# UPPER MISSOURI RIVER

# ARCTIC GRAYLING

# CONSERVATION STRATEGY

Prepared by

MONTANA ARCTIC GRAYLING WORKGROUP

2022

**Upper Missouri River Arctic Grayling Conservation Plan**

**August 1, 2022**

Contents

# Introduction and Overview

Upper Missouri River Arctic grayling *Thymallus arcticus* are a unique native species and important part of Montana's history and natural heritage. Arctic grayling (grayling) were once widespread in the Missouri River Basin upstream of Great Falls, MT (UMR), but indigenous populations are now restricted to the upper Big Hole River near Wisdom, Miner, Mussigbrod, and Pintler lakes in the Big Hole River Valley, and the Centennial Valley near Lima. Conservation populations, which were developed using Montana-origin UMR grayling, have been and are currently being established in historically occupied habitats including the Ruby River, several tributaries to the Big Hole, and the upper Madison River drainage. Additionally, 12 populations representing the ancestral genetic variation of UMR grayling have been established in historically unoccupied mountain lakes within the UMR.

UMR grayling are currently listed as *Species of Concern* by the State of Montana and a *Sensitive Species* by the U. S. Bureau of Land Management (BLM) and the U. S. Forest Service (USFS). Though previously identified as a *Candidate Species* under the Federal Endangered Species Act (ESA), in August of 2014 the U. S. Fish and Wildlife Service (USFWS) found that UMR grayling were *not warranted* for listing as threatened or endangered, which was confirmed in 2020 following legal challenge (USFWS 2020).

This Conservation Strategy was prepared by the Montana Arctic Grayling Workgroup (AGW) to update past conservation plans and describe contemporary conservation approaches and programmatic needs. The AGW was formed in 1987 following declines in the abundance and distribution of the Big Hole River Arctic grayling population and concerns about indigenous lake populations. AGW is chaired by Montana Fish, Wildlife & Parks (FWP), and includes representatives from FWP, Montana Department of Natural Resources (DNRC), Natural Resources Conservation Service (NRCS), USFWS, BLM, USFS and Yellowstone National Park (YNP) as management agencies with an interest in conservation and restoration of UMR grayling. AGW developed an initial *Montana Fluvial Arctic Grayling Restoration Plan* in 1995 that established a timeline to achieve recovery goals including establishing at least five fluvial conservation populations among at least three of the subbasins within the historic range; however, a 2016 MOU formalized commitment among AGW members to develop an updated conservation plan that addresses all UMR grayling populations (AGW 2017). This strategy incorporates the most promising elements of established conservation approaches, techniques, and research and builds upon the success and knowledge gained since the establishment of the AGW.

Numerous approaches have been used to conserve and restore Arctic grayling in Montana since the formation of AGW in 1987. Conservation actions have improved habitat, conserved genetic diversity, expanded distributions through introductions to suitable habitats, and developed a better understanding of grayling biology, life history requirements, interactions with nonnative trout, and habitat needs. This diverse program requires support and collaboration among a large group of partners, which includes the Montana Chapter of the American Fisheries Society, Montana State University, Montana Trout Unlimited (TU), The Arctic Grayling Recovery Program (a 501 (c) nonprofit), The Nature Conservancy (TNC), The University of Montana, The University of Montana Western, Big Hole River Foundation, Big Hole Watershed Committee, Centennial Valley Association (CVA) and numerous private landowners who provide access to the habitats where many Arctic grayling conservation and restoration efforts occur.

This Strategy is intended to provide guidance for conservation of UMR grayling. The Strategy updates grayling status and includes objectives, goals, strategies, and measures for all conservation populations. It prioritizes conservation programs in the Big Hole and Centennial valleys, including implementation of Candidate Conservation Agreement with Assurances (CCAA) programs in both watersheds and an adaptive management plan for Centennial Valley grayling. Resource agencies are encouraged to collaborate with partners to meet the intent of this Strategy and direct UMR grayling restoration efforts within their authority. The AGW anticipates that the actions described in this Strategy will be implemented, leading to the long-term conservation and persistence of UMR grayling in Montana. This Strategy incorporates new information to revise the original 1995 Restoration Plan and will be updated hereafter every five years.

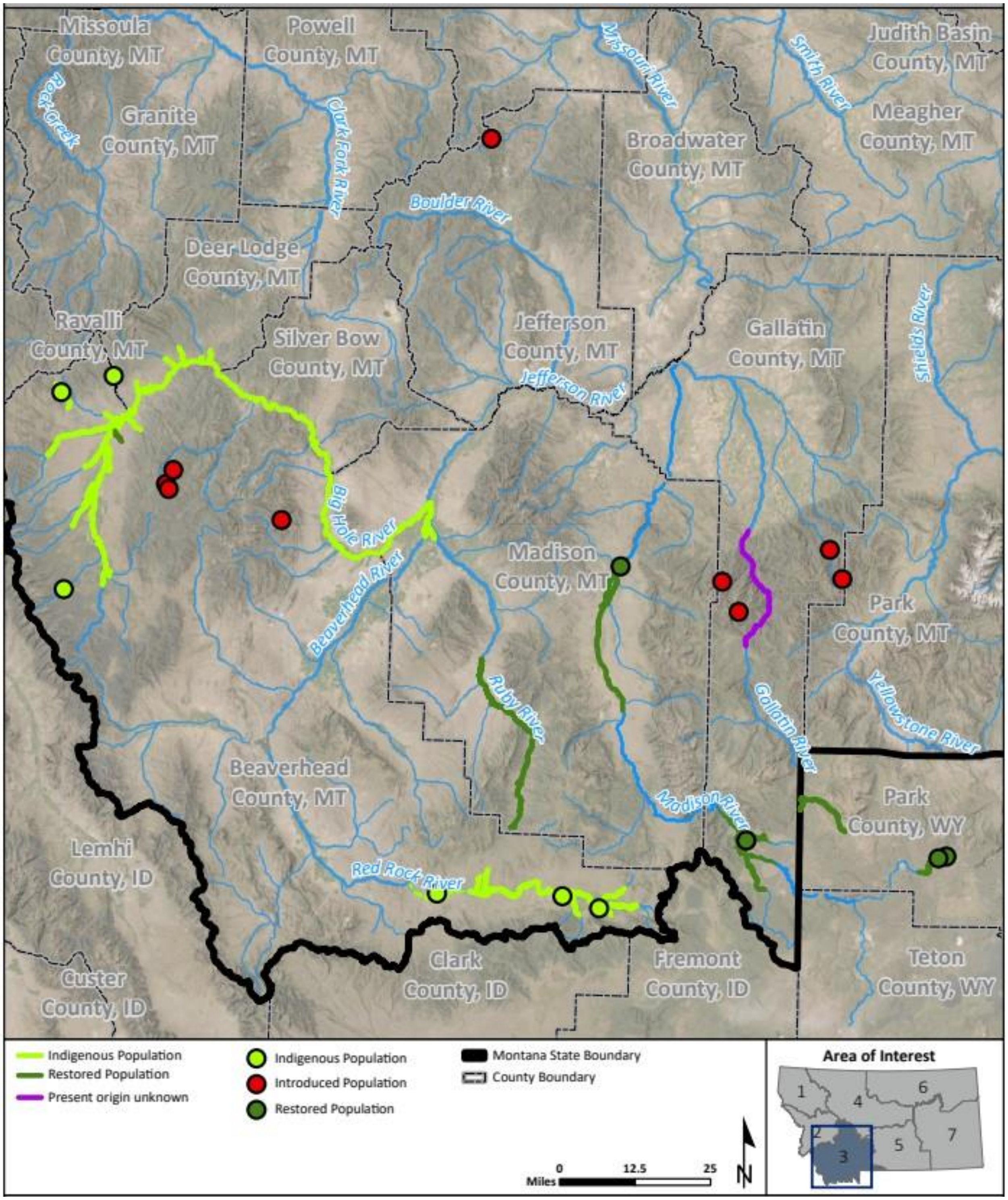


**Figure 1.** Current distribution of Arctic grayling in the upper Missouri River Basin. Map includes distribution of extant native populations (bright green), introduced conservation populations (red dots), native populations which have been reintroduced (dark green), and the Gallatin River (purple) which contains grayling of unknown origin. (Lake Levale in the Sun River is not shown due to proximity).

## Historical and Current Distribution

UMR grayling distribution and abundance has declined over the past 150 years and is presently comprised of indigenous and introduced populations. Grayling were patchily distributed throughout the UMR drainage prior to the mid-1850s and inhabited the mainstem Missouri River and the Smith, Sun, Jefferson, Madison, Gallatin, Big Hole, Beaverhead, Red Rock, and Ruby rivers as well as several Centennial Valley streams (Vincent 1962; Kaya 1992). Grayling also occupied Red Rock and Elk lakes in the Centennial Valley (Vincent 1962; Kaya 1992) and Miner, Mussigbrod, and Pintler lakes in the upper Big Hole River drainage (Leary et al. 2015). The distribution of native grayling in the UMR has declined since the late 1800s, particularly in riverine habitats (Vincent 1962; Kaya 1992).

