YY} into their management strategy. Therefore, while managers may be willing to use these novel methods, incorporating them into management decisions is complicated and these external socio-political elements are important influences on a manager's decision-making process.

Risk propensity was shown to be a good indicator of managers' willingness to implement novel strategies. Managers categorized as risk-takers indicated a higher willingness to implement both an M_{YY} eradication strategy and a genetic rescue strategy in all scenarios than managers

categorized as risk-averse. Many managers stated that these populations are already imperiled and therefore there is nothing to lose by implementing these methods, while others indicated that because they were imperiled, they couldn't risk implementing something that was untested. Though we understand that a manager's decision-making is complex and involves many different factors, this result suggests that a manager's risk tolerance can impact overall conservation outcomes by influencing the types of management actions they are willing to implement. This is the first study to our knowledge to show that a manager's risk tolerance impacts their willingness to implement certain management strategies and could be a key to understanding how and why novel methods of conservation management are used.

Our results show that understanding the individual characteristics of managers is important for identifying factors that hinder the implementation of novel methods into the conservation of species. Changes in culture, external socio-political factors, and individual manager attitudes may need to occur before novel methods may be used. Most managers disagreed that it was never ok to use M_{YY}'s or genetic rescue as a management tool, suggesting a positive attitude towards the use of these novel methods. However, the overall lack of certainty in implementing these methods for the different scenarios suggests that the characteristics of managers may override the ability to implement novel strategies. Though it is noted that none of these results is likely to impact a manager's decision-making in isolation, these results provide a more comprehensive understanding of the complex decision-making that managers trying to conserve native species must go through.

Table 5-1. Mean and standard deviation (SD) of attitude questions for all respondents. Attitude questions were asked on a 5-point scale of strongly disagree (1) to strongly agree (5).

Question	Mean	SD
Brook trout are a major threat to native cutthroat	4.01	0.81
Current management of cutthroat trout populations in my state is excessive	2.15	0.81
Current management of cutthroat trout populations in my state is lacking	2.97	0.98
I need more information regarding the use of genetic rescue to consider using it as a management approach to conserve native species	3.48	1.03
I need more information regarding the use of YYs to consider using it as a management approach to conserve native species	3.48	1.07
Inbreeding is a cause of extinctions of native fish populations	3.45	0.81
It is acceptable to have inbred populations of native cutthroat trout	2.74	0.87
It is more important to focus on non-native fish management than native fish management	2.15	0.86
It is never ok to use biotechnology when managing for native species conservation	1.96	0.69
It is never ok to use genetic rescue as a method for conserving native populations of cutthroat trout	1.68	0.66
It is never ok to use YYs as a method to remove non-native trout in order to conserve native populations of cutthroat trout	1.69	0.63
Non-native fish are a cause of extinctions of native fish populations	4.09	0.84
Public opinion influences my willingness to accept genetic rescue as a conservation tool	2.13	0.91
Public opinion influences my willingness to accept YYs as a conservation tool	2.22	0.96
Rainbow trout hybridization is a major threat to native cutthroat	4.10	0.83
How familiar on a scale of not at all familiar to extremely familiar are you with genetic rescue?	3.08	0.83
How familiar on a scale of not at all familiar to extremely familiar are you with YY implementation?	2.98	0.89

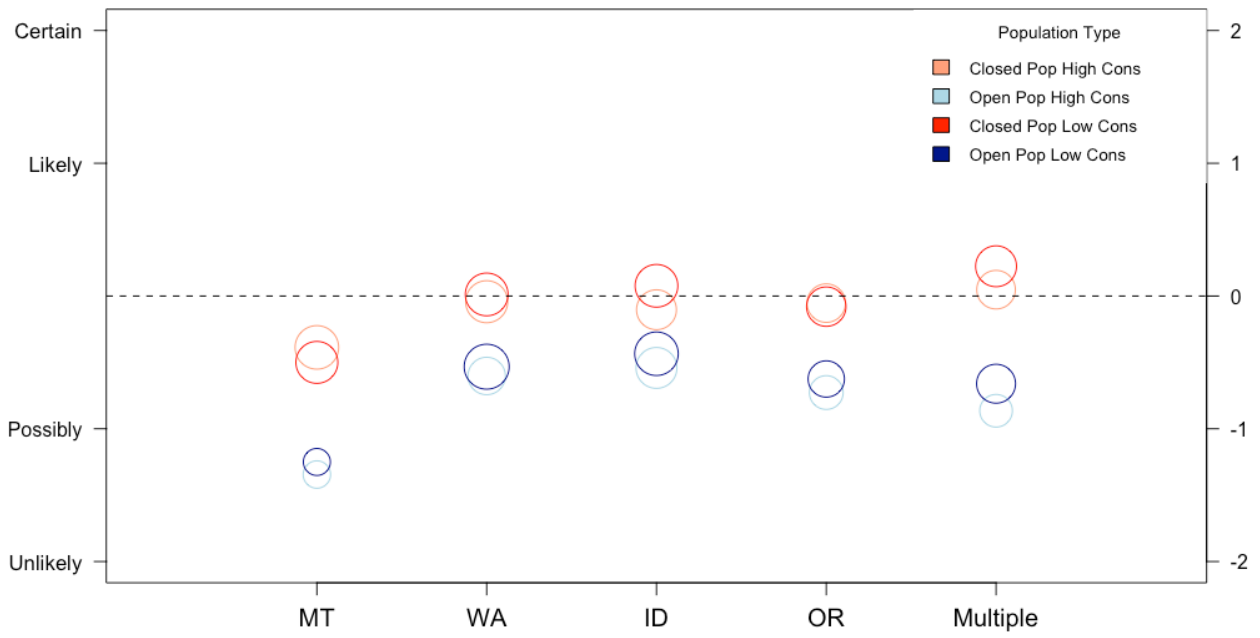


Figure 5-1. PCI results for the likelihood of managers from different states implementing an M_{YY} management approach based on whether the cutthroat trout population is a high conservation priority closed population (closed pop high cons), a high conservation priority open population (open pop high cons), a low conservation priority closed population (closed pop low cons), or a low conservation priority open population (open pop low cons). In PCI graphs placement along the Y-axis indicates the mean of the response within the group, size of the circle indicates the spread of the data. Values within the circles range from 0 to 1, where 0 indicates agreement among all respondents and 1 indicates no agreement.

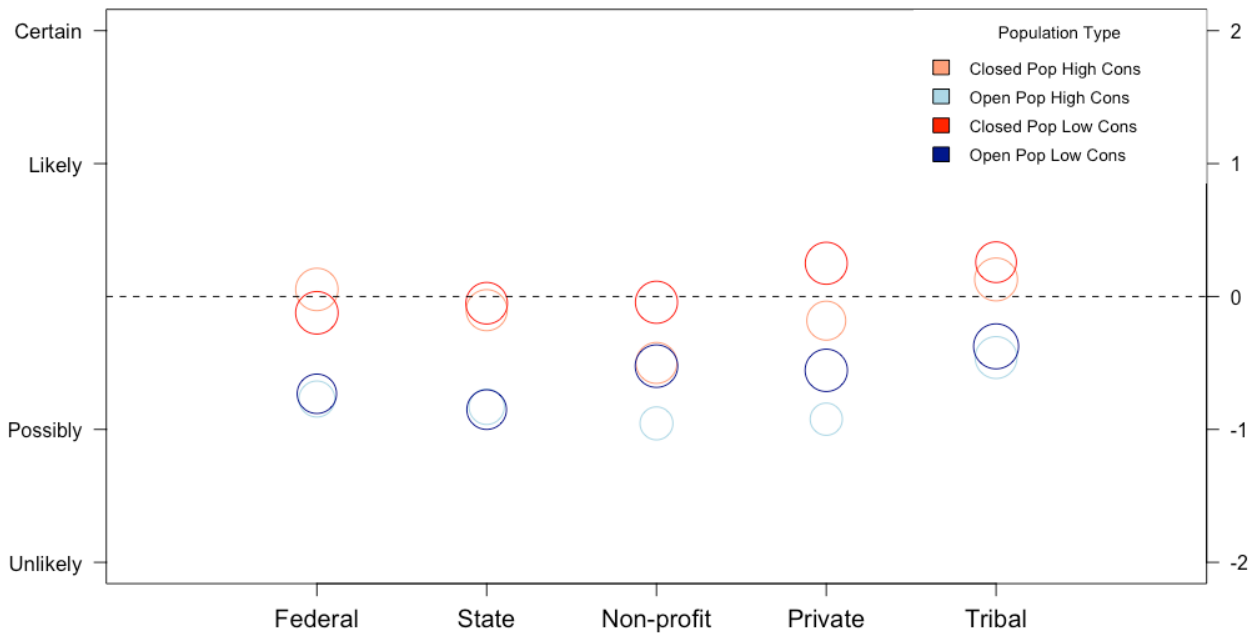


Figure 5-2. PCI results for the likelihood of managers from different agency types implementing an M_{YY} management approach based on whether the cutthroat trout population is a high conservation priority closed population (closed pop high cons), a high conservation priority open population (open pop high cons), a low conservation priority closed population (closed pop low cons), or a low conservation priority open population (open pop low cons).

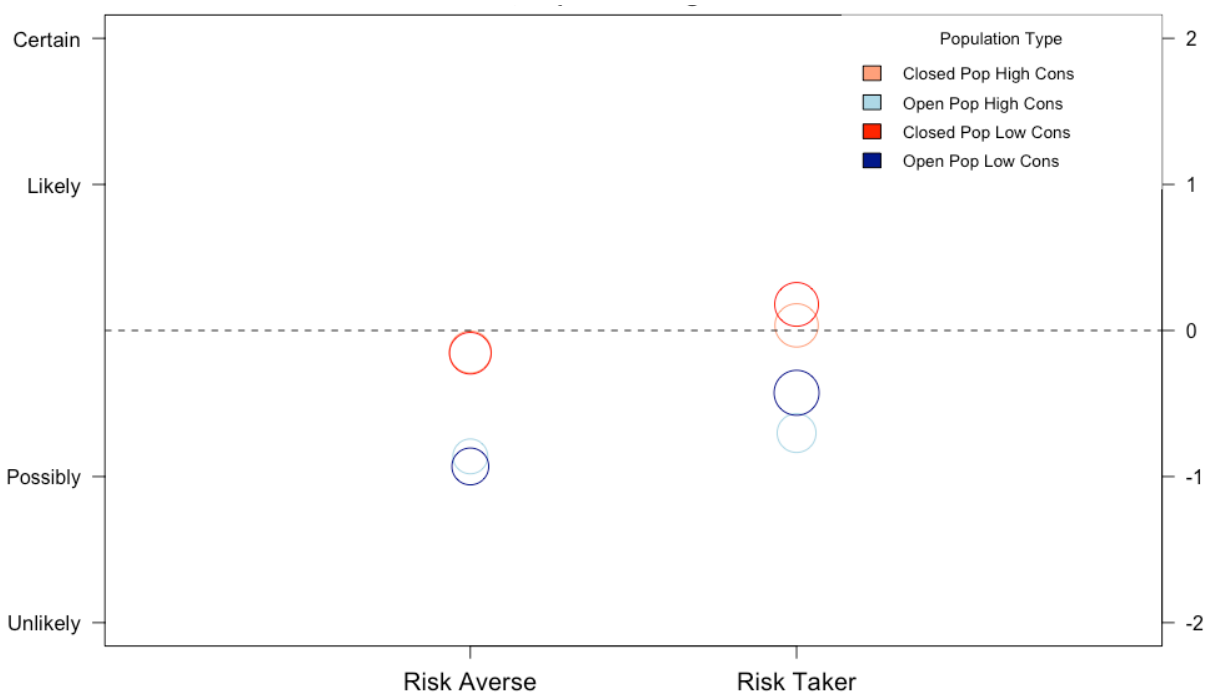


Figure 5-3. PCI results for the likelihood of managers that were categorized as risk-averse or risk-takers implementing an M_{YY} management approach based on whether the cutthroat trout population is a high conservation priority closed population (closed pop high cons), a high conservation priority open population (open pop high cons), a low conservation priority closed population (closed pop low cons), or a low conservation priority open population (open pop low cons).

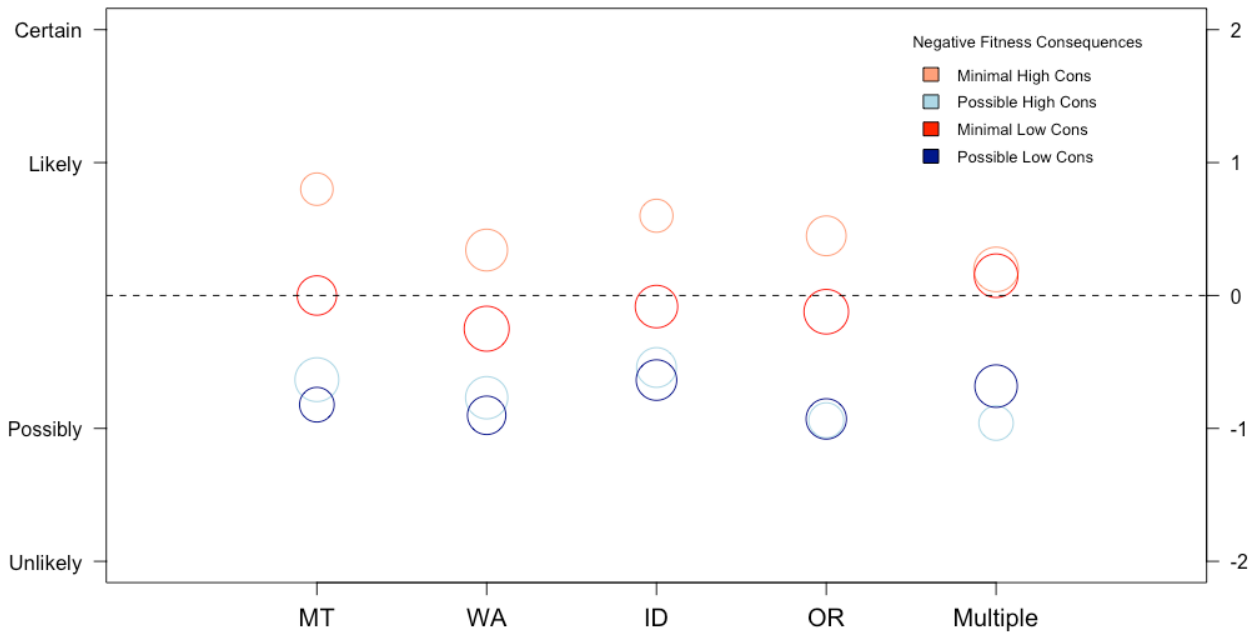


Figure 5-4. PCI results for the likelihood of managers from different states implementing a genetic rescue management approach based on whether the negative fitness consequences of the translocation are minimal in a population that is a high conservation priority population (minimal high cons), possible in a population that is a high conservation priority population (possible high cons), minimal in a population that is a low conservation priority population (minimal low cons), or possible in a population that is a low conservation priority population (possible low cons).