Currently there are 19 extant UMR grayling populations. Riverine populations of grayling exist in the Big Hole River and its tributaries, Red Rock Creek and its tributaries, the upper Ruby River, and the headwaters of the Gibbon River in the Madison drainage (Kaya 1992; Byorth 1997; Liermann 2001; Gander et al. 2019, Puchani 2021). Extant lake populations in the UMR reside in Miner, Mussigbrod, and Pintler lakes in the upper Big Hole River drainage (Leary et al. 2015). Twelve additional mountain lake populations in the Gallatin, Madison, Big Hole, Missouri, and Sun rivers drainages were the result of historical stockings yet are important for genetic conservation of Arctic grayling in the UMR (Leary et al. 2015; USFWS 2020). Contemporary abundance and genetic status of each population is described below.

## Threats

***Habitat Degradation:*** Habitat degradation reduced the distributions of native fishes within the UMR, beginning with European settlement of western Montana in the 1800s (Vincent 1962). Dam construction on the Madison and Red Rock rivers inundated essential spawning habitats and blocked fish movements (Kaya 1990, Vincent 1962). Early agricultural practices contributed to population declines of grayling in several large rivers of Montana through dewatering, degradation of riparian habitats, and siltation (Vincent 1962). Extensive irrigation occurred from most tributary streams by the early 1900s and complete dewatering of streams for irrigation, especially during periods of drought, likely had a large influence on distribution, abundance, and life history strategies of grayling through time (Deeds and White 1926, Vincent 1962, Randall 1978). In addition to dewatering streams, irrigation diversions can also entrain grayling and restrict their movements (FWP 2021). Overgrazing and the introduction of nonnative grasses have negatively affected riparian communities by replacing deep-rooted native willows, sedges and grasses with shallow-rooted species, which resulted in increased streambank erosion, over-widened channels, decreased pool habitat, and increased water temperatures (Hansen et al. 1995). Extensive cattle grazing in the Centennial Valley resulted in excessive siltation, which may have contributed to reduced depths in Upper Red Rock Lake (Upper Lake) from a maximum of over 20 feet to less than six feet (Gangloff 1996). In the Centennial Valley, aggressive waterfowl management and construction of instream ponds to benefit the once endangered trumpeter swan disconnected spawning tributaries, blocked migrations, and inundated spawning gravels with silt (Vincent 1962; Gillin 2001). Improved grazing and irrigation practices and targeted habitat programs and restoration projects have improved instream flows, riparian health, fish passage, and grayling abundances (USFWS 2020).

***Nonnative Species:*** The influence of nonnative fish on grayling varies by species. Nonnative trout species have replaced native salmonids, except for mountain whitefish, in much of the UMR (Miller et al. 1989; Nico and Fuller 1999; Schade et al. 2005). Sympatric populations of introduced brook *Salvelinus fontinalis*, rainbow *Oncorhynchus mykiss*, and brown trout *Salmo trutta* may compete with and prey on UMR grayling (Nelson 1954; Miller et al. 1989; Streu 1990; Fausch 1998; Katzman 1998; Schade et al. 2005). Brook trout, which are the most widespread nonnative fish in the UMR, may prey on grayling eggs (Katzman 1998). However, in the Big Hole River, there is little evidence that brook trout limit grayling (Magee and Byorth 1994). Conversely, high brown trout densities were identified as a primary limiting factor for age-0 grayling (McCullough 2017). Brown trout were also considered to be a primary factor in the decline and unsuccessful grayling reintroductions in the Madison River near Ennis (Vincent 1962, Clancey 1997, Liermann 2001, FWP 2014). In the Centennial Valley, nonnative Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* are not primary drivers of grayling abundances, including Upper Red Rock Lake (Katzman 1998; Warren et al. 2022).

***Overharvest:*** Although overharvest and early propagation programs contributed to historical grayling declines, restrictive angling regulations have reduced this threat. Grayling are generally more susceptible to angling than nonnative trout, and liberal historical creel limits allowed for overharvest of grayling (Henshall 1907; Beal 1953). High fishing pressure in the early 1900s caused declines in grayling abundances in parts of the UMR, which was exacerbated when overall angling pressure increased throughout Montana following the establishment of nonnative trout fisheries (Vincent 1962). Additionally, initial propagation programs removed millions of gametes per year from the Centennial and Madison River grayling populations as other threats emerged in the UMR (FWP 1907, Beal 1953). Restrictive regulations (e.g., catch-and-release for grayling on all rivers, spawning closures in Red Rock Creek) and increased enforcement have reduced this threat (FWP 2014). However, high fishing pressure, particularly during warm summer months, may still result in incidental grayling mortality.

***Climate Change:*** Systemic habitat improvement and targeted intervention will improve grayling resiliency to climate change. Freshwater ecosystems are generally the most sensitive to deviations in global temperature and shifting habitat conditions (Comte et al. 2013). Climate change has reduced overall precipitation, decreased mean annual snowpack, diminished the magnitude of spring runoff, and increased water temperatures in Montana (Lohr et al. 1996; Gillian and Boyd 2009; Vatland 2015). A warming climate could have negative consequences for grayling through increasing water temperatures and exacerbation of impacts from agricultural practices (Vincent 1962). In the Centennial Valley, declining precipitation and snowpack have decreased stream flows into Upper Lake, which has contributed to reduced suitable overwinter habitat for grayling because of reduced water depths and increased retention times (Davis 2016; Warren et al. 2022). Elk Lake contained a viable population of grayling until the 1990s when climate change and geologic activity disrupted connectivity of spawning tributaries (Lund 1974; Gillian and Boyd 2009). Targeted intervention and unconventional projects may be required to address these threats (e.g., Flynn et al. 2019). In the Big Hole River, ongoing drought combined with early runoff has decreased mean summer discharges (Vatland 2015). However, the Big Hole CCAA has improved irrigation practices, stream flows, and riparian habitats, which provide a critical buffer against climate change (FWP unpublished data; Seavey et al. 2009).

## Conservation Objective, Goals, and Measures

***Conservation Objective:*** The conservation objective is to ensure the long-term, self-sustaining persistence of UMR grayling.

***Conservation Goals:*** The conservation objective for UMR grayling will be achieved when the following goals are met:

1. The indigenous Big Hole River, Centennial Valley, Miner Lake, Mussigbrod Lake, Pintler Lake and viable introduced populations (see below) exhibit a stable or increasing genetic effective population size ($N_e$) over multiple generations that is sufficiently large to avoid inbreeding depression and maintain evolutionary potential.
2. The geographic distribution of extant UMR grayling is maintained or increased.
3. Self-sustaining conservation populations of UMR grayling (defined below) are restored in historically occupied drainages.

***Conservation Strategy:*** This Strategy emphasizes the conservation of genetic variation in UMR grayling, and thus, the evolutionary legacy of the species. Conservation success of the Strategy is safeguarded by managing for local population sizes sufficiently large to avoid loss of genetic variation and maintain evolutionary potential (Goal 1), and in the event of local population decline or extirpation, genetic variation is replicated across multiple, independent populations throughout the UMR (Goals 2 and 3). Drainage or population-specific strategies to meet each conservation goal and, resultantly, the overarching conservation objective are described in detail below.

***Measures of Success and Monitoring:*** Attainment of conservation goals will be objectively determined by genetic (Goal 1) and presence/absence (Goals 2 and 3) monitoring.

Status of indigenous and viable introduced UMR grayling populations will be assessed by directly estimating or considering genetic metrics relevant to $N_e$. A genetically viable conservation population of UMR grayling is defined as one in which $N_e$ is 1) sufficient to maintain adaptive genetic variation; 2) sufficient to minimize risk of inbreeding depression; and 3) either stable or increasing over time. $N_e$ controls the rate at which populations lose genetic variation (i.e., evolutionary potential) due to genetic drift and the rate at which inbreeding accumulates in small populations. $N_e$ is a critical measure in conservation biology (Allendorf et al. 2022), but one of the most difficult metrics to accurately estimate in wild populations (e.g., Waples et al. 2014). Accurate estimates of $N_e$ require extensive sampling that is not realistic for most extant UMR grayling populations. As such, $N_e$ will only be monitored explicitly for the Big Hole and Centennial Valley populations because they 1) have the highest priority conservation status in the UMR, 2) are most strongly limited by human actions or the legacy of human actions, and 3) are the specific targets of large-scale conservation programs where annual estimates of population success are critical for communicating the efficacy of conservation actions with stakeholders (Table 1). $N_e$ will be estimated using annual estimates of the effective number of breeders ($N_b$) that produce a given cohort and multiple life history traits (Waples et al. 2013; Waples et al. 2014). In the Big Hole drainage, $N_b$ estimates will be produced by collecting samples from at least 120 young-of year (YOY) grayling from known spawning locations across the drainage each fall. In the Centennial Valley, $N_b$ will be estimated by sampling spawning fish from Red Rocks and Elk Springs creeks and assigning cohort based on age determined from scales. In all other indigenous and viable introduced UMR grayling populations, inference about $N_e$ will be based on temporal trends in genetic variation measured every 6-8 years with allelic richness ($A_r$) and average heterozygosity ($H_{e;}$ Tables 1 and 2). $A_r$ is the number of alleles present in a population, which is a surrogate for standing genetic variation. $H_e$ is a fundamental measure of genetic variation and represents the proportion of genes that have two different alleles within a population. Allelic richness provides information about adaptive potential (e.g., Caballero and García-Dorado 2013; Allendorf et al. 2014) whereas $H_e$ assesses the risk of contemporary and future inbreeding depression. Both measures are directly influenced by $N_e$, where populations with increasingly small $N_e$ experience increasingly rapid loss of $H_e$ and $A_r$, with $A_r$ being particularly sensitive to rapid declines in $N_e$ (Allendorf 1986; Luikart et al. 1998). However, neither metric increases with increasing $N_e$ over contemporary management time-scales. Broadly speaking, temporal stability in measures of genetic variation over a few generations provides evidence that $N_e$ is relatively large, whereas decreases provide evidence that $N_e$ is small. Thus, losses of genetic variation can be used to trigger management actions that can help ensure conservation goals are met.

Distribution of extant UMR grayling populations and self-sustaining conservation status of introduced grayling populations will be confirmed by presence/absence sampling with electrofishing, eDNA, or gill netting. Frequency of distributional sampling for UMR grayling populations will occur on a case-by-case basis as dictated by genetic trends. Introduced grayling will be designated as self-sustaining UMR conservation populations when they are located within the UMR, founded using UMR grayling, and persist for 10 years with no artificial propagation and observation of wild fish from each cohort. Reintroduction approach will consider both demographic and genetic criteria. Each introduced population will be established using source populations selected to maximize their conservation value and genetic diversity and founded by at least fifty breeding pairs to reduce genetic bottlenecks. Successful establishment of non-indigenous lake populations in Montana typically involved stocking 1,500-2,000 grayling per acre for at least a three-year period. Based on results from the Ruby River reintroduction, onsite incubation of fertilized eggs will be the primary introduction tool for fluvial populations. All stream reintroductions should utilize at least 130,000 eggs per year for five years with at least two years exceeding 200,000 eggs (Gander et al. 2019). In lakes, a combination of stocking fry, fingerlings, and onsite incubation will be used. Monitoring to assess whether a population is self-sustaining will begin after stocking ceases and natural reproduction of introduced fish is first expected. When an introduced population meets criteria to be designated as a self-sustaining UMR conservation population its genetic status ($A_r$, $H_e$) will be assessed every 6-8 years as described above.

**Table 1.** Genetic sampling intervals for estimates of the number of effective breeders ($N_b$), genetic effective population size ($N_e$), allelic richness ($A_r$), and expected heterozygosity ($H_e$) for indigenous Arctic grayling populations in the upper Missouri River Basin.

| Population | Genetic Metric | Sampling Interval |
|---|---|---|
| Big Hole | $N_b$, $N_e$, $A_r$, $H_e$ | Annual |
| Centennial Valley | $N_b$, $N_e$, $A_r$, $H_e$ | Annual |
| Miner Lake | $A_r$, $H_e$ | 6-8 years |
| Mussigbrod Lake | $A_r$, $H_e$ | 6-8 years |
| Pintler Lake | $A_r$, $H_e$ | 6-8 years |

**Table 2.** Self-sustaining, introduced Arctic grayling populations in the upper Missouri River Basin, by subbasin.

| Subbasin | Populations |
|---|---|
| Big Hole | Agnes, Bobcat, Schwinegar, Odell |
| Beaverhead | Ruby |
| Madison | Gibbon |
| Gallatin | Hyalite, Deer, Emerald, Grayling |
| Sun | Levale, Gibson, Diversion, |
| Missouri | Park |

## Conservation Strategy

Individual conservation strategies have been developed for each of the 19 extant UMR grayling populations. These strategies describe all measures that are planned for each population over the next five years to achieve the overarching conservation goals and objective for UMR grayling. The operations cost to implement the UMR conservation strategy over the next five years is about $2.7 million and will require at least 34 staff. Costs to implement conservation measures include on-the-ground projects, contracted services, general operations, and genetic analyses. Staff refer to the total number personnel that are required to implement each conservation measure, with multiple conservation measures being implemented by the same personnel each year over the duration of this strategy. The requisite staffing is presently provided by FWP, DNRC, FWS, NRCS, USFS, BLM, YNP, CVA, TU, and TNC.

***Big Hole River:*** The Big Hole River contains an indigenous population of fluvial Arctic grayling which exist in the mainstem river, seasonally connected side channels, and the lower ends of eleven tributaries (Figure 2). The Big Hole River is a moderate-sized, free-flowing river system that originates in Skinner Meadows near Jackson and flows about 100 miles to its confluence with the Beaverhead River to form the Jefferson River near Twin Bridges. The upper Big Hole Valley is wide and flat, fed by X tributaries, and supports numerous cattle ranches dependent on irrigated hay and pasture grounds. The Big Hole River grayling population is the last indigenous, entirely fluvial grayling population in the lower 48 states (Shepard and Oswald 1989) and has been the center of focused research (Lohr et al. 1996, Byorth and Magee 1998, Vatland 2015, McCullough 2017); management and conservation (Kaya 1990, Kovach et al. 2019, USFWS 2020, FWP 2021), since the 1970s.

Big Hole grayling distribution and abundance declined to historic lows by the late 1990's but have since improved following targeted conservation. Consistent monitoring of the Big Hole River grayling began in the 1970s (Liknes 1981) and declines were observed at most monitoring sites by the late-1980s (Shepard and Oswald 1989). Declines were likely triggered by low-flow conditions that resulted in weak cohorts of grayling (Shepard and Oswald 1988, Kaya 1990). Historically, irrigation and stock water withdrawals completely dewatered the Big Hole River during drought years. Most recently, in 1988 near Wisdom the Big Hole River was dry for 24 days. Age-1 and older grayling abundances in the Wisdom Section decreased from 69/km in 1983 to 14/km in 1989 and the population continued to decline until the early 2000s (Magee et al. 2005; Kovach et al. 2019). Following these declines, the USFWS was petitioned to list grayling as threatened in 1991. FWP first hired staff to research grayling biology and develop management actions to address threats to the species. The Big Hole Watershed Committee was created in 1995 to address landowner concerns about grayling declines and develop strategies to prevent ESA listing, and the Big Hole CCAA program was created in 2006 to focus FWP, NRCS, DNRC, USFWS, and private landowner resources towards developing grayling conservation strategies on private lands and providing assurances against additional regulations if grayling become listed (USFWS 2006). Following drainage-wide conservation measures to improve instream flows, riparian health, fish passage, and entrainment in irrigation diversions, mean $N_b$ increased by over 160% (2012-2021) compared with past sampling (2006-2011) and genetic diversity has remained high and stable (Figure 3; Kovach et al. 2019). Currently, grayling occupy at least 100 miles of the mainstem river, select side channels, and tributaries, but are most concentrated between Divide and Wisdom (FWP unpublished data). Occupied tributaries include (upstream to downstream) Steel Creek, Swamp Creek, McVey Creek, North Fork Big Hole River, Plimpton Creek, Pintler Creek, York Gulch, Fish Trap Creek, Seymour Creek, LaMarche Creek, and Deep Creek.