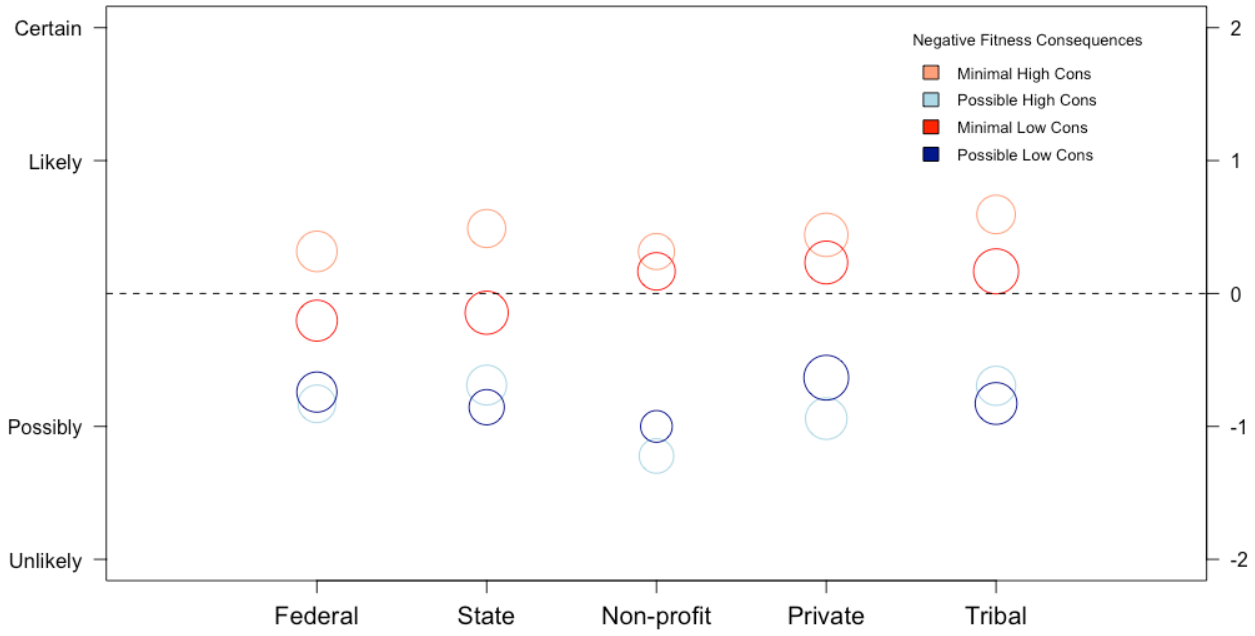


Figure 5-5. PCI results for the likelihood of managers from different agency types implementing a genetic rescue management approach based on whether the negative fitness consequences of the translocation are minimal in a population that is a high conservation priority population (minimal high cons), possible in a population that is a high conservation priority population (possible high cons), minimal in a population that is a low conservation priority population (minimal low cons), or possible in a population that is a low conservation priority population (possible low cons).

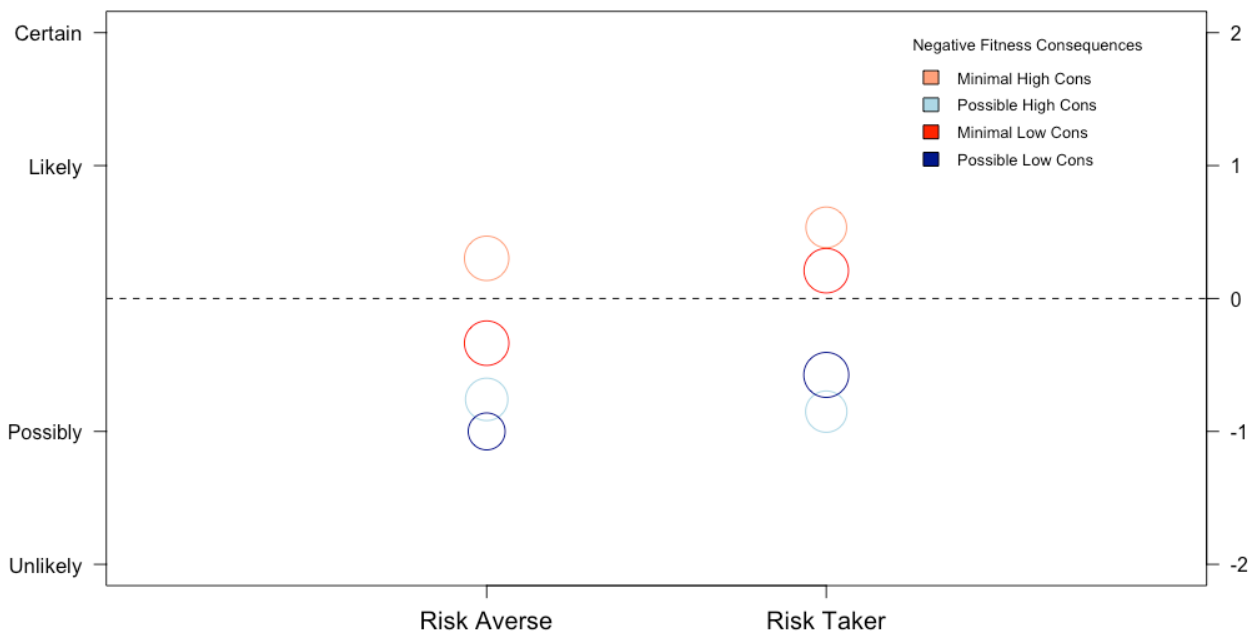


Figure 5-6. PCI results for the likelihood of managers that were categorized as risk-averse or risk-takers implementing a genetic rescue management approach based on whether the negative fitness consequences of the translocation are minimal in a population that is a high conservation priority population (minimal high cons), possible in a population that is a high conservation priority population (possible high cons), minimal in a population that is a low conservation priority population (minimal low cons), or possible in a population that is a low conservation priority population (possible low cons).

REFERENCES

Allendorf, F.W., Leary, R.F., Spruell, P., and Wenburg, J.K., 2001. The problems with hybrids: setting conservation guidelines. Trends in ecology & evolution 16, 613–622.

Allendorf, F.W., Luikart, G.H., and Aitken, S.N., 2012. Conservation and the genetics of populations. John Wiley & Sons.

Ali, O.A., O'Rourke, S.M., Amish, S.J., Meek, M.H., Luikart, G., Jeffres, C., and Miller, M.R., 2016. RAD capture (Rapture): flexible and efficient sequence-based genotyping. Genetics 202, 389–400.

Araki, H., Berejikian, B.A., Ford, M.J., and Blouin, M.S., 2008. Fitness of hatchery-reared salmonids in the wild. Evolutionary applications 1, 342–355.

Archer, F.I., Adams, P.E., and Schneiders, B.B., 2017. stratag: An r package for manipulating, summarizing and analysing population genetic data. Molecular Ecology Resources 17, 5–11.

Armstrong, B.A., Caldwell, C.A., Ruhl, M.E., and Bohling, J.H., 2022. Streamwide Evaluation of Survival and Reproduction of MYY and Wild Brook Trout Populations. North American Journal of Fisheries Management 42, 1398–1413.

Armstrong, D.P., and Cassey, P., 2007. Estimating the effect of inbreeding on survival. Animal Conservation 10, 487–492.

Bearlin, A. R., and Simmons, R. K. 2015. Estimation of Salmonid densities in the three watershed tributaries to Boundary Reservoir in Eastern Washington, USA. Seattle, WA.

Behnke, R.J., 1979. The native trouts of the genus Salmo of western North America. Report to US Fish and Wildlife Service, Denver, Colorado.

Beirão, J., Egeland, T.B., Purchase, C.F., and Nordeide, J.T., 2019. Fish sperm competition in hatcheries and between wild and hatchery origin fish in nature. Theriogenology 133, 201–209.

Bell, D.A., Robinson, Z.L., Funk, W.C., Fitzpatrick, S.W., Allendorf, F.W., Tallmon, D.A., and Whiteley, A.R., 2019. The exciting potential and remaining uncertainties of genetic rescue. Trends in Ecology & Evolution 34, 1070–1079.

Bell, D.A., Kovach, R.P., Muhlfeld, C.C., Al-Chokhachy, R., Cline, T.J., Whited, D.C., Schmetterling, D.A., Lukacs, P.M., and Whiteley, A.R., 2021. Climate change and expanding invasive species drive widespread declines of native trout in the northern Rocky Mountains, USA. Science Advances 7, eabj5471.

Bérénos, C., Ellis, P.A., Pilkington, J.G., and Pemberton, J.M., 2016. Genomic analysis reveals depression due to both individual and maternal inbreeding in a free-living mammal population. Molecular ecology 25, 3152–3168.

Bobe, J., 2015. Egg quality in fish: Present and future challenges. Animal Frontiers 5, 66–72.

Bongers, A., Zandieh-Doulabi, B., Richter, C.J., and Komen, J., 1999. Viable androgenetic YY genotypes of common carp (Cyprinus carpio L.). Journal of Heredity 90, 195–198.

Britton, J.R., and Brazier, M., 2006. Eradicating the invasive topmouth gudgeon, Pseudorasbora parva, from a recreational fishery in northern England. Fisheries Management and Ecology 13, 329–335.

Britton, J.R., Gozlan, R.E., and Copp, G.H., 2011. Managing non-native fish in the environment. Fish and Fisheries 12, 256–274.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., and Bolker, B.M., 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. The R Journal 9, 378–400

Buckley, M.R., Ferris, G.R., Bernardin, H.J., and Harvey, M.G., 1998. The disconnect between the science and practice of management. Business horizons 41, 31–38.

Buckwalter, J.D., Frimpong, E.A., Angermeier, P.L., and Barney, J.N., 2018. Seventy years of stream-fish collections reveal invasions and native range contractions in an Appalachian (USA) watershed. Diversity and Distributions 24, 219–232.

Buktenica, M.W., Hering, D.K., Girdner, S.F., Mahoney, B.D., and Rosenlund, B.D., 2013. Eradication of nonnative Brook Trout with electrofishing and antimycin-A and the response of a remnant Bull Trout population. North American Journal of Fisheries Management 33, 117–129.

Cambray, J.A., 2003. Impact on indigenous species biodiversity caused by the globalisation of alien recreational freshwater fisheries. Hydrobiologia 500, 217–230.

Campbell, N.R., Harmon, S.A., and Narum, S.R., 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. Molecular ecology resources 15, 855–867.

Campton, D.E., 2004. Sperm competition in salmon hatcheries: the need to institutionalize genetically benign spawning protocols. Transactions of the American Fisheries Society 133, 1277–1289.

Casselman, S.J., Schulte-Hostedde, A.I., and Montgomerie, R., 2006. Sperm quality influences male fertilization success in walleye (Sander vitreus). Canadian Journal of Fisheries and Aquatic Sciences 63, 2119–2125.

Christie, M.R., French, R.A., Marine, M.L., and Blouin, M.S., 2014. How much does inbreeding contribute to the reduced fitness of hatchery-born steelhead (Oncorhynchus mykiss) in the wild? Journal of Heredity 105, 111–119.

Clark, L.B., González, E., Henry, A.L., and Sher, A.A., 2020. A Solution to Treat Mixed-Type Human Datasets from Socio-Ecological Systems. Journal of Environmental Geography 13, 51–60.

Clavero, M., and García-Berthou, E., 2006. Homogenization dynamics and introduction routes of invasive freshwater fish in the Iberian Peninsula. Ecological Applications 16, 2313–2324.

Cockburn, A., Peñalba, J.V., Jaccoud, D., Kilian, A., Brouwer, L., Double, M.C., Margraf, N., Osmond, H.L., Kruuk, L.E., and van de Pol, M., 2021. hiphop: Improved paternity assignment among close relatives using a simple exclusion method for biallelic markers. Molecular Ecology Resources 21, 1850–1865.

Cook, C.N., and Sgrò, C.M., 2019. Conservation practitioners' understanding of how to manage evolutionary processes. Conservation Biology 33, 993–1001.

Coster, A., and Coster, M.A., 2010. Package 'pedigree.' R package version 1.

Cotton, S., and Wedekind, C., 2007. Control of introduced species using Trojan sex chromosomes. Trends in ecology & evolution 22, 441–443.

Crawford, B.A, 1979. The origin and history of the trout brood stocks of the Washington Department of Game. Washington State Game Department. Fisheries Research Report, Olympia.

Crooks, J.A., Soulé, M.E., and Sandlund, O.T., 1999. Lag times in population explosions of invasive species: causes and implications. Invasive species and biodiversity management 24, 103–125.

Crooks, J.A., 2005. Lag times and exotic species: The ecology and management of biological invasions in slow-motion1. Ecoscience 12, 316–329.

Crystal-Ornelas, R., and Lockwood, J.L., 2020. Cumulative meta-analysis identifies declining but negative impacts of invasive species on richness after 20 yr. Ecology 101, e03082.

Day, C.C., Landguth, E.L., Bearlin, A., Holden, Z.A., and Whiteley, A.R., 2018. Using simulation modeling to inform management of invasive species: A case study of eastern brook trout suppression and eradication. Biological Conservation 221, 10–22.

Day, C.C., Landguth, E.L., Simmons, R.K., Baker, W.P., Whiteley, A.R., Lukacs, P.M., and Bearlin, A., 2020. Simulating effects of fitness and dispersal on the use of Trojan sex chromosomes for the management of invasive species. Journal of Applied Ecology 57, 1413–1425.

Day, C.C., Landguth, E.L., Simmons, R.K., Baker, W.P., Whiteley, A.R., Lukacs, P.M., Davenport, K.A., and Bearlin, A.R., 2021. Evaluation of management factors affecting the relative success of a brook trout eradication program using YY male fish and electrofishing suppression. Canadian Journal of Fisheries and Aquatic Sciences 78, 1109–1119.

Day, C.C., Landguth, E.L., Simmons, R.K., and Bearlin, A.R., CDMetaPOP2: a multispecies, eco-evolutionary framework for landscape demogenetics and connectivity. Ecography *in press*.

Decker, D.J., and Chase, L.C., 1997. Human dimensions of living with wildlife: A management challenge for the 21st century. Wildlife Society Bulletin 788–795.

Dickerson, B.R., Brinck, K.W., Willson, M.F., Bentzen, P., and Quinn, T.P., 2005. Relative importance of salmon body size and arrival time at breeding grounds to reproductive success. Ecology 86, 347–352.

Dillman, D.A., 2011. Mail and Internet surveys: The tailored design method--2007 Update with new Internet, visual, and mixed-mode guide. John Wiley & Sons.

Donaldson, L.A., and Cooke, S.J., 2016. The effectiveness of non-native fish eradication techniques in freshwater ecosystems: a systematic review protocol. Environmental Evidence 5, 1–10.

Downs, C.C., White, R.G., and Shepard, B.B., 1997. Age at sexual maturity, sex ratio, fecundity, and longevity of isolated headwater populations of westslope cutthroat trout. North American Journal of Fisheries Management 17, 85-92.

Dunham, J.B., Adams, S.B., Schroeter, R.E., and Novinger, D.C., 2002. Alien invasions in aquatic ecosystems: toward an understanding of brook trout invasions and potential impacts on inland cutthroat trout in western North America. Reviews in Fish Biology and Fisheries 12, 373–391.

Edmands, S., 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular ecology 16, 463–475.

Edwards, A., 1971. Distances between populations on the basis of gene frequencies. Biometrics 873–881.

Emlen, J.M., 1991. Heterosis and outbreeding depression: a multi-locus model and an application to salmon production. Fisheries Research 12, 187–212.

Enck, J.W., and Decker, D.J., 1997. Examining assumptions in wildlife management: A contribution of human dimensions inquiry. Human Dimensions of Wildlife 2, 56-72.

Facon, B., Pointier, J.-P., Jarne, P., Sarda, V., and David, P., 2008. High genetic variance in life-history strategies within invasive populations by way of multiple introductions. Current biology 18, 363–367.

Fantle-Lepczyk, J.E., Haubrock, P.J., Kramer, A.M., Cuthbert, R.N., Turbelin, A.J., Crystal-Ornelas, R., Diagne, C., and Courchamp, F., 2022. Economic costs of biological invasions in the United States. Science of the Total Environment 806, 151318.

Fausch, K.D., Rieman, B.E., Dunham, J.B., Young, M.K., and Peterson, D.P., 2009. Invasion versus isolation: trade-offs in managing native salmonids with barriers to upstream movement. Conservation Biology 23, 859–870.

Finlayson, B.J., Schnick, R.A., Cailteux, R.L., DeMong, L., Horton, W.D., McClay, W., Thompson, C.W., and Tichacek, G., 2000. Rotenone use in fisheries management: administrative and technical guidelines manual. American Fisheries Society.

Fleming, I.A., and Gross, M.R., 1993. Breeding success of hatchery and wild coho salmon (Oncorhynchus kisutch) in competition. Ecological Applications 3, 230–245.

Frankham, R., 2005. Genetics and extinction. Biological Conservation 126, 131–140.

Frankham, R., 2015. Genetic rescue of small inbred populations: Meta-analysis reveals large and consistent benefits of gene flow. Molecular ecology 24, 2610–2618.

Friggens, M., Raish, C., Finch, D., and McSweeney, A., 2015. The influence of personal belief, agency mission and city size on open space decision making processes in three southwestern cities. Urban Ecosystems 18, 577–598.

Fuller, A.K., Decker, D.J., Schiavone, M.V., and Forstchen, A.B., 2020. Ratcheting up rigor in wildlife management decision making. Wildlife Society Bulletin 44, 29–41.

Fuller, P., and Neilson, M., 2019. Salvelinus fontinalis (Mitchill, 1814): U.S. Geological Survey, Nonindigenous Aquatic Species Database [WWW Document]. URL https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=939 (accessed 3/20/23).

Geissinger, E.A., Gregory, R.S., Laurel, B.J., and Snelgrove, P.V., 2021. Food and initial size influence overwinter survival and condition of a juvenile marine fish (age-0 Atlantic cod). Canadian Journal of Fisheries and Aquatic Sciences 78, 472–482.

Gelman, A., and Hill, J., 2006. Data analysis using regression and multilevel/hierarchical models. Cambridge university press.

Gharrett, A.J., Smoker, W.W., Reisenbichler, R.R., and Taylor, S.G., 1999. Outbreeding depression in hybrids between odd-and even-broodyear pink salmon. Aquaculture 173, 117–129.

Gore, M.L., Wilson, R.S., Siemer, W.F., Wieczorek Hudenko, H., Clarke, C.E., Sol Hart, P., Maguire, L.A., and Muter, B.A., 2009. Application of risk concepts to wildlife management: Special issue introduction. Human Dimensions of Wildlife 14, 301–313.

Goudet, J., 2005. Hierfstat, a package for R to compute and test hierarchical F-statistics. Molecular ecology notes 5, 184–186.

Gozlan, R.E., 2008. Introduction of non-native freshwater fish: is it all bad? Fish and Fisheries 9, 106–115.

Gozlan, R.E., Britton, J.R., Cowx, I., and Copp, G.H., 2010. Current knowledge on non-native freshwater fish introductions. Journal of fish biology 76, 751–786.

Gunckel, S.L., Hemmingsen, A.R., and Li, J.L., 2002. Effect of bull trout and brook trout interactions on foraging habitat, feeding behavior, and growth. Transactions of the American Fisheries Society 131, 1119–1130.

Gutierrez, J.B., and Teem, J.L., 2006. A model describing the effect of sex-reversed YY fish in an established wild population: the use of a Trojan Y chromosome to cause extinction of an introduced exotic species. Journal of theoretical biology 241, 333–341.

Gutierrez, J.B., Hurdal, M.K., Parshad, R.D., and Teem, J.L., 2012. Analysis of the Trojan Y chromosome model for eradication of invasive species in a dendritic riverine system. Journal of mathematical biology 64, 319–340.

Hedrick, P.W., Peterson, R.O., Vucetich, L.M., Adams, J.R., and Vucetich, J.A., 2014. Genetic rescue in Isle Royale wolves: genetic analysis and the collapse of the population. Conservation Genetics 15, 1111–1121.

Helfman, G.S., 2007. Fish conservation: a guide to understanding and restoring global aquatic biodiversity and fishery resources. Island Press.

Hoxmeier, R.J.H., Dieterman, D.J., and Miller, L.M., 2015. Brook Trout distribution, genetics, and population characteristics in the Driftless Area of Minnesota. North American Journal of Fisheries Management 35, 632–648.

Huisman, J., Kruuk, L.E., Ellis, P.A., Clutton-Brock, T., and Pemberton, J.M., 2016. Inbreeding depression across the lifespan in a wild mammal population. Proceedings of the National Academy of Sciences 113, 3585–3590.

Hung, K.-T., and Tangpong, C., 2010. General risk propensity in multifaceted business decisions: Scale development. Journal of Managerial Issues 88–106.

Hürlimann, C., 2019. Valuation of Renewable Energy Investments: Practices Among German and Swiss Investment Professionals. Springer.

Jelks, H.L., Walsh, S.J., Burkhead, N.M., Contreras-Balderas, S., Diaz-Pardo, E., Hendrickson, D.A., Lyons, J., Mandrak, N.E., McCormick, F., and Nelson, J.S., 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33, 372–407.

Jeschke, J.M., Bacher, S., Blackburn, T.M., Dick, J.T., Essl, F., Evans, T., Gaertner, M., Hulme, P.E., Kühn, I., and Mrugała, A., 2014. Defining the impact of non-native species. Conservation Biology 28, 1188–1194.

Johnson, J.A., and Dunn, P.O., 2006. Low genetic variation in the heath hen prior to extinction and implications for the conservation of prairie-chicken populations. Conservation Genetics 7, 37–48.

Jombart, T., Devillard, S., and Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC genetics 11, 1–15.

Jones, O.R., and Wang, J., 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular ecology resources 10, 551–555.

Kallis, J.L., and Marschall, E.A., 2014. How body size and food availability influence first-winter growth and survival of a stocked piscivore. Transactions of the American Fisheries Society 143, 1434–1444.

Kamvar, Z.N., Tabima, J.F., and Grünwald, N.J., 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2, e281.

Kanda, N., Leary, R.F., and Allendorf, F.W., 2002. Evidence of introgressive hybridization between bull trout and brook trout. Transactions of the American Fisheries Society 131, 772–782.

Kanno, Y., Vokoun, J.C., and Letcher, B.H., 2011. Fine-scale population structure and riverscape genetics of brook trout (Salvelinus fontinalis) distributed continuously along headwater channel networks. Molecular ecology 20, 3711–3729.

Kanno, Y., Letcher, B.H., Coombs, J.A., Nislow, K.H., and Whiteley, A.R., 2014. Linking movement and reproductive history of brook trout to assess habitat connectivity in a heterogeneous stream network. Freshwater Biology 59, 142–154.

Karas, Nick, 1997. Brook Trout. Lyons and Buford, New York.

Kardos, M., Luikart, G., and Allendorf, F.W., 2015. Measuring individual inbreeding in the age of genomics: marker-based measures are better than pedigrees. Heredity 115, 63–72.

Kardos, M., Armstrong, E.E., Fitzpatrick, S.W., Hauser, S., Hedrick, P.W., Miller, J.M., Tallmon, D.A., and Funk, W.C., 2021. The crucial role of genome-wide genetic variation in conservation. Proceedings of the National Academy of Sciences 118, e2104642118.

Kazyak, D.C., Lubinski, B.A., Kulp, M.A., Pregler, K.C., Whiteley, A.R., Hallerman, E., Coombs, J.A., Kanno, Y., Rash, J.M., and Morgan, R.P., 2022. Population Genetics of Brook Trout in the Southern Appalachian Mountains. Transactions of the American Fisheries Society 151, 127–149.

Kennedy, B.M., Peterson, D.P., and Fausch, K.D., 2003. Different life histories of brook trout populations invading mid-elevation and high-elevation cutthroat trout streams in Colorado. Western North American Naturalist 215–223.

Kennedy, P., Schill, D.J., Meyer, K.A., Campbell, M.R., Vu, N., and Hansen, M.J., 2017. Production and evaluation of YY-Male brook trout to nradicate Nonnative wild brook trout populations. Presented at the Proceedings of the Wild Trout Symposium XII—Science, Politics, and Wild Trout Management: Who's Driving and Where Are We Going, pp. 251–260.

Kennedy, P.A., Meyer, K.A., Schill, D.J., Campbell, M.R., and Vu, N.V., 2018. Survival and reproductive success of hatchery YY male Brook Trout stocked in Idaho streams. Transactions of the American Fisheries Society 147, 419–430.

Kloempken, K., 1996. Washington Department of Game planting records. Washington Dept.of Game, Olympia, Wash.

Kurtz, R.S., 2003. Organizational culture, decision-making, and integrity: The National Park Service and the Exxon Valdez. Public Integrity 5, 305–317.

Lahoz-Monfort, J.J., and Magrath, M.J., 2021. A comprehensive overview of technologies for species and habitat monitoring and conservation. Bioscience 71, 1038–1062.

Landguth, E. L., and S. Cushman., 2010. CDPOP: a spatially explicit cost distance population genetics program. Molecular Ecology Resources 10, 156-161.

Landguth, E., Hand, B., Glassy, J., Cushman, S., and Sawaya, M., 2012. UNICOR: a species connectivity and corridor network simulator. Ecography 35:9-14.

Landguth, E.L., Bearlin, A., Day, C.C., and Dunham, J., 2017. CDMetaPOP: an individual-based, eco-evolutionary model for spatially explicit simulation of landscape demogenetics.Methods in Ecology and Evolution, 8 (1), 4–11.

Landguth, E.L., Forester, B.R., Eckert, A.J., Shirk, A.J., Menon, M., Whipple, A., Day, C.C., and Cushman, S.A., 2020. Modelling multilocus selection in an individual-based, spatially-explicit landscape genetics framework. Molecular ecology resources 20, 605–615.

Letcher, B.H., Schueller, P., Bassar, R.D., Nislow, K.H., Coombs, J.A., Sakrejda, K., Morrissey, M., Sigourney, D.B., Whiteley, A.R., and O'Donnell, M.J., 2015. Robust estimates of environmental effects on population vital rates: an integrated capture–recapture model of seasonal brook trout growth, survival and movement in a stream network. Journal of Animal Ecology 84, 337–352.

Likert, R., 1932. A technique for the measurement of attitudes. Archives of psychology.

Little, L.R., Parslow, J., Fay, G., Grafton, R.Q., Smith, A.D., Punt, A.E., and Tuck, G.N., 2014. Environmental derivatives, risk analysis, and conservation management. Conservation Letters 7, 196–207.

Liu, H., Guan, B., Xu, J., Hou, C., Tian, H., and Chen, H., 2013. Genetic manipulation of sex ratio for the large-scale breeding of YY super-male and XY all-male yellow catfish (Pelteobagrus fulvidraco (Richardson)). Marine biotechnology 15, 321–328.

Lockwood, J.L., Cassey, P., and Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. Trends in ecology & evolution 20, 223–228.

Lovich, J.E., and Lovich, R.E., 1996. The decline of native brook trout (Salvelinus fontinalis) populations along the upper west branch of the Susquehanna River: canaries outside the coal mine. Journal of the Pennsylvania Academy of Science 55–60.

Lynch, M., 1991. The genetic interpretation of inbreeding depression and outbreeding depression. Evolution 45, 622–629.

MacCrimmon, H.R., and Campbell, J.S., 1969. World distribution of brook trout, Salvelinus fontinalis. Journal of the Fisheries Board of Canada 26, 1699–1725.

MacCrimmon, H.R., Gots, B.L., and Campbell, J.S., 1971. World distribution of brook trout, Salvelinus fontinalis: further observations. Journal of the Fisheries Board of Canada 28, 452–456.

Mair, G.C., Abucay, J.S., Abella, T.A., Beardmore, J.A., and Skibinski, D., 1997. Genetic manipulation of sex ratio for the large-scale production of all-male tilapia Oreochromis niloticus. Canadian Journal of Fisheries and Aquatic Sciences 54, 396–404.

Manchester, S.J., and Bullock, J.M., 2000. The impacts of non-native species on UK biodiversity and the effectiveness of control. Journal of Applied Ecology 37, 845–864.

Manfredo, M., Vaske, J., and Teel, T., 2003. The potential for conflict index: A graphic approach to practical significance of human dimensions research. Human Dimensions of Wildlife 8, 219–228.

McClelland, E.K., and Naish, K.A., 2007. What is the fitness outcome of crossing unrelated fish populations? A meta-analysis and an evaluation of future research directions. Conservation Genetics 8, 397.