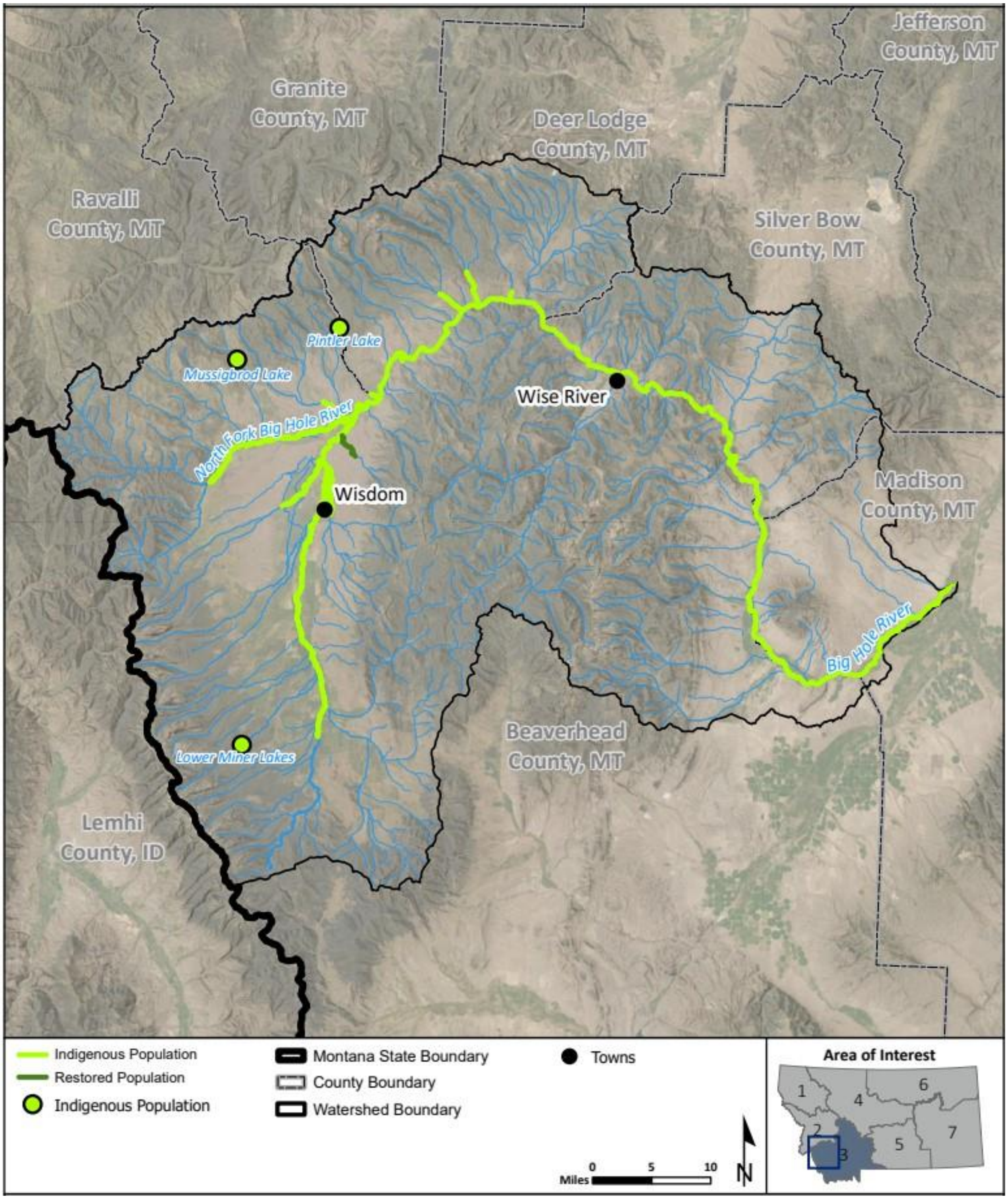


Figure 2. Distribution of Arctic grayling in the Big Hole River including three lakes with indigenous populations of grayling.

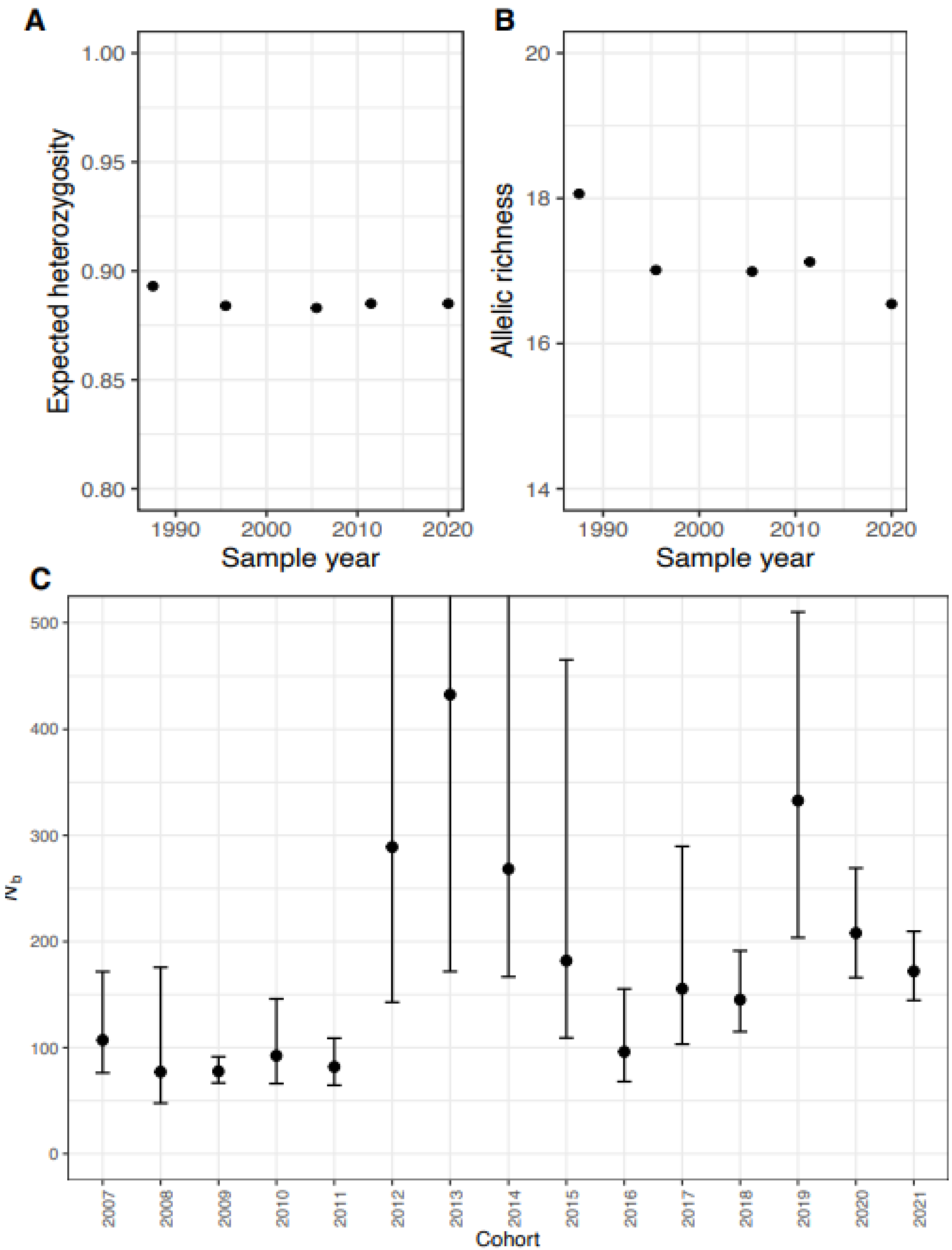


**Figure 3.** Genetic Status of Big Hole River grayling; A) Expected heterozygosity from 1990-2020, B) Allelic richness from 1990-2020, C) Number of effective breeders ($N_b$) in Arctic grayling from the Big Hole River from 2007-2021. Error bars are 95% confidence intervals.

The conservation strategy for Big Hole grayling is to 1) implement all conservation measures prescribed by the Big Hole CCAA program, 2) manage nonnative trout to benefit grayling, and 3) expand the distribution of grayling in conjunction with Westslope Cutthroat Trout restoration projects within the Big Hole drainage.

Implementation of the Big Hole CCAA is the primary grayling conservation strategy in the Big Hole drainage. The Big Hole CCAA explicitly addresses four threats to grayling persistence: 1) low instream flows, 2) degraded riparian habitat, 3) barriers to fish passage, and 4) entrainment in irrigation ditches (Table 3; USFWS 2006). The Big Hole CCAA has 32 landowners and over 160,000 acres of private and 5000 acres of State of Montana DNRC trust lands enrolled as of January 1st, 2022 (FWP 2021). Enrolled lands contain 245 miles of river and streams that are either occupied by grayling or contribute to grayling habitat. As part of their enrollment in the Big Hole CCAA, landowners working with agency personnel develop site-specific plans (SSP) for their lands (USFWS 2006). Each SSP includes conservation measures to provide adequate instream flow (i.e., irrigation management), improve riparian habitat, remove fish passage barriers, and reduce grayling entrainment in irrigation ditches. The Big Hole CCAA established instream flow targets that rely on voluntary reductions in water use by private water right holders. Coordinated implementation efforts have helped to achieve instream flow targets 77% of the time during May 1st – October 1st since 2006 and prevented the Big Hole River from going dry during the severe drought of 2021 as it had in previous years (DNRC unpublished data). Additionally, drainage-wide riparian conditions, which were characterized as “at risk” when the Big Hole CCAA was created, have improved 14% since 2006 and are now characterized as “sustainable” (USDA 2012; FWP 2021). Additional CCAA conservation measures to date have included 153 instream flow improvement projects such as diversions and water control or measurement structures, 84 stock water projects to conserve instream flows and improve riparian health, 71 projects involving about 100 miles of riparian fence, 17 livestock crossings, 80 fish ladders and 15 grade control structures to improve passage, and 43 stream restoration projects to improve grayling habitat across 40 miles of channel (FWP 2021). In 2020, the USFWS found that grayling did not warrant listing under the ESA specifically because of increasing abundances and habitat improvements resulting from the Big Hole CCAA (USFWS 2020). Implementation of all SSP’s and the conservation measures contained therein is expected to result in stable or increasing $N_b$, $N_e$, $A_r$, and $H_e$ and to maintain or increase grayling distribution in all reaches of the Big Hole CCAA (Table 3).