McEachern, M.B., Van Vuren, D.H., Floyd, C.H., May, B., and Eadie, J.M., 2011. Bottlenecks and rescue effects in a fluctuating population of golden-mantled ground squirrels (Spermophilus lateralis). Conservation Genetics 12, 285–296.

McGinnity, P., Prodöhl, P., Ferguson, A., Hynes, R., Maoiléidigh, N. ó, Baker, N., Cotter, D., O'Hea, B., Cooke, D., and Rogan, G., 2003. Fitness reduction and potential extinction of wild

populations of Atlantic salmon, Salmo salar, as a result of interactions with escaped farm salmon. Proceedings of the Royal Society of London.Series B: Biological Sciences 270, 2443–2450.

Meyer, K.A., Lamansky Jr, J.A., and Schill, D.J., 2006. Evaluation of an unsuccessful brook trout electrofishing removal project in a small Rocky Mountain stream. North American Journal of Fisheries Management 26, 849–860.

Miller, L.M., Close, T., and Kapuscinski, A.R., 2004. Lower fitness of hatchery and hybrid rainbow trout compared to naturalized populations in Lake Superior tributaries. Molecular ecology 13, 3379–3388.

Miller, R.R., Williams, J.D., and Williams, J.E., 1989. Extinctions of North American fishes during the past century. Fisheries 14, 22–38.

Mollot, G., Pantel, J.H., and Romanuk, T.N., 2017. The effects of invasive species on the decline in species richness: a global meta-analysis, Advances in ecological research. Elsevier.

Mooney, H.A., and Cleland, E.E., 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences 98, 5446–5451.

Narum, S.R., Gallardo, P., Correa, C., Matala, A., Hasselman, D., Sutherland, B.J., and Bernatchez, L., 2017. Genomic patterns of diversity and divergence of two introduced salmonid species in Patagonia, South America. Evolutionary Applications 10, 402–416.

Nei, M., 1972. Genetic distance between populations. The American Naturalist 106, 283–292.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.

Neville, H.M., and Bernatchez, L., 2013. Coding gene single nucleotide polymorphism population genetics of nonnative Brook Trout: the ghost of introductions past. Transactions of the American Fisheries Society 142, 1215–1231.

Nietlisbach, P., Keller, L.F., Camenisch, G., Guillaume, F., Arcese, P., Reid, J.M., and Postma, E., 2017. Pedigree-based inbreeding coefficient explains more variation in fitness than heterozygosity at 160 microsatellites in a wild bird population. Proceedings of the Royal Society B: Biological Sciences 284, 20162763.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., and Wagner, H., 2013. Package 'vegan.' Community ecology package, version 2, 1–295.

Pejchar, L., and Mooney, H.A., 2009. Invasive species, ecosystem services and human well-being. Trends in ecology & evolution 24, 497–504.

Peterson, D.P., Fausch, K.D., and White, G.C., 2004. Population ecology of an invasion: effects of brook trout on native cutthroat trout. Ecological Applications 14, 754–772.

Peterson, D.P., Fausch, K.D., Watmough, J., and Cunjak, R.A., 2008. When eradication is not an option: modeling strategies for electrofishing suppression of nonnative brook trout to foster persistence of sympatric native cutthroat trout in small streams. North American Journal of Fisheries Management 28, 1847–1867.

Pimentel, D., Zuniga, R., and Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52, 273–288.

Pluess, T., Jarošík, V., Pyšek, P., Cannon, R., Pergl, J., Breukers, A., and Bacher, S., 2012. Which factors affect the success or failure of eradication campaigns against alien species? PloS one 7, e48157.

Pregler, K.C., Kanno, Y., Rankin, D., Coombs, J.A., and Whiteley, A.R., 2018. Characterizing genetic integrity of rear-edge trout populations in the southern Appalachians. Conservation Genetics 19, 1487–1503.

Primack, R.B., Sher, A.A., Maas, B., and Adams, V.M., 2021. Manager characteristics drive conservation success. Biological Conservation 259, 109169.

Pritchard, J.K., Stephens, M., and Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.

Puechmaille, S.J., 2016. The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Molecular ecology resources 16, 608–627.

Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T., Dawson, W., Essl, F., Foxcroft, L.C., and Genovesi, P., 2020. Scientists' warning on invasive alien species. Biological Reviews 95, 1511–1534.

R2 Resource Consultants, I. 2014. Boundary Hydroelectric Project Tributary Management Plan. License Article 404, Prepared for: City of Seattle, Seattle City Light.

Rahel, F.J., and Olden, J.D., 2008. Assessing the effects of climate change on aquatic invasive species. Conservation Biology 22, 521–533.

Ribeiro, F., and Leunda, P.M., 2012. Non-native fish impacts on Mediterranean freshwater ecosystems: current knowledge and research needs. Fisheries Management and Ecology 19, 142–156.

Ricciardi, A., and MacIsaac, H.J., 2011. Impacts of biological invasions on freshwater ecosystems. Fifty years of invasion ecology: the legacy of Charles Elton 1, 211–224.

Rieman, B.E., Peterson, J.T., and Myers, D.L., 2006. Have brook trout displaced bull trout along longitudinal gradients in central Idaho streams. Canadian Journal of Fisheries and Aquatic Sciences 63, 63–78.

Robinson, Z.L., Coombs, J.A., Hudy, M., Nislow, K.H., Letcher, B.H., and Whiteley, A.R., 2017. Experimental test of genetic rescue in isolated populations of brook trout. Molecular ecology 26, 4418–4433.

Rousset, F., 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular ecology resources 8, 103–106.

Rytwinski, T., Taylor, J.J., Donaldson, L.A., Britton, J.R., Browne, D.R., Gresswell, R.E., Lintermans, M., Prior, K.A., Pellatt, M.G., and Vis, C., 2019. The effectiveness of non-native fish removal techniques in freshwater ecosystems: a systematic review. Environmental Reviews 27, 71–94.

Sala, O.E., Stuart Chapin, F., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., and Kinzig, A., 2000. Global biodiversity scenarios for the year 2100. Science 287, 1770–1774.

Salirrosas, D., Leon, J., Arqueros-Avalos, M., Sanchez-Tuesta, L., Rabanal, F., and Prieto, Z., 2017. YY super males have better spermatic quality than XY males in red tilapia Oreochromis niloticus. Scientia Agropecuaria 8, 349–355.

Sammarco, P.W., Porter, S.A., Genazzio, M., and Sinclair, J., 2015. Success in competition for space in two invasive coral species in the western Atlantic–Tubastraea micranthus and T. coccinea. Plos one 10, e0144581.

Schade, C.B., and Bonar, S.A., 2005. Distribution and abundance of nonnative fishes in streams of the western United States. North American Journal of Fisheries Management 25, 1386–1394.

Schill, D.J., Heindel, J.A., Campbell, M.R., Meyer, K.A., and Mamer, E.R., 2016. Production of a YY male brook trout broodstock for potential eradication of undesired brook trout populations. North American Journal of Aquaculture 78, 72–83.

Schill, D.J., Meyer, K.A., and Hansen, M.J., 2017. Simulated effects of YY-male stocking and manual suppression for eradicating nonnative brook trout populations. North American Journal of Fisheries Management 37, 1054–1066.

Schneider, C.A., Rasband, W.S., and Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nature methods 9, 671–675.

Schrieber, K., and Lachmuth, S., 2017. The genetic paradox of invasions revisited: the potential role of inbreeding× environment interactions in invasion success. Biological Reviews 92, 939–952.

Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M., Pagad, S., Pyšek, P., Winter, M., and Arianoutsou, M., 2017. No saturation in the accumulation of alien species worldwide. Nature communications 8, 14435.

Shepard, B.B., Nelson, L.M., Taper, M.L., and Zale, A.V., 2014. Factors influencing successful eradication of nonnative Brook Trout from four small Rocky Mountain streams using electrofishing. North American Journal of Fisheries Management 34, 988–997.

Sher, A.A., Clark, L., Henry, A.L., Goetz, A.R., González, E., Tyagi, A., Simpson, I., and Bourgeois, B., 2020. The human element of restoration success: Manager characteristics affect vegetation recovery following invasive Tamarix control. Wetlands 40, 1877–1895.

Sitkin, S.B., and Weingart, L.R., 1995. Determinants of risky decision-making behavior: A test of the mediating role of risk perceptions and propensity. Academy of management Journal 38, 1573–1592.

Tallmon, D.A., Luikart, G., and Waples, R.S., 2004. The alluring simplicity and complex reality of genetic rescue. Trends in Ecology & Evolution 19, 489–496.

Teem, J.L., Gutierrez, J.B., and Parshad, R.D., 2014. A comparison of the Trojan Y chromosome and daughterless carp eradication strategies. Biological Invasions 16, 1217–1230.

Thomas, R., Kane, A., and Bierwagen, B.G., 2008. Effects of climate change on aquatic invasive species and implications for management and research.

Thrower, F.P., and Hard, J.J., 2009. Effects of a single event of close inbreeding on growth and survival in steelhead. Conservation Genetics 10, 1299–1307.

Tulloch, A.I., Maloney, R.F., Joseph, L.N., Bennett, J.R., Di Fonzo, M.M., Probert, W.J., O'Connor, S.M., Densem, J.P., and Possingham, H.P., 2015. Effect of risk aversion on prioritizing conservation projects. Conservation Biology 29, 513–524.

United States Department of Agriculture (USDA), 2019. Nonnative Trout. https://www.fs.fed.us/research/invasive-species/fish-aquatic/nonnative-trout.php. Accessed August 6 2019.

United States Department of the Interior, 2021. U.S. Department of the Interior Invasive Species Strategic Plan, Fiscal Years 2021-2025. Washington, D.C

United States Fish and Wildlife Service (USFWS), 1999a. Status Review for Westslope Cutthroat Trout in the United States.

United States Fish and Wildlife Service (USFWS), 1999b. Endangered and threatened wildlife and plants; determination of threatened status for Bull Trout in the coterminous United States; final rule. Federal register 64, 58909–58933.

United States Geological Survey, (USGS), 2023. Nonindigenous Aquatic Species [WWW Document]. URL https://nas.er.usgs.gov/queries/SpeciesList.aspx?Group=Fishes (accessed 3/20/23).

Van Harlow, W., and Brown, K.C., 1990. The role of risk tolerance in the asset allocation process: A new perspective. Research Foundation of the Institute of Chartered Financial Analysts.

Vandewoestijne, S., Schtickzelle, N., and Baguette, M., 2008. Positive correlation between genetic diversity and fitness in a large, well-connected metapopulation. Bmc Biology 6, 1–11.

Vaske, J.J., Needham, M.D., Newman, P., Manfredo, M.J., and Petchenik, J., 2006. Potential for conflict index: Hunters' responses to chronic wasting disease. Wildlife Society Bulletin 34, 44–50.

Vitousek, P.M., 1990. Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. Oikos 7–13.

Von Bertalanffy, L., 1938. A quantitative theory of organic growth (inquiries on growth laws. II). Human biology 10, 181–213.

Walling, C.A., Nussey, D.H., Morris, A., Clutton-Brock, T.H., Kruuk, L.E., and Pemberton, J.M., 2011. Inbreeding depression in red deer calves. BMC Evolutionary Biology 11, 1–13.

Walston, J. 2018. Sullivan Creek non-native fish suppression project, 2017 report, Boundary Hydroelectric Project (FERC No. 2144). Kalispel Tribe of Indians, Usk, WA, USA.

Wang, X., Walton, J.R., and Parshad, R.D., 2016. Stochastic models for the Trojan Y-Chromosome eradication strategy of an invasive species. Journal of biological dynamics 10, 179–199.

Waples, R.S., 2015. Testing for Hardy–Weinberg proportions: have we lost the plot? Journal of heredity 106, 1–19.

Waples, R.S., and Anderson, E.C., 2017. Purging putative siblings from population genetic data sets: a cautionary view. Molecular Ecology 26, 1211-1224.

Ward, J.M., and Ricciardi, A., 2007. Impacts of Dreissena invasions on benthic macroinvertebrate communities: a meta-analysis. Diversity and Distributions 13, 155–165.

Warnock, W.G., and Rasmussen, J.B., 2013. Abiotic and biotic factors associated with brook trout invasiveness into bull trout streams of the Canadian Rockies. Canadian Journal of Fisheries and Aquatic Sciences 70, 905–914.

Westemeier, R.L., Brawn, J.D., Simpson, S.A., Esker, T.L., Jansen, R.W., Walk, J.W., Kershner, E.L., Bouzat, J.L., and Paige, K.N., 1998. Tracking the long-term decline and recovery of an isolated population. Science 282, 1695–1698.

West Fork Environmental, 2012. Salmonid tissue sampling in the Boundary Hydroelectric Project Area, NE Washington. Seattle City Light, Seattle, WA.

Whiteley, A.R., Fitzpatrick, S.W., Funk, W.C., and Tallmon, D.A., 2015. Genetic rescue to the rescue. Trends in ecology & evolution 30, 42–49.

Wiegmann, D.D., Baylis, J.R., and Hoff, M.H., 1997. Male fitness, body size and timing of reproduction in smallmouth bass, Micropterus dolomieui. Ecology 78, 111–128.

Wilcove, D.S., Rothstein, D., Dubow, J., Phillips, A., and Losos, E., 1998. Quantifying threats to imperiled species in the United States. Bioscience 48, 607–615.

Williamson, K.S., Murdoch, A.R., Pearsons, T.N., Ward, E.J., and Ford, M.J., 2010. Factors influencing the relative fitness of hatchery and wild spring Chinook salmon (Oncorhynchus tshawytscha) in the Wenatchee River, Washington, USA. Canadian Journal of Fisheries and Aquatic Sciences 67, 1840–1851.

Xu, C.L., Letcher, B.H., and Nislow, K.H., 2010. Size-dependent survival of brook trout Salvelinus fontinalis in summer: effects of water temperature and stream flow. Journal of fish biology 76, 2342–2369.

Zabel, R.W., and Achord, S., 2004. Relating size of juveniles to survival within and among populations of Chinook salmon. Ecology 85, 795–806.

Zhou, R., Xiao, J., Qin, Q., Zhu, B., Zhao, R., Zhang, C., Tao, M., Luo, K., Wang, J., and Peng, L., 2015. YY super sperm lead to all male triploids and tetraploids. BMC genetics 16, 1–9.

APPENDIX A. Chapter 2 Supplementary Materials

Table S2-1. Pilot pairwise F_{ST} values for brook trout sampled in tributaries to the Lower Pend Oreille River. Red values equal statistical significance $P < 0.001$, orange values equal statistical significance $P < 0.05$, light yellow values indicate a lack of statistical significance $P > 0.05$.

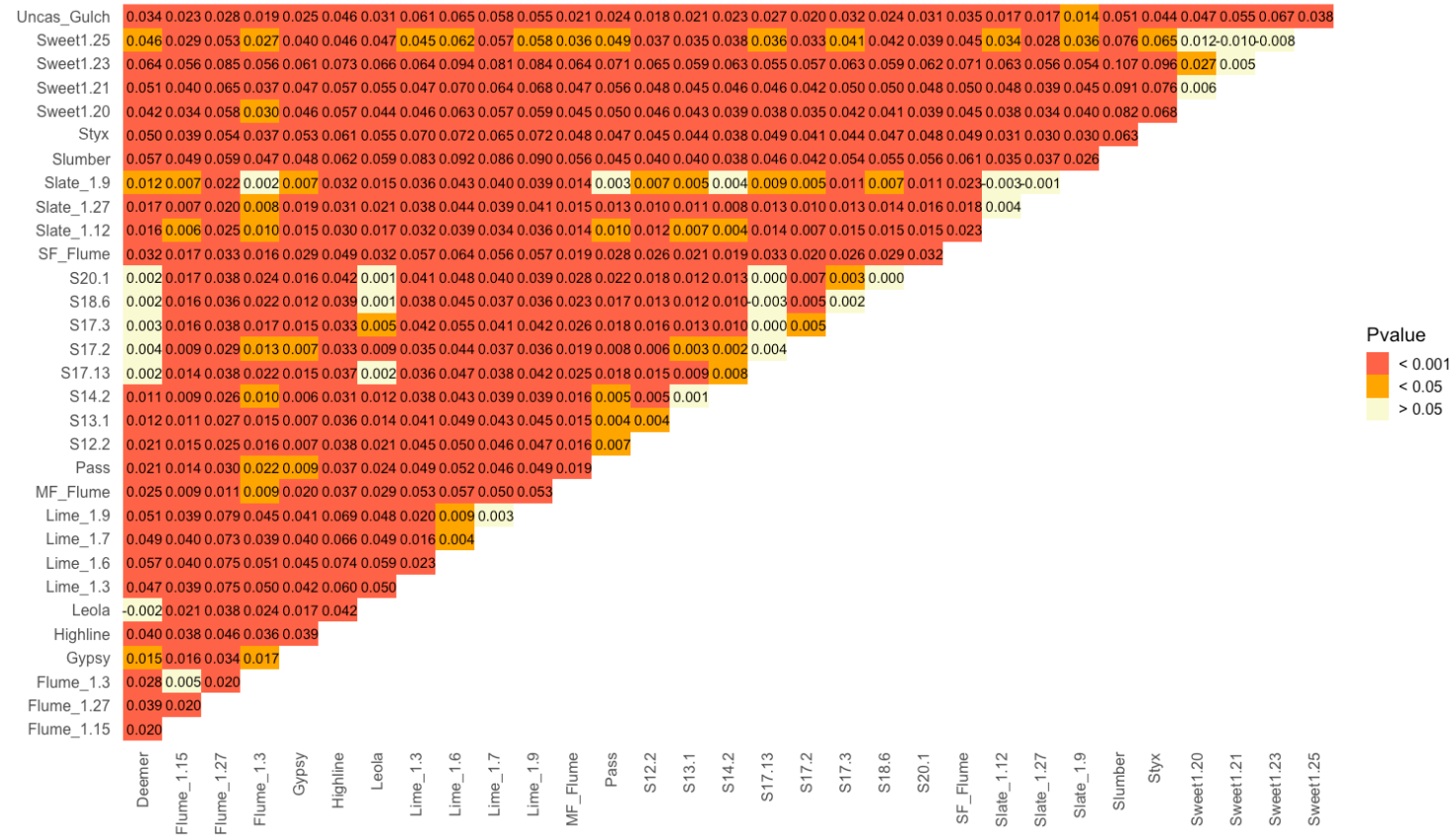
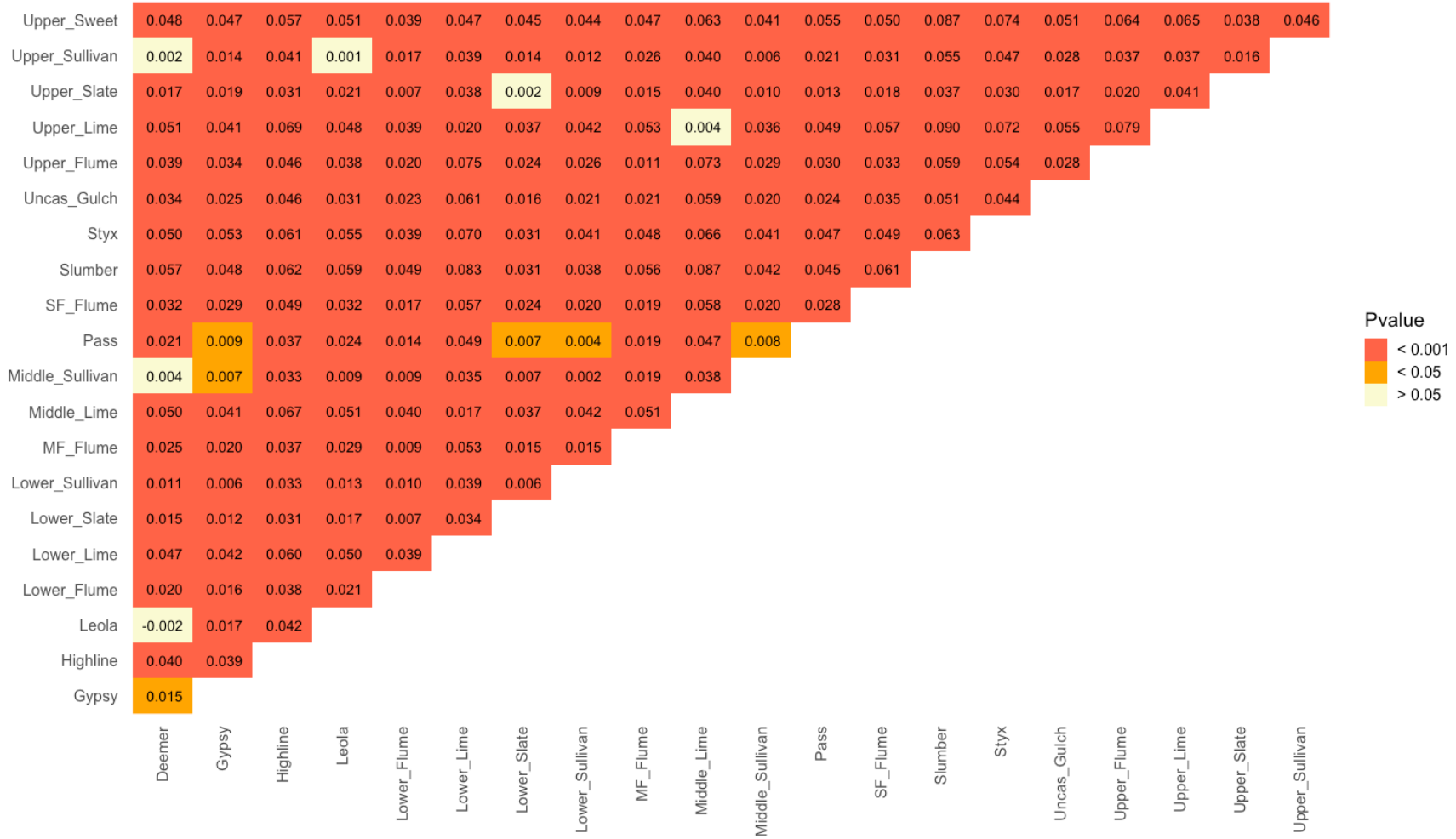


Table S2-2. Pairwise F_{ST} values for brook trout sampled in tributaries to the Lower Pend Oreille River where samples were grouped into populations based on the FST analysis from supplemental Table 1. Red values equal statistical significance $P < 0.001$, yellow values equal statistical significance $P < 0.05$, light yellow values indicate a lack of statistical significance $P > 0.05$.



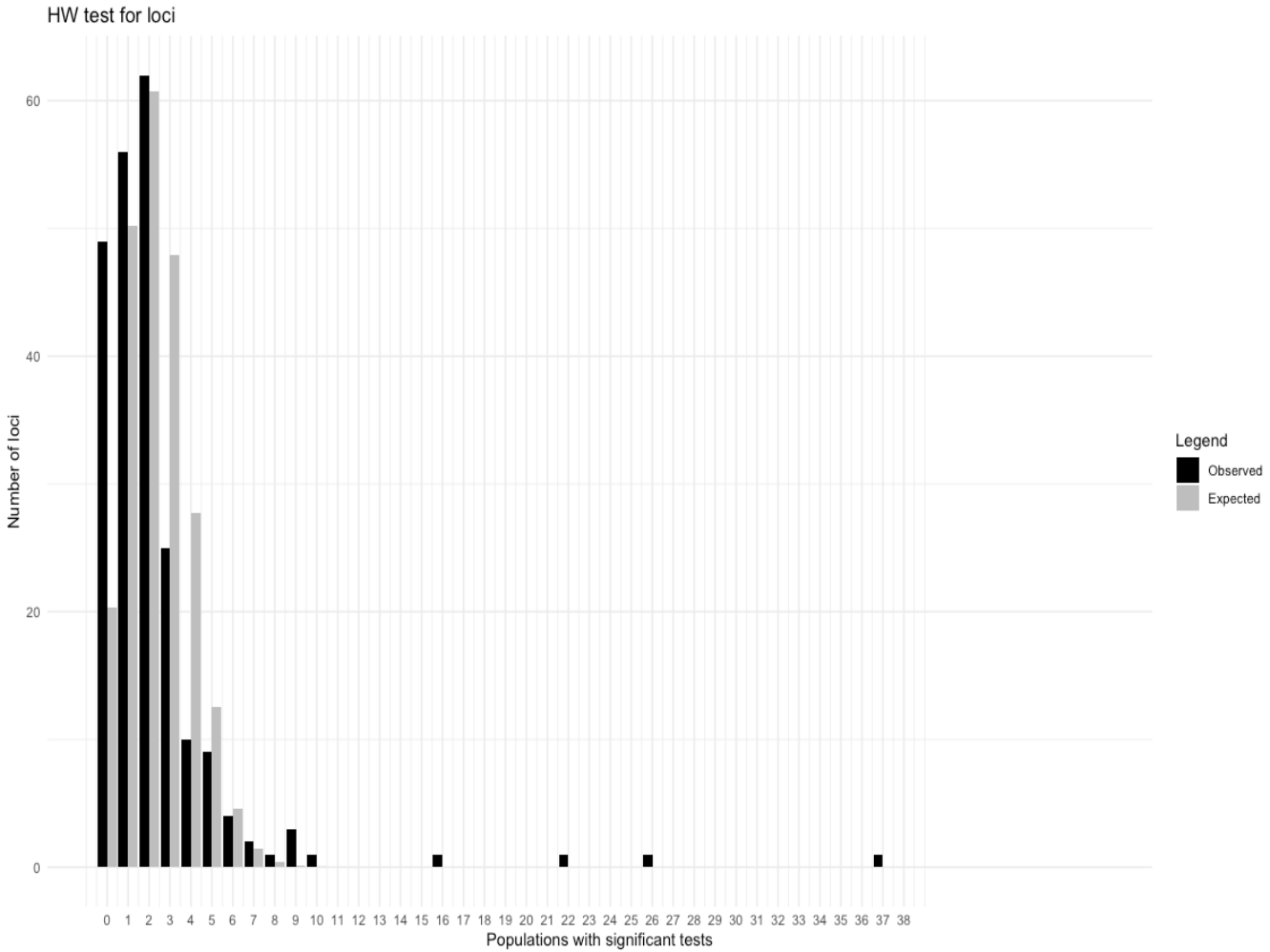


Figure S2-1. Histogram of observed vs expected HW significance tests across populations. The expected distribution is based on a binomial distribution, following Waples (2014). Loci that were significant in seven or more populations were removed from the analysis.

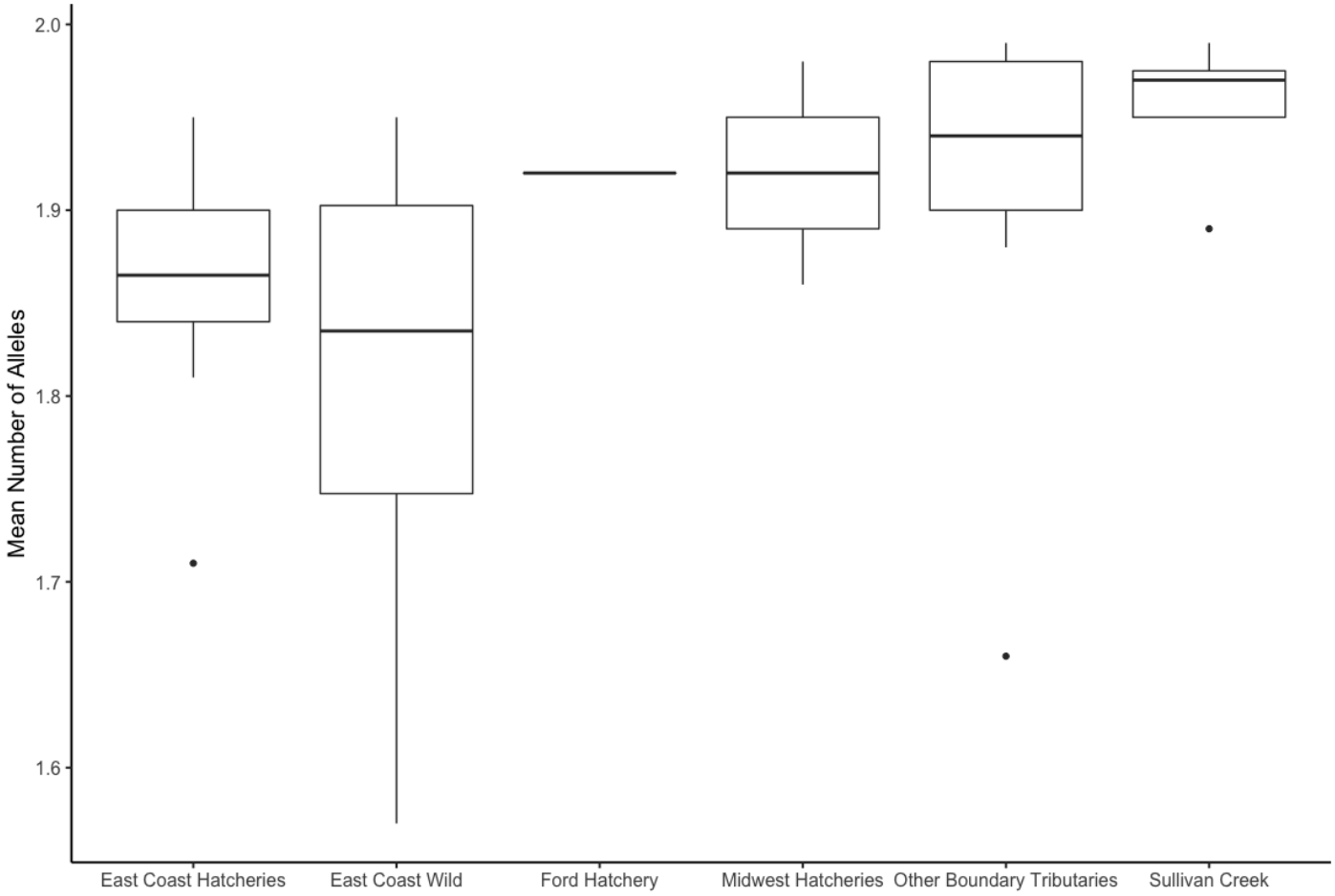


Figure S2-2. Boxplot of mean number of alleles per population. LPO = lower Pend Oreille River system populations and SLVN= Sullivan River system populations according to Table 2-1. Within each box, horizontal black lines denote median values; lower (Q1) and upper (Q3) quantiles represent the 25th to the 75th percentile of each group's distribution of values; data falling outside the Q1 –Q3 range are plotted but are considered outliers of the data.