Managing nonnative fish is a secondary strategy for Big Hole grayling conservation. Nonnative Brook, Rainbow and Brown Trout are well-established with locally abundant populations throughout the Big Hole River and its tributaries. Although nonnative Brook Trout do not appear to affect habitat use or growth of juvenile Arctic grayling (Byorth and Magee 1998), a negative relationship exists between Brown Trout and grayling abundances (Kaya 1992; McCullough 2017). Long-term sampling near Jackson indicated a shift in the fishery from predominantly Brook Trout to Brown Trout over the past 15 years (McCullough 2017), which could lead to changes in the grayling population. Therefore, fishing regulations to encourage harvest of nonnative trout were established for the Big Hole River as a conservation measure to protect grayling. The entire Big Hole River is catch-and-release for grayling and the Brook and Brown Trout creel limits are 20 and 5, respectively, with no size restrictions upstream of Dickie Bridge (FWP 2022). A potential study design to evaluate the effects of nonnative trout interactions with grayling could include the suppression of nonnative trout in select tributaries that are known to be used by grayling while maintaining nonnative populations in other streams. If nonnative trout are a significant limiting factor of grayling distributions and abundances, management options for reducing or eliminating nonnative trout may be limited given their wide distribution and the social tolerance of management actions that might reduce popular sport fisheries.

Big Hole grayling distribution will be expanded by experimental introductions in conjunction with Westslope Cutthroat Trout (WCT) restoration projects. The WCT Conservation Strategy for the Missouri River Headwaters of Southwest Montana outlines potential projects to isolate WCT from nonnative species with constructed fish barriers and nonnative fish removal over large reaches of Big Hole River tributaries not presently occupied by grayling (FWP 2022b). Grayling introductions to tributaries of the Big Hole River with abundant nonnative fish populations have largely been unsuccessful (Olsen 2020); however, grayling became abundant and widespread in McVey Creek when experimentally introduced following removal of nonnative fish for WCT reintroduction. Therefore, streams where nonnative species have or will be removed may provide better opportunities for successful grayling reintroduction. Grayling will next be experimentally introduced in French Creek, where nonnative trout were removed from 40 miles of stream.

Finally, supplementing the Big Hole River grayling population with fish from a genetic reserve brood in Axolotl Lake may be necessary if the grayling $N_e$ declines below 50 for one or more generations in the Big Hole River. However, such measures should only be a last resort as genetic variation of the Axolotl Lake population is lower than the Big Hole population, and supplementation often has negative effects on fitness of wild-born salmonid fish (e.g., Christie et al. 2014). Management of the Big Hole genetic reserve grayling broods are described below.

Table 3. Measures to achieve Big Hole River Arctic grayling conservation strategies. Participating agencies or organizations are Montana Fish, Wildlife & Parks (FWP), U. S. Fish and Wildlife Service (USFWS), Montana Department of Natural Resources (DNRC), USFWS Partners for Fish and Wildlife Program (PFWLP), and USFWS Ecological Services.

| Waterbody | Conservation Measure | Lead Agencies | Cost |
|---|---|---|---|
| Big Hole and Tributaries | 200 days/year flow compliance & monitoring | FWP, DNRC | $25,000 operations & 2 existing staff |
| Big Hole Ditches | Improve functionality of headgates (~4 per year) | FWP, USFWS | $40,000 operations & 2 existing staff |
| Big Hole Ditches | Modify existing irrigation infrastructure (≈ 2/year) | FWP, USFWS | $10,000 |
| Big Hole Ditches | 7-10 miles entrainment surveys annually | FWP | $2,500 operations & 2 existing staff |
| Big Hole Ditches | Install one fish screen every five years as needed | FWP | $150,000 |
| Spawning Tributaries | Collect at least 120 YOY grayling genetic samples annually | FWP | $1,000 operations & 3 existing staff |
| Big Hole and Tributaries | Install fish ladders or step pools (≈ 3/year) | FWP, USFWS | $5,000 |
| Big Hole and Tributaries | Remove perched/nonfunctioning culverts (≈ 1/year) | FWP, USFWS | $5,000 |
| Big Hole and Tributaries | Install bridges (≈ 1/3 years) | FWP, USFWS | $25,000 |
| Big Hole and Tributaries | Install rock grade control diversions (≈ 1/year) | FWP, USFWS | $10,000 |
| Big Hole and Tributaries | Complete 70 miles of riparian assessments on enrolled property | FWP | $5,000 operations & 2 existing staff |
| Big Hole and Tributaries | Update and modify grazing plans (≈ 6/year) | FWP | 2 existing staff |
| Big Hole and Tributaries | Livestock stock water projects (≈ 2/year) | FWP, PFWLP | $30,000 |
| Big Hole and Tributaries | Fencing for riparian pasture management (≈ 3 miles/year) | FWP, CVA | $10,000 operations & 3 existing staff |
| Big Hole and Tributaries | Streambank and channel restoration (≈ 0.75 miles/year) | FWP, PFWLP | $20,000 |

***Centennial Valley:*** An indigenous grayling population resides in the Centennial Valley, which includes all tributaries of the Red Rock River and associated drainages upstream of Lima Dam (Figure 4). Landownership is divided among USFWS Red Rock Lakes National Wildlife Refuge (12%; Refuge), Beaverhead-Deerlodge National Forest (17%; USFS), DNRC (18%), BLM (26%), and private (27%). Centennial Valley geology (Sonderegger 1981), hydrology (Deeds and White 1926; FWP 1989; Montana Code Annotated 2000), fisheries (Nelson 1954; Randall 1978; Gillin 2001; Oswald et al. 2008; Gander et al. 2019a), and contemporary administrative status (USFWS 2009) are well-described elsewhere. Several historical summaries of Centennial Valley aquatic resources (Randall 1978; Gillin 2001), and specifically grayling (Vincent 1962; Unthank 1989), have been assembled. Centennial Valley grayling have been the focus of propagation (Henshall 1906; Kaeding and Boltz 1999; Gander et al. 2019a), management and conservation (Nelson 1954; Boltz 2000; Warren and Jaeger 2017), and research (Lund 1974; Gangloff 1996; Mogen 1996; Katzman 1998; Levine 2007; Paterson 2013; Davis 2016; Gander et al. 2019a) efforts in the Centennial Valley for over 100 years.