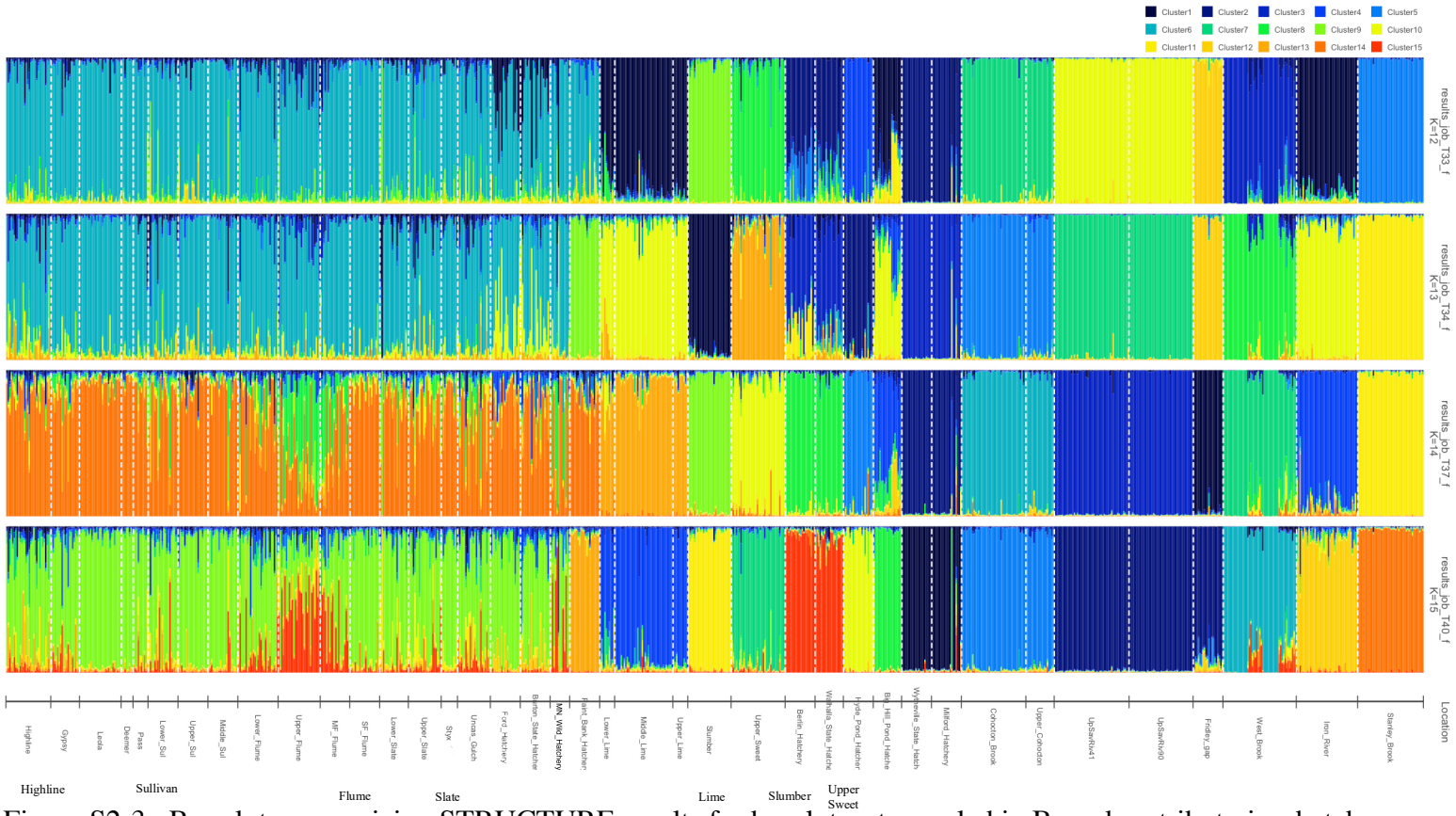


Figure S2-3. Bar plot summarizing STRUCTURE results for brook trout sampled in Boundary tributaries, hatchery samples, and eastern wild collections. Panels are shown for each of K = 12, 13, 14, and 15. Clusters are shown as separate colors. The *x*-axis is divided into collection samples and within each population sample, each individual is shown as a single bar. The *y*-axis is individual *Q*-values, or the partitioning of each individual's genome among the clusters. Sullivan collections were grouped into upper, middle and lower based on the pilot F_{ST} and IBD analysis. Red outlines show grouped collections within the Boundary tributaries including Highline, Sullivan, Flume, Slate, Lime, Slumber and Upper Sweet Creek collections.

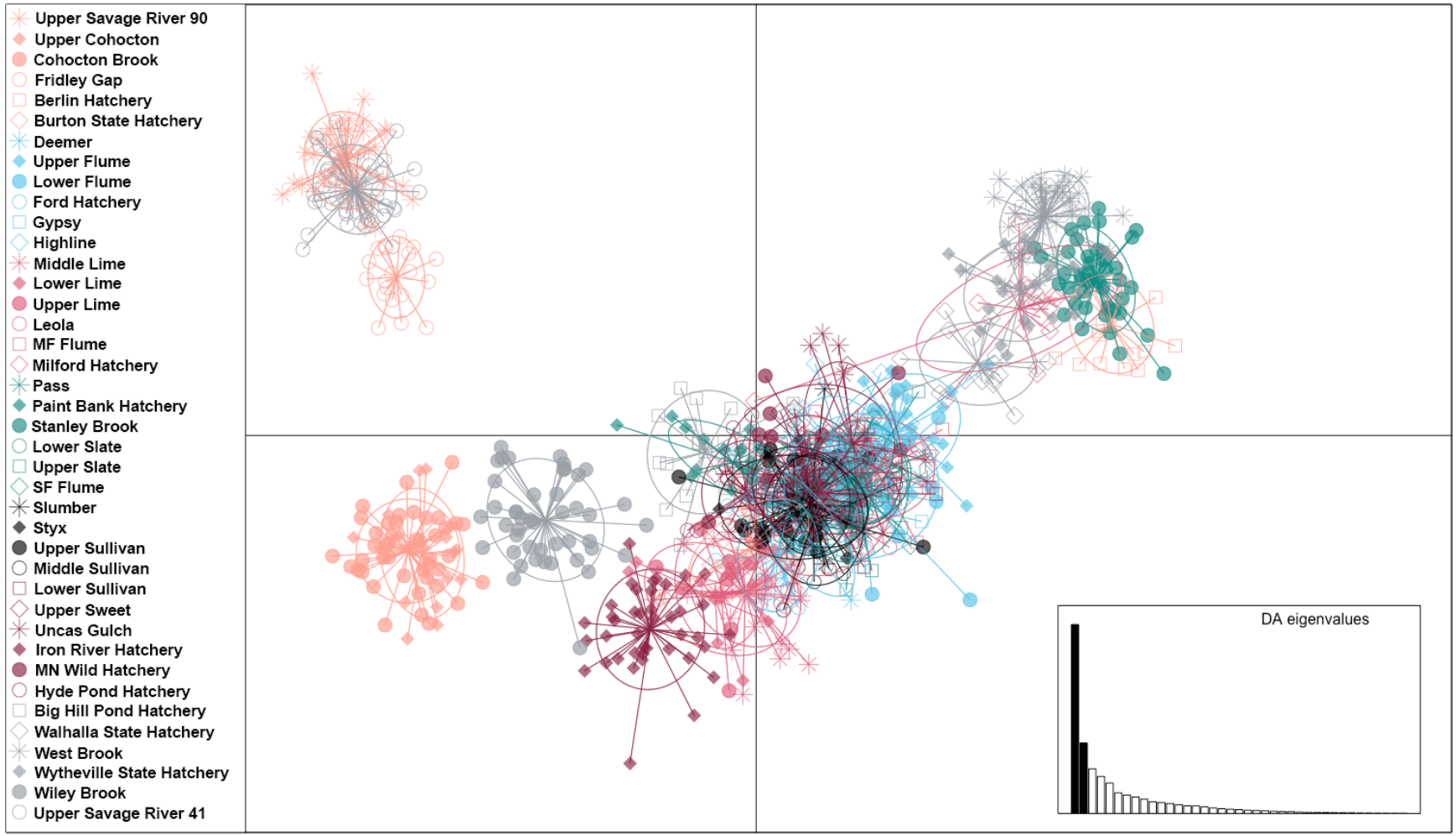


Figure S2-4. DAPC analysis including all sampled populations.

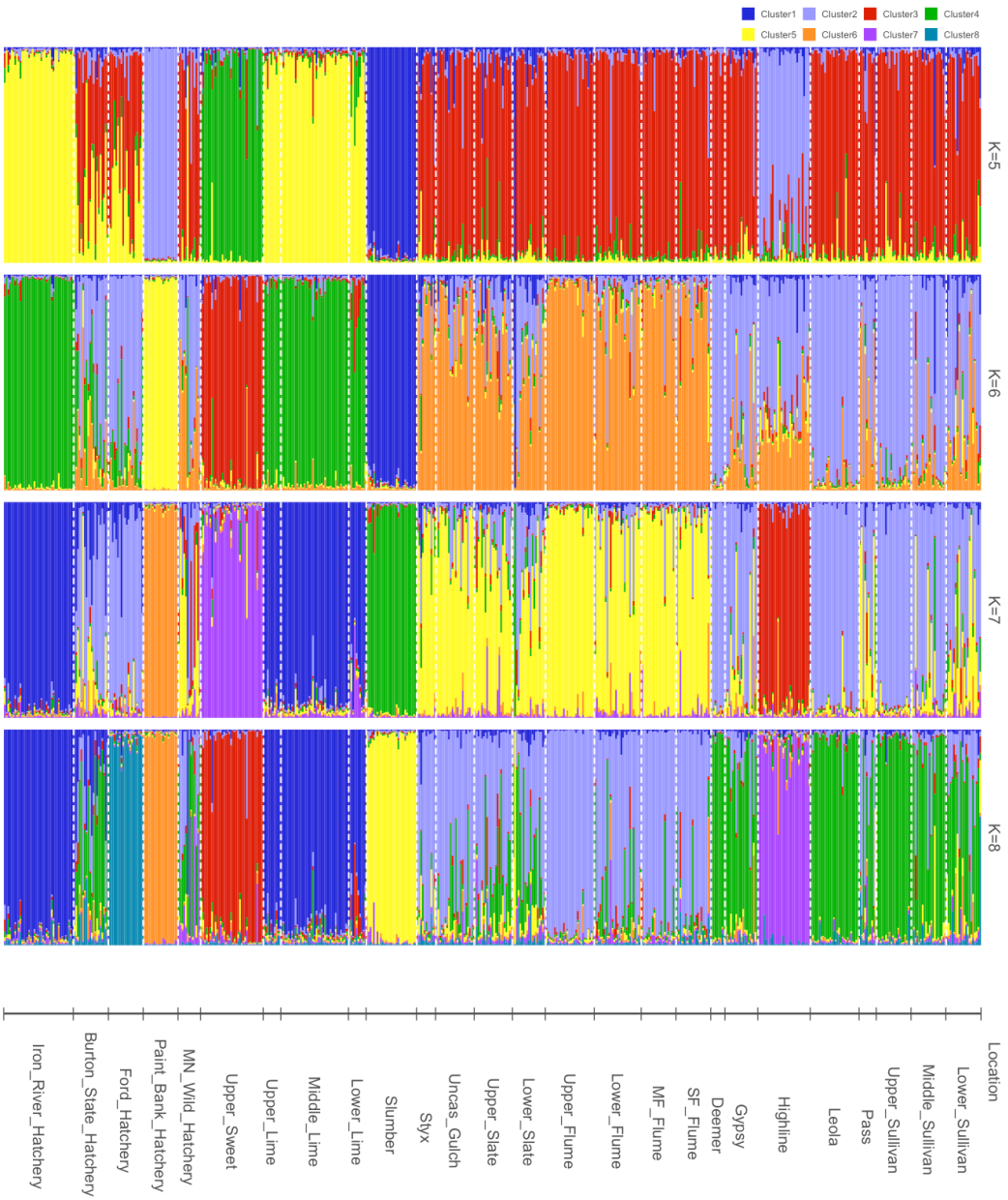


Figure S2-5. Bar plot summarizing STRUCTURE results for brook trout sampled in tributaries from the Lower Pend Oreille River and the five most closely related hatchery strains. Panels are shown for each of K = 4, 5, and 6. Clusters are shown as separate colors. The *x*-axis is divided into population samples and within each population sample, each individual is shown as a single bar. The *y*-axis is individual *Q*-values, or the partitioning of each individual's genome among the clusters. Individual Sullivan collections were grouped into a lower, middle and upper (Sul) based on the pilot F_{ST} and IBD analysis.

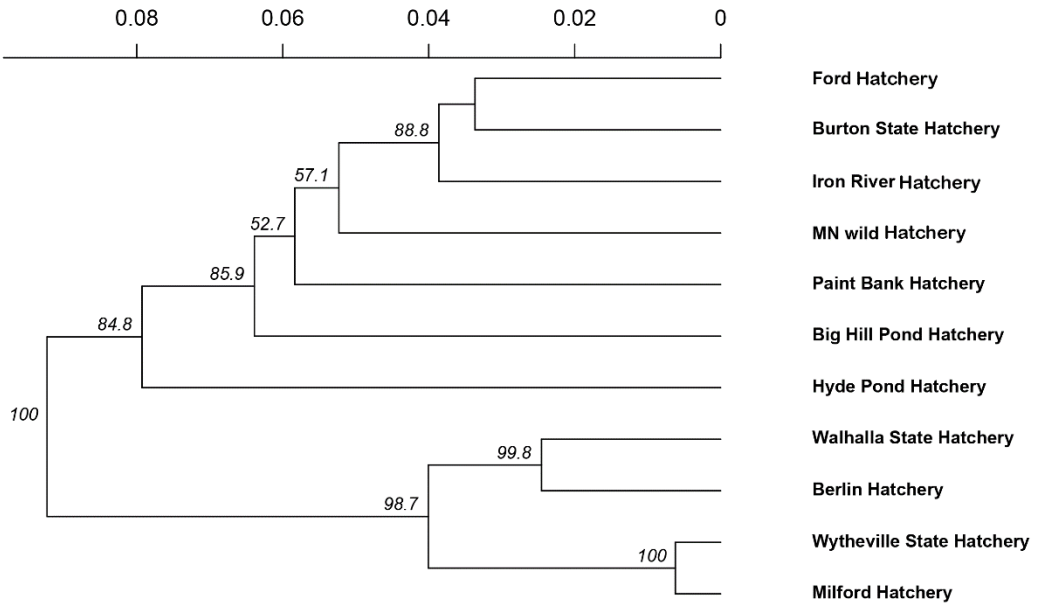


Figure S2-6. UPGMA dendrogram analysis of samples from all hatcheries using Nei's distance. horizontal axis is representative of the allele frequency distance between clusters. Bootstrap values > 50 (percent out of 1000 iterations) are shown.

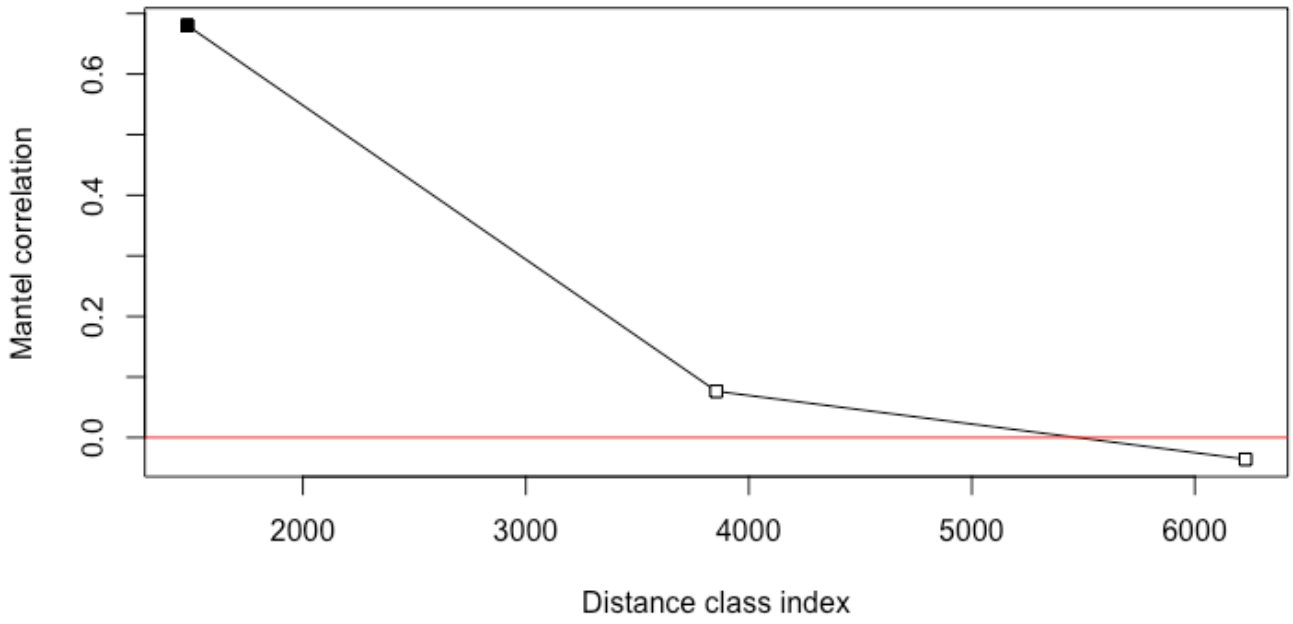


Figure S2-7. Mantel correlogram of isolation by distance within Sullivan Creek where Sullivan Creek sampling units were grouped based on the FST results from Table S2-1. Brook trout were sampled from Leola, Deemer, Pass, and Gypsy Creeks along with adjacent mainstem Sullivan Creek locations. Geographic distances are riverine distances measured between each pair of collections.

APPENDIX B. Chapter 3 Supplementary Materials

Table S3-1. Number of females spawned per date.

Date	# Spawned
October 19 2021	4
October 25 2021	14
November 2 2021	11
November 3 2021	11
November 4 2021	3

Table S3-2. Estimated effect sizes for survival to each developmental stage for each cross-type.

	1-Day Post-Fertilization Survival		Eyed-Egg Survival		Hatch Survival		Swim-up Survival		Post Swim-up Survival		Fry Survival	
Predictors	Estimate ± SE	*P*	Estimate ± SE	*P*	Estimate ± SE	*P*	Estimate ± SE	*P*	Estimate ± SE	*P*	Estimate ± SE	*P*
Cross-Type 1 (XY)	2.64	< 2e-16	0.62 ± 0.18	0.19	0.31 ± 0.17	0.08	-0.07 ± 0.17	0.68	-0.66 ± 0.16	0.18	3.87 ± 0.20	0.01
Cross-Type 2 (Age-0 MYY)	1.48	3E-13	0.39 ± 0.18	0.81	0.16 ± 0.18	0.36	0.01 ± 0.17	0.97	-0.48 ± 0.16	0.06	4.53 ± 0.26	0.01
Cross-Type 3 (Age-1 MYY)	1.9	< 2e-16	0.43 ± 0.18	0.99	0.21 ± 0.17	0.23						

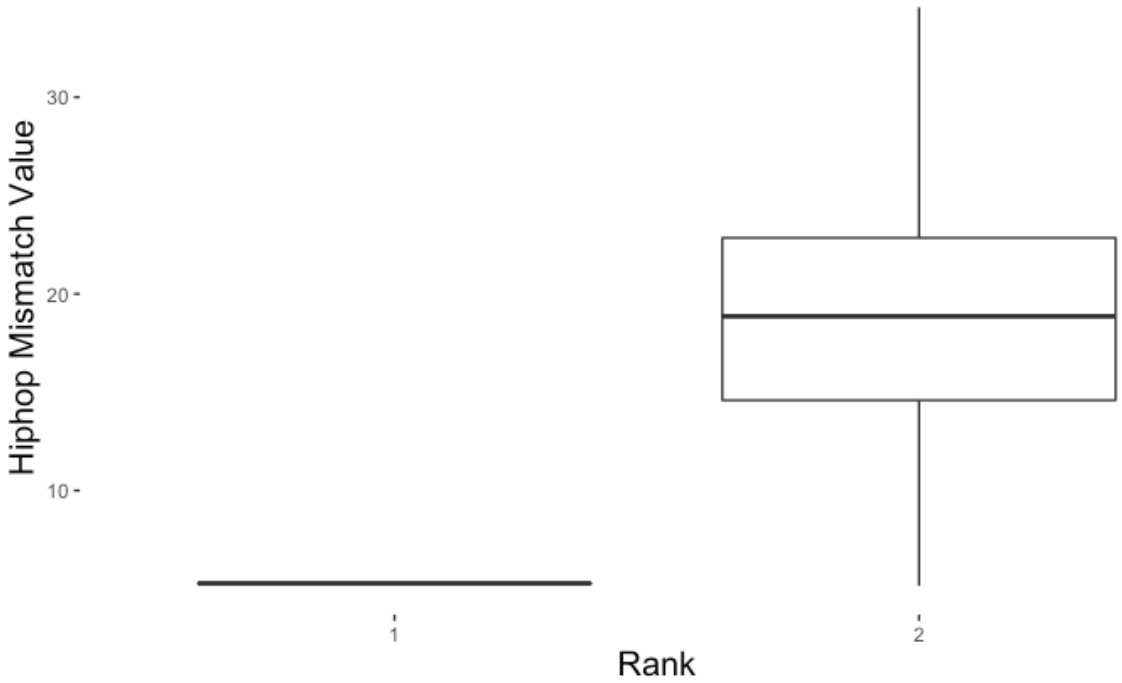


Figure S3-1. Number of genotypic mismatches for male parents that are ranked as either the highest likely parent (rank=1) or the least likely parent (rank= 2) using the Hiphop package in R.

APPENDIX C. Chapter 4 Supplementary Materials

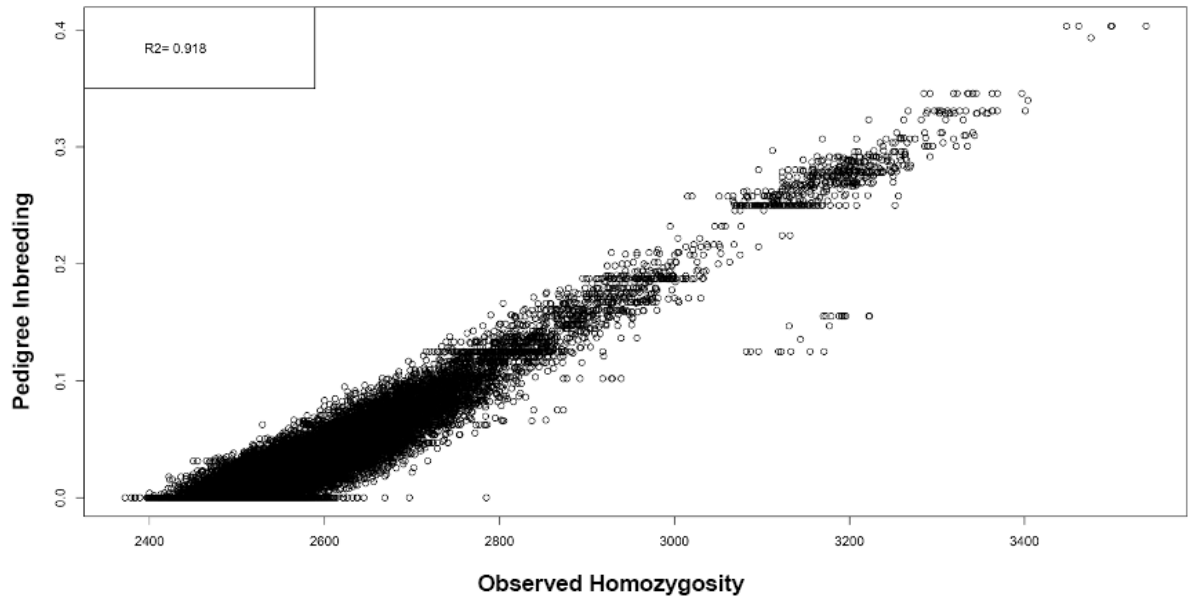


Figure S4-1. Correlation between pedigree inbreeding calculated from the package *pedigree* in R and individual observed homozygosity based on 5000 loci.

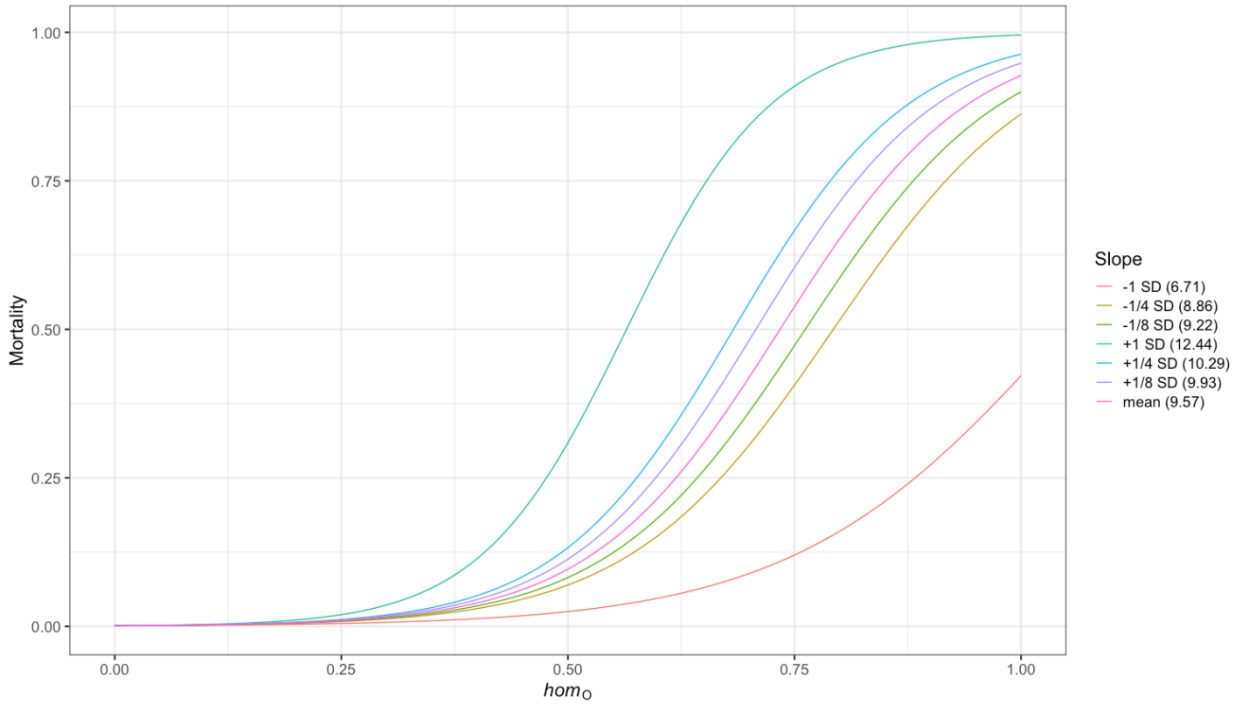


Figure S4-2. Logistic regression of the probability of mortality based on hom_O for all slope values.