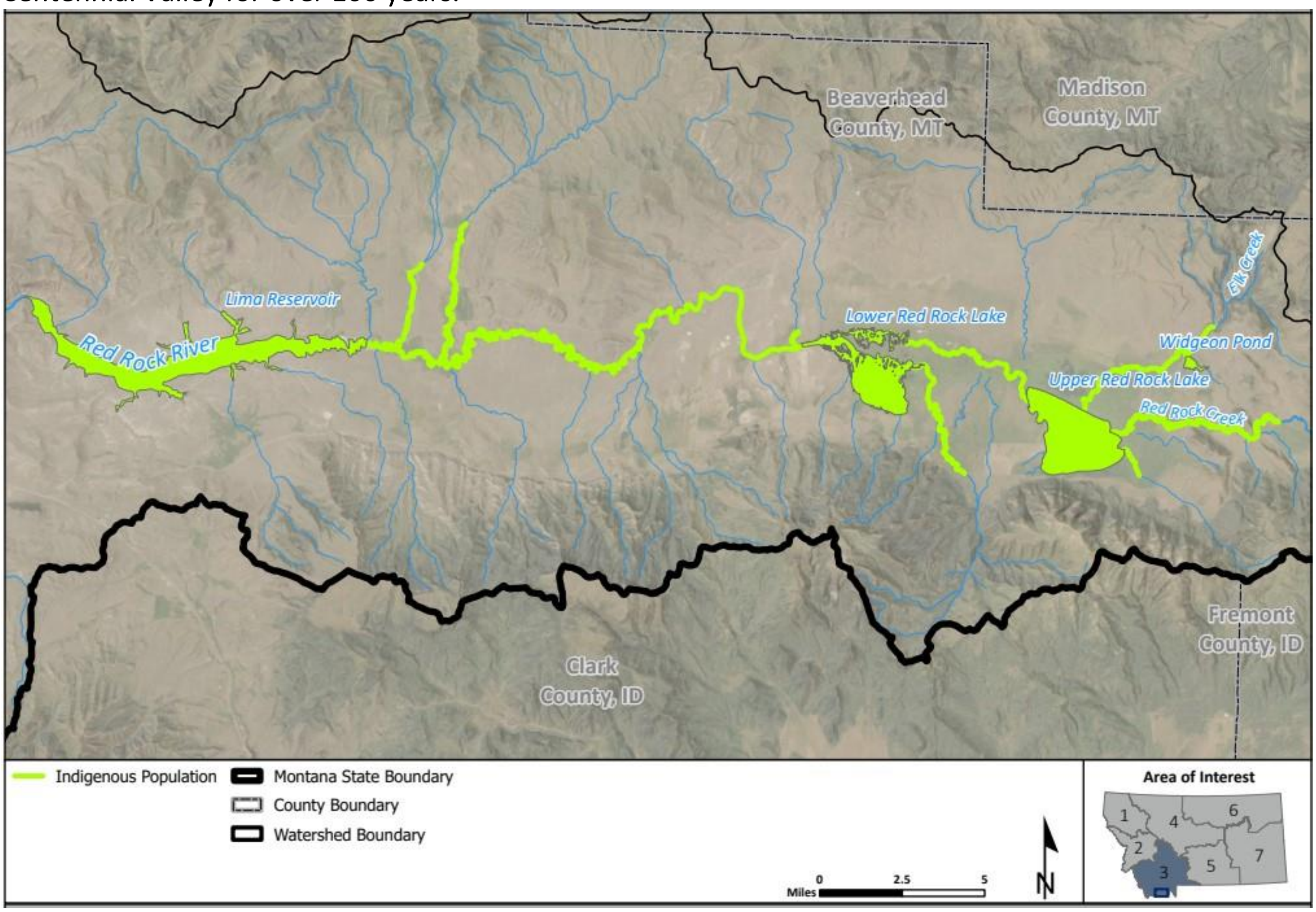


**Figure 4.** Arctic grayling in the Centennial Valley including the extirpated population in Elk Lake.

Centennial Valley grayling distribution has contracted and expanded over the past 100 years. Historical distribution is unknown but it is generally accepted that most suitable Centennial Valley tributaries and downstream portions of the Red Rock and Beaverhead watersheds were occupied by grayling (Evermann 1893; Nelson 1954). Specific and recurrent mention of large numbers of spawning grayling in Elk Springs (Henshall 1906) and Red Rock (Nelson 1954) creeks indicate they historically supported the highest abundances and most frequent use among Centennial Valley streams. Grayling remained relatively abundant and presumably widely distributed throughout the Centennial Valley until 1930 when a major decline in distribution occurred coincident with drought and chronic valley-wide dewatering; grayling that had previously occurred in streams year-round now only occupied Upper Red Rock Lake (Upper Lake) outside of spawning periods (Harding 1915; Deeds and White 1926; Vincent 1962). Failure of grayling to return to previous distributions and abundances during improved hydrologic conditions prompted the first extensive and focused fisheries survey of Centennial Valley waters in 1951, which documented grayling in 11 streams and 2 lakes (Nelson 1954). From the 1950s to 1990s, disparate sampling unevenly assessed and described Centennial Valley grayling distribution, which by 1999 declined to only two tributaries, Red Rock and O'dell creeks, and two lake populations, Upper and Lower Red Rock lakes (Lund 1974; Myers 1977; Randall 1978; USFWS 1985; Unthank 1989; Mogen 1996; Boltz 2000). Presence-absence electrofishing and gill netting surveys of historically occupied waters between 2010 and 2013 found grayling in seven streams (Red Rock, Elk Springs, Odell, Corral, Picnic, Tom, and Long creeks) and three lakes (Upper Red Rock and Elk lakes and Lima Reservoir; Gander et al. 2019a).

Centennial Valley grayling abundance and genetic variation have significantly declined in the past six years. Most Centennial Valley grayling spawn in Red Rock Creek and occupy Upper Lake the rest of the year, thus demographic and genetic population surveys in Red Rock Creek during spawning periods are used to assess population status (Paterson 2013; Gander et al. 2019). The grayling population significantly declined following near anoxic conditions in Upper Lake during the winter of 2016 and is presently at historically low abundances (Figure 5; Warren et al. 2022). Metrics of genetic diversity have similarly declined to historic lows, demonstrating the population is experiencing an increasingly severe genetic bottleneck (Figure 6; Kovach et al. 2019; Kovach et al. 2022). Immediate conservation intervention is necessary because each additional year of small population size erodes the evolutionary legacy and resilience of Centennial Valley grayling and increases the likelihood of stochastically driven extirpation.

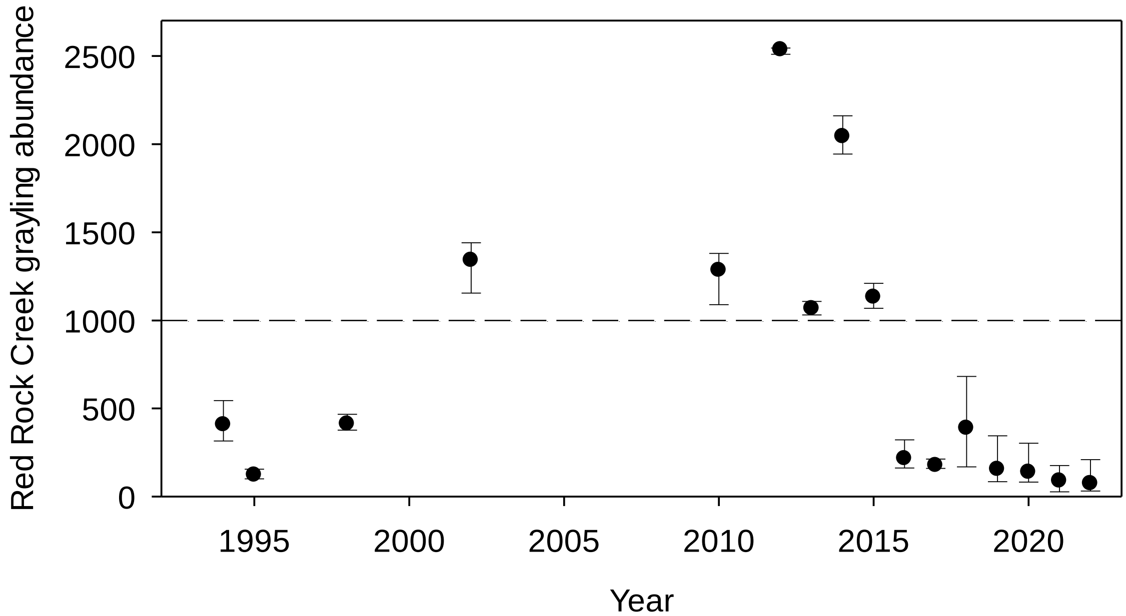


**Figure 5.** Abundances of spawning grayling in Red Rock Creek, 1994 to 2022. Error bars are the 95% confidence intervals. The dashed line represents the conservation goal of a population that supports at least 1000 spawning grayling.

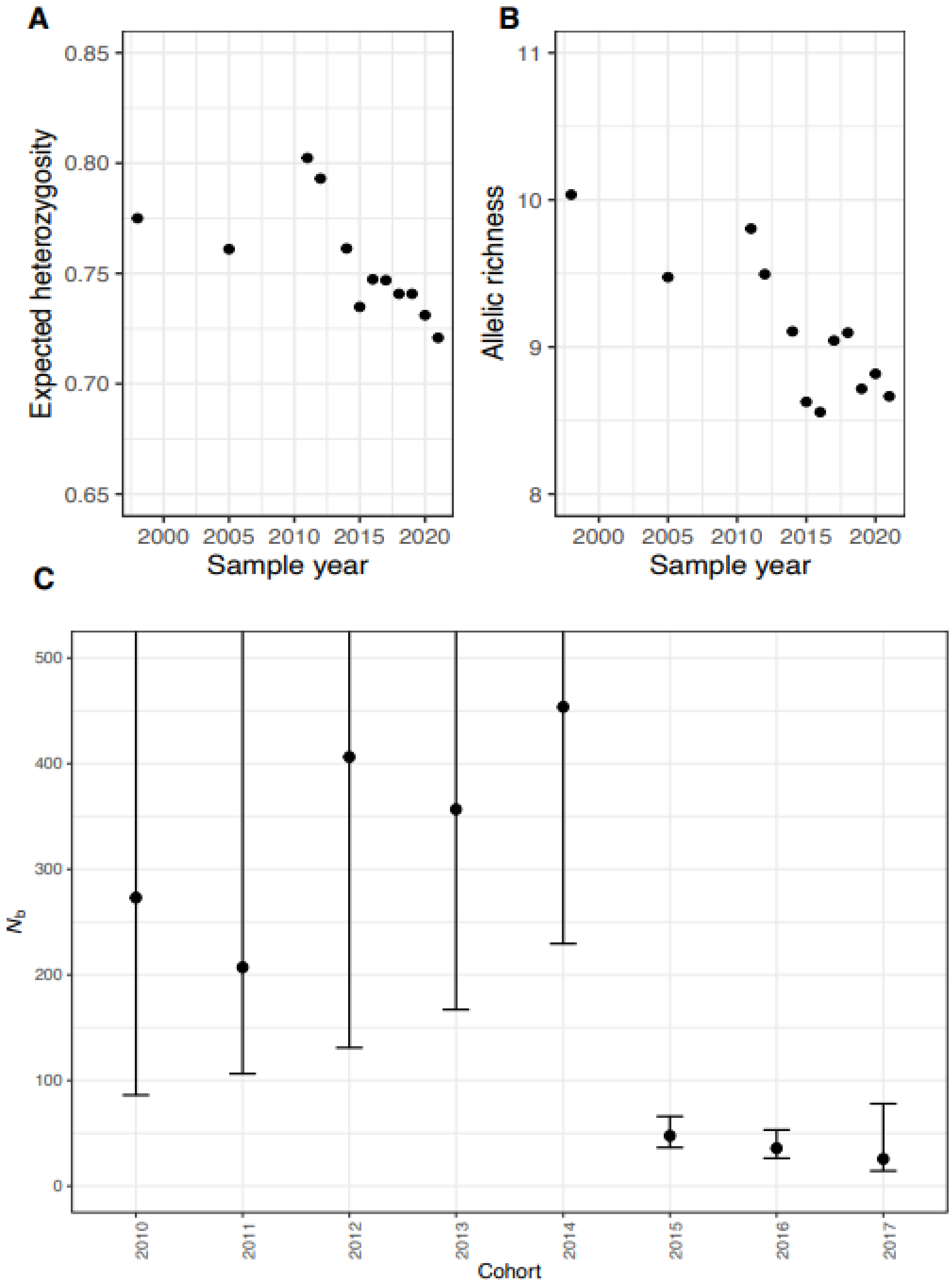


**Figure 6.** Genetic Status of Centennial Valley grayling; A) Expected heterozygosity from 2000-2020, B) Allelic richness from 2000-2020, C) Number of effective breeders ($N_b$) in Arctic grayling from Upper Red Rock Lake from 2010-2017. Error bars are 95% confidence intervals.

The Centennial Valley grayling conservation strategy was developed by a stakeholder workgroup comprised of agencies and NGOs that have direct grayling or land management authority in the Centennial Valley. The workgroup met annually between 2011 and 2021 to develop and reaffirm grayling conservation measures and supporting monitoring and science. The workgroup developed the following three strategies with the belief that, if collectively met, they will satisfy the species-wide population objective and goals within the Centennial Valley: 1) conserve existing Centennial Valley grayling genetic diversity, 2) establish or maintain grayling spawning and/or refugia in at least two tributaries up and downstream of Upper Lake and connectivity among tributaries, and 3) manage for at least 1000 spawning fish in the Upper Lake grayling population.

Genetic diversity of Centennial Valley grayling will be conserved by maintaining at least 1000 fish in the Upper Lake spawning population and creating a genetic reserve in Handkerchief Lake. Centennial Valley grayling genetic variation was high and stable when over 1000 fish were present in Upper Lake spawning population. However, grayling abundances have declined to historic lows following near anoxic conditions during the winter of 2016, which also led to decreases in $N_b$, $H_e$, and $A_r$ (Figure 6; Leary et al. 2015; Kovach et al 2022). Accordingly, highest emphasis will be placed on maintaining a robust, indigenous grayling population in the Centennial Valley to achieve this strategy, which will allow it to respond to native selective forces and most effectively build inherent, long-term resiliency against extirpation. Because the primary conservation strategy has not been achieved recently, a Centennial Valley genetic reserve grayling brood is being created in Handkerchief Lake by amalgamating stocked lake populations that most closely represent the ancestral Centennial Valley genetic legacy (Kovach 2021). Brood creation and management is described below. The genetic reserve population may be used to infuse genetic variation or restore Centennial Valley grayling to streams where they have been extirpated. Because founding populations have been isolated and genetically drifted from the Centennial Valley population, use of this population would only occur under extreme circumstances (e.g., genetic variability of extant population was lower than the brood) and as a last resort.

Grayling spawning and refugia habitats will be prioritized upstream of Upper Lake in Red Rock and Elk Springs creeks. Red Rock and Elk Springs creeks occur primarily on Refuge lands and have received considerable conservation focus. The Red Rock Compact and Jefferson River Basin closure ensures maintenance of instream flows on Red Rock Lakes tributaries (Montana Code Annotated 2000). A Comprehensive Conservation Plan stipulates numerous actions to benefit Arctic grayling and their habitats on the Refuge, including restoring formerly occupied Arctic grayling habitat and minimizing deleterious effects of grazing, dewatering, and habitat degradation (USFWS 2009). A CCAA was developed for Centennial Valley grayling that addresses riparian habitat, entrainment, fish passage, and instream flows on private lands (USFWS 2018). Conservative angling regulations (e.g., catch-and-release, spawning closure from May 1-June 15) are in place on both streams (FWP 2022). Elk Springs Creek restoration included MacDonald Pond removal in 2009, direct reconnection to Upper Lake in 2016, and headwaters and former lakebed spawning habitat restoration in 2016 and 2021, respectively. Red Rock Creek headwater and tributary habitat restoration occurred in 2010 and 2020 and, as part of CCAA site-specific plans, irrigation diversions were replaced and measuring devices installed to improve water control and fish passage. Additional habitat restoration and CCAA site-specific and grazing plan development are needed on Red Rock Creek, but this conservation strategy will otherwise be satisfied by annual population, fish barrier, and flow compliance and periodic entrainment monitoring (Table 4; Warren and Jaeger 2017; USFWS 2018; Warren et al. 2019).

Arctic grayling spawning and refugia habitat downstream of Upper Lake will be prioritized in Long, Middle, and West creeks. Long, Middle, and West creeks occur almost entirely on private lands. The Centennial Valley CCAA directly addresses the primary threats to grayling spawning and refugia habitat on those streams (degradation of riparian habitats, entrainment, fish passage, and instream flows; USFWS 2018). Restoration projects to improve riparian habitats, floodplain connectivity, and spawning and rearing habitats occurred between 2010 and 2021 on Long Creek. Reaches of Middle Creek captured by irrigation ditches were restored to their channels in 2019. Four irrigation diversions and five measuring devices were replaced to improve water control and fish passage among the streams and a hardened livestock crossing was installed on West Creek to improve riparian habitats between 2019 and 2021. Three headgates, measuring devices, and irrigation diversion modifications to improve fish passage and water control, one stock tank, about 0.5 miles/year of stream restoration, one grazing plan, two CCAA site-specific plans, and 40 days/year flow and compliance monitoring are needed to fulfill this conservation strategy (Table 4). Periodic population and entrainment monitoring is also required (Table 4; USFWS 2018).

The Centennial Valley Arctic Grayling Adaptive Management Plan (AMP) will be implemented to manage for at least 1000 spawning fish in the Upper Lake grayling population. The AMP is a tool intended to facilitate achievement of Centennial Valley grayling conservation goals in perpetuity (Warren and Jaeger 2017). The AMP was collaboratively developed by the Centennial Valley workgroup between 2012 and 2017 to resolve structural uncertainty about population drivers and long-standing disagreement among resource managers about what actions would most effectively conserve grayling (Warren and Jaeger 2017). The workgroup set a threshold for management intervention at 1000 spawning fish using an expert elicitation process that defined the population size where long-term self-sustaining persistence of grayling was expected (Boyd 2014). If the spawning population is less than 1000 fish, management actions predicted to restore the population to objective most quickly will be implemented (Warren and Jaeger 2017). The AMP investigated 1) reduction and alteration of spawning habitat, 2) predation by and competition with nonnative fishes, and 3) limited winter habitat in Upper Lake as potential population drivers by implementing sequential, large-scale management experiments. Overwinter habitat in Upper Lake was identified as and continues to be the primary population driver; a threshold level of 10–25 ha of habitat > 1 m deep and > 4mg/L $O_2$ appears necessary to overwinter 1000 grayling (Warren et al. 2022). An alternatives analysis was completed in 2019 to assess costs, logistical and legal feasibilities, and likely effects on grayling of winter habitat enhancement approaches (Flynn et al. 2019). Following piloting of several alternatives, tributary outlet modification, electric aeration, and building a berm to route Elk Springs Creek effluent to deep parts of the lake remain as viable options to create oxygenated deep-water habitat throughout winter (Flynn 2022). Selection and implementation of management alternatives to improve overwinter habitat in Upper Lake is necessary to fulfill this strategy and the overall conservation objective for Centennial Valley grayling; the other two conservation strategies are contingent on this strategy being successful to collectively preserve long-term self-sustaining persistence of grayling.