APPENDIX D. Chapter 5 Supplementary Materials

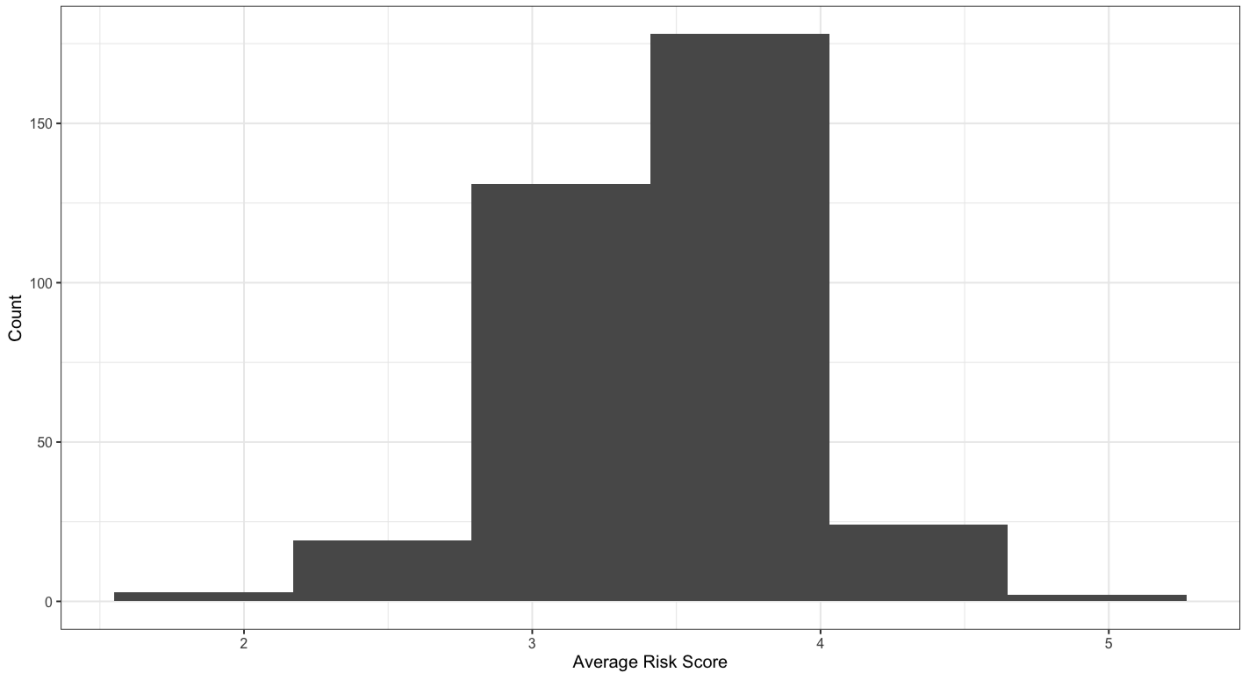


Figure S5-1. Histogram of average risk score based on respondents' answers to the risk propensity scale.

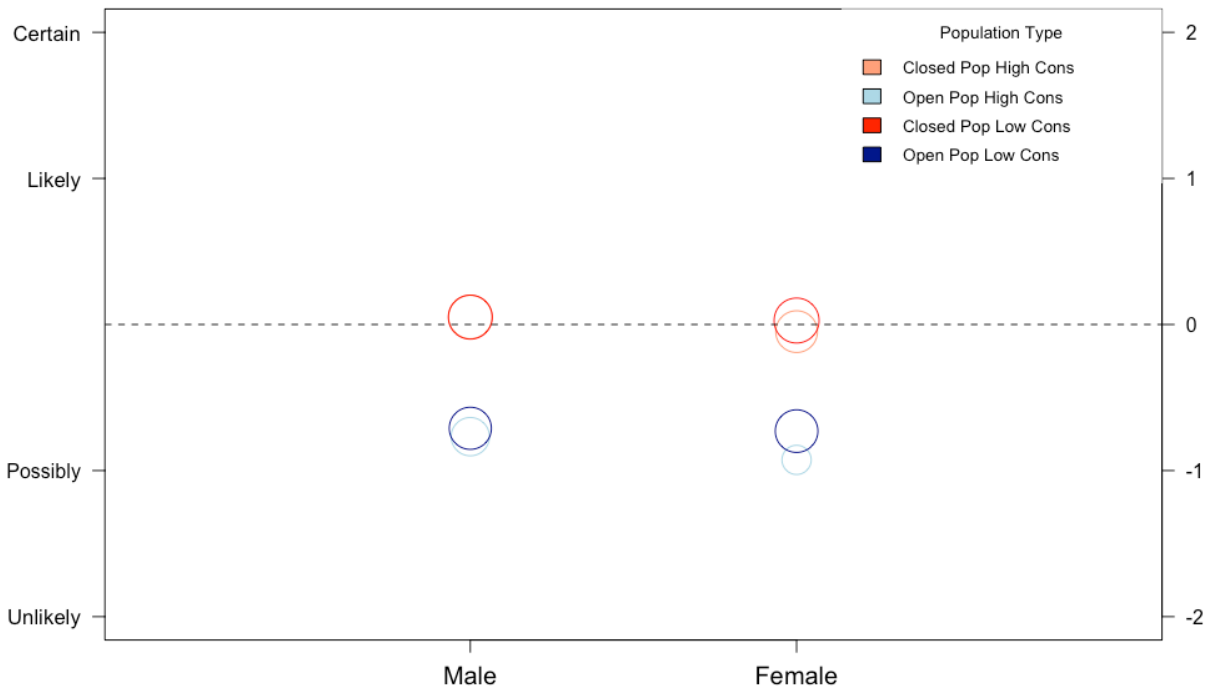


Figure S5-2. PCI results for the likelihood of managers of different genders implementing an M_{YY} management approach based on whether the cutthroat trout population is a high conservation priority closed population (closed pop high cons), a high conservation priority open population (open pop high cons), a low conservation priority closed population (closed pop low cons), or a low conservation priority open population (open pop low cons).

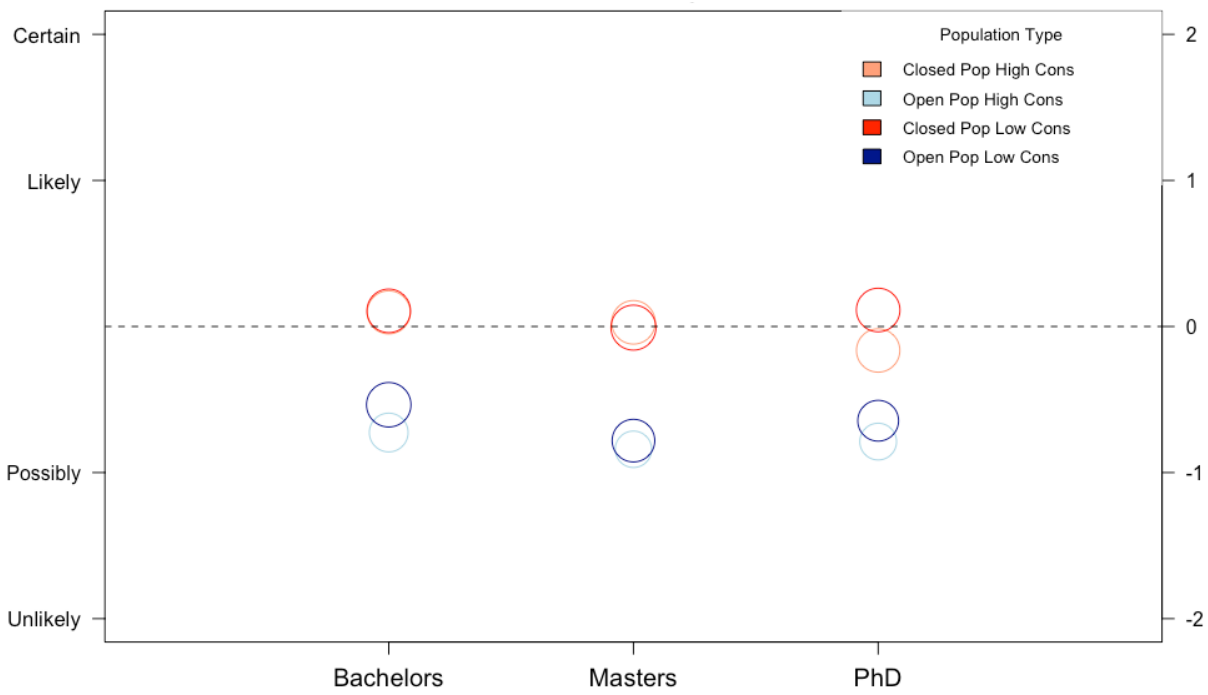


Figure S5-3. PCI results for the likelihood of managers with different education backgrounds implementing an M_{YY} management approach based on whether the cutthroat trout population is a high conservation priority closed population (closed pop high cons), a high conservation priority open population (open pop high cons), a low conservation priority closed population (closed pop low cons), or a low conservation priority open population (open pop low cons).

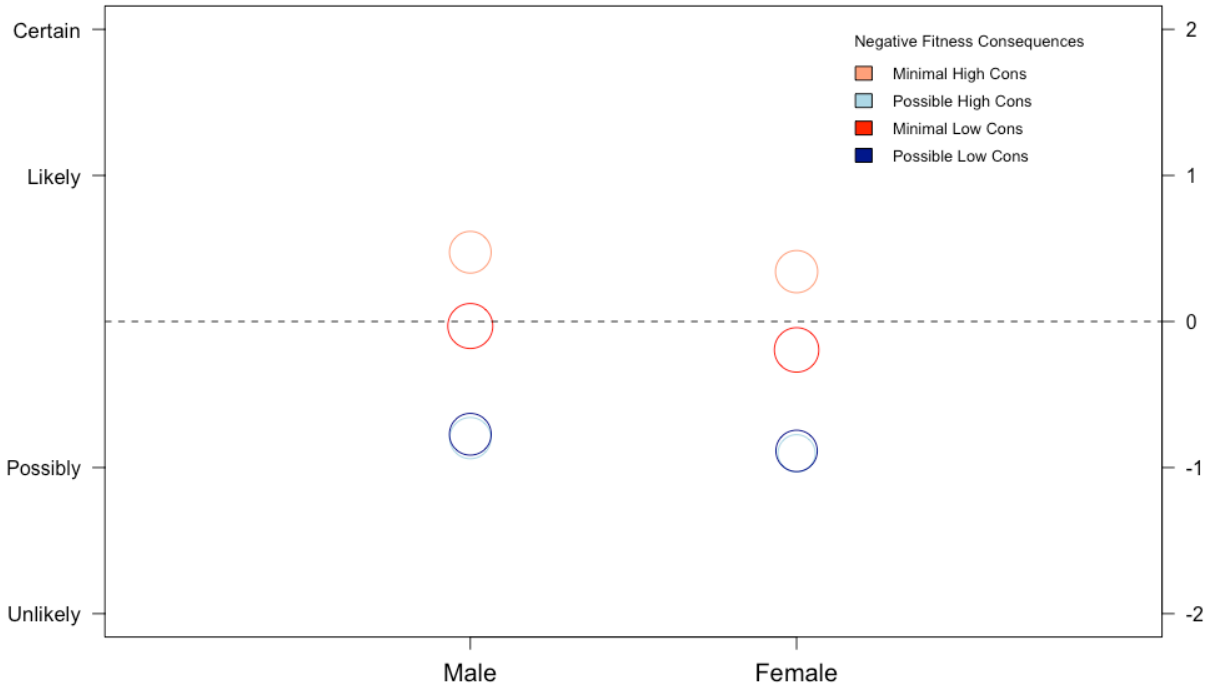


Figure S5-4. PCI results for the likelihood of managers of different genders implementing a genetic rescue management approach based on whether the negative fitness consequences of the translocation are minimal in a population that is a high conservation priority population (minimal high cons), possible in a population that is a high conservation priority population (possible high cons), minimal in a population that is a low conservation priority population (minimal low cons), or possible in a population that is a low conservation priority population (possible low cons).

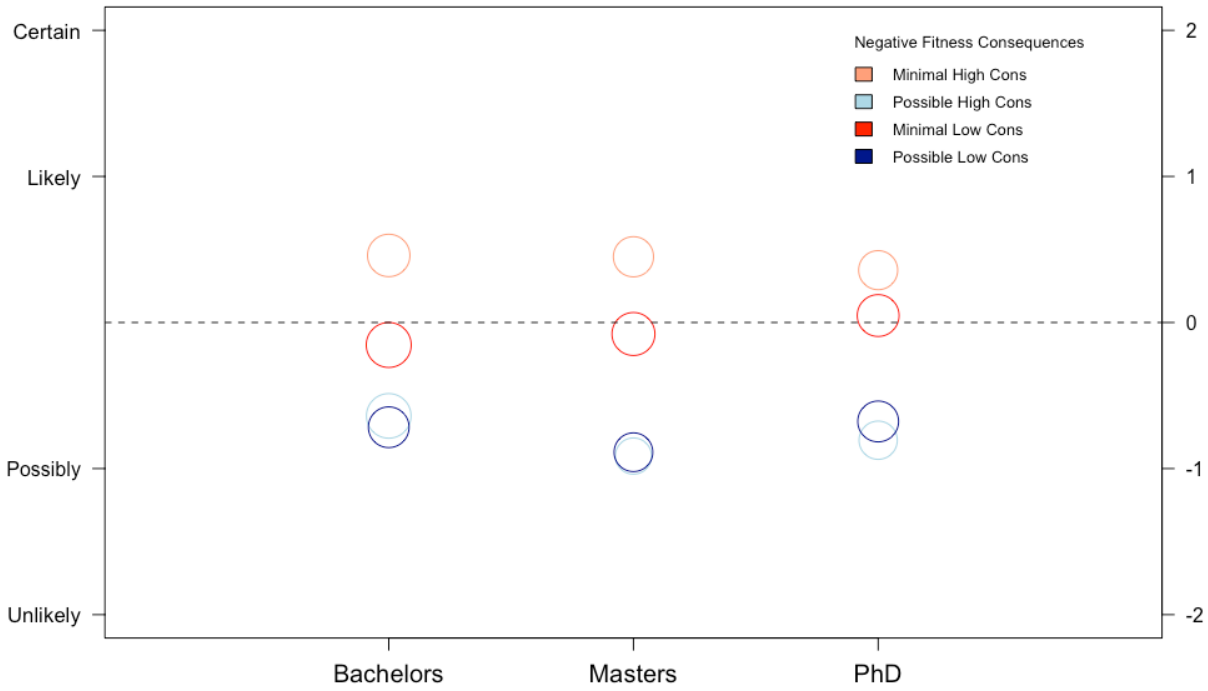


Figure S5-5. PCI results for the likelihood of managers with different education backgrounds of implementing a genetic rescue management approach based on whether the negative fitness consequences of the translocation are minimal in a population that is a high conservation priority population (minimal high cons), possible in a population that is a high conservation priority population (possible high cons), minimal in a population that is a low conservation priority population (minimal low cons), or possible in a population that is a low conservation priority population (possible low cons).