**Table 3.** Measures to achieve Centennial Valley Arctic grayling conservation strategies. Participating agencies or organizations are Montana Fish, Wildlife & Parks (FWP), U. S. Fish and Wildlife Service (USFWS), Red Rock Lakes National Wildlife Refuge (Refuge), USFWS Partners for Fish and Wildlife Program (PFWLP), USFWS Ecological Services, Centennial Valley Association (CVA), The Nature Conservancy (TNC), and Montana Trout Unlimited (TU).

| Waterbody | Conservation Measure | Lead Agencies | Cost |
|---|---|---|---|
| Red Rock Creek | At least 1.5 miles/year population surveys | FWP | $4,000 operations & 3 existing staff |
| Red Rock Creek | 11 miles/year provide fish passage at beaver dams | FWP, Refuge | $1,000 operations & 3 existing staff |
| Red Rock Creek | 1 mile stream restoration | FWP, PFWLP | $500,000 |
| Red Rock Creek | 1 CCAA site-specific and grazing plans | FWP, ES | Existing staff (5 FTE) |
| Red Rock Creek | 15 days/year flow compliance & monitoring | FWP, CVA | $5,000 operations & 3 existing staff |
| Red Rock Creek | 5 miles entrainment surveys every 10 years | FWP | $1,000 operations & 2 existing staff |
| Elk Springs Creek | 1 mile/year population surveys | FWP | $2,000 operations & 3 existing staff |
| Long, West, Middle Cr. | 2 miles population surveys every 5 years | FWP | $2,000 operations & 3 existing staff |
| Long, West, Middle Cr | 0.5 miles stream restoration/year | FWP, PFWLP, TNC | $40,000 |
| Long, West, Middle Cr | 2 CCAA site-specific plans | FWP, ES | 5 existing staff |
| Long, West, Middle Cr | 10 miles/year riparian assessments | FWP | $1,000 operations & 2 existing staff |
| Long, West, Middle Cr | 1 grazing plans/year | FWP | 2 existing staff |
| Long, West, Middle Cr | 1 stock tank | FWP, PFWLP | $30,000 |
| Long, West, Middle Cr | 40 days/year flow compliance & monitoring | FWP, CVA | $10,000 operations & 3 existing staff |
| Long, West, Middle Cr | 3 headgates | FWP, PFWLP | $20,000 |
| Long, West, Middle Cr | 3 diversion passage modifications | FWP, PFWLP | $20,000 |
| Long, West, Middle Cr | 5 miles entrainment surveys every 10 years | FWP | $1,000 operations & 2 existing staff |
| Upper Red Rock Lake | 10-25 ha habitat >1 m deep and > 4mg/L $O_2$ | FWP, Refuge, ES, TU | $650,000 |
| CV AMP | Substrate and winter habitat monitoring, data analysis, reporting | FWP,Refuge,CVA,TNC | $10,000 operations & 8 existing staff |

***Miner, Mussigbrod, and Pintler Lakes:*** Miner, Mussigbrod, and Pintler lakes in the Big Hole River drainage support native populations of grayling (Figure 2). Although each lake was stocked from the 1930s through the early 1950s with millions of grayling that were likely of Red Rock Lakes origin, genetics analyses indicate the grayling populations in these lakes are predominantly of Big Hole River ancestry (Peterson and Arden 2009; Leary et al. 2015, Kovach et al. 2021). Nonnative Brook Trout are common in the lakes as well as several native species including Burbot, Longnose Sucker, and White Sucker.

Mussigbrod Lake is the largest (116 acres) and one of the deepest (> 70 ft) lakes in the Big Hole drainage. Historically, Mussigbrod Creek was well-connected to Mussigbrod Lake; however, a dam across the historical outlet of the lake was constructed, which raised lake elevation 10-15 ft and moved the outlet several hundred feet to the west at full pool. The new outlet is a concrete spillway with a 3-ft drop to a constructed boulder channel that connects to the natural stream channel. The altered outlet serves as an upstream barrier to fish passage into Mussigbrod Lake except during exceptionally high flow events. The lake surface elevation typically decreases to its historical level as irrigation demand increases in the summer months; however, the lake often refills by the following June. Despite habitat modifications and lake elevation fluctuations, negligible effects on grayling have resulted and the population remains abundant (Olsen 2014). Spawning occurs in the new outlet immediately upstream of the spillway and in other areas of the lake in mid-May. Grayling occur in high densities near the inlet of Mussigbrod Creek during the same timeframe indicating the inlet stream may also serve as spawning habitat. Grayling are rarely captured in Mussigbrod Creek upstream and downstream of the lake outside of the spawning season despite abundant, high quality spawning habitats in those reaches. Oswald et al. (2007) and Olsen (2014) provide additional information dating back to the early 1970s for the Mussigbrod Lake grayling population.

Miner Lake is 66 acres, has a maximum depth of 34 ft (Olsen 2014), and is well-connected to Miner Creek. The lake consists of large, shallow (< 3 ft) silt flats with two distinct depressions in the upper lobe of the lake. Recent data collected at the Miner Lake suggests the grayling population is abundant (Olsen 2014). Grayling spawn in the narrows between the upper and lower lobes of the lake. Although grayling have been captured in high abundances near the inlet, no spawning has been observed there. Spawning has not been documented in the outlet stream either, despite it having high-quality habitat. Similar to Mussigbrod Lake population, grayling are rare in Miner Creek upstream and downstream of the lake. Oswald et al. (2007) and Olsen (2014) provide additional information dating back to the early 1960s for the Miner Lake grayling population.

Pintler Lake is the smallest of the three lakes that support native grayling populations in the Big Hole River drainage. The lake is about 39 acres with a maximum depth of 21 ft. Although depths exceed 10 ft near the inlet, about 75% of Pintler Lake is < 4 ft deep with extensive silt flats and aquatic macrophytes. Pintler Creek above the lake flows through a large wetland with extensive beaver activity, which may restrict fish passage. Pintler Falls, located approximately 1.3 miles upstream of Pintler Lake, is a complete upstream barrier to fish passage. The stream below the lake is low gradient and highly sinuous with abundant high quality spawning habitat. Sampling in 1964, 2006, and 2009 indicated that suckers are the most common fish in Pintler Lake followed by grayling and brook trout (Olsen 2014). Grayling abundances in Pintler Lake have been stable or slightly increasing since initial sampling efforts in 1964. The average size of grayling in Pintler Lake is greater than that observed in either Miner or Mussigbrod lakes with some fish approaching 15 inches.

The conservation strategy for Mussigbrod, Miner and Pintler lakes grayling is to monitor genetic variation to maintain a stable or increasing genetic effective population size and amalgamate the populations in Twin Lakes. The primary conservation goal for the Mussigbrod, Miner and Pintler lakes grayling populations is to maintain their genetic variation. Genetic variation of the Miner and Mussigbrod grayling populations is stable (Table 5), a result of high grayling abundances and suitable habitats in both lakes. Updated genetic assessment of Pintler Lake grayling is needed to determine the status of that population (Table 5). If future declines in genetic variation are observed, then lake-specific conservation strategies will be developed based on factors limiting the population. Conservation strategies would attempt to improve genetic variation by implementing measures likely to increase grayling abundances and/or simulate geneflow by translocating grayling from one of the other two native Big Hole lake populations. Replicating the Miner, Mussigbrod and potentially Pintler lakes grayling populations in Twin Lakes is a secondary conservation strategy. Twin Lakes is a low elevation lake in the Big Hole River drainage that was potentially occupied by grayling prior to 1900 (Shields 1897) and is also home to a native population of Lake Trout. About 150,000 eggs from Mussigbrod Lake and 50,000 eggs from Miner Lake were incubated in the narrows between Twin Lakes in 2017 and 2019. No subsequent monitoring of the lake has been completed, so the success of the introduction is presently uncertain. If monitoring indicates the previous introduction did not produce a self-sustaining population then native Big Hole lakes grayling will be stocked at a density of 1,500-2,000 fish per acre for at least a three-year period. If the Twin Lakes introduction is unsuccessful, 1-2 populations will be established in lakes elsewhere in the Big Hole River drainage. This conservation measure will increase the geographic distribution of native Big Hole lakes grayling and replicating these populations will create a genetic reserve that could be used for subsequent grayling introductions or to improve genetic variation of the native Big Hole lake populations as necessary. Big Hole lakes conservation measures are expected to cost $1,000 to $10,000 each and will be completed by existing FWP staff (Table 6).

**Table 4.** Genetic metrics of Arctic grayling from native Big Hole River lakes ($H_e$ = average heterozygosity; $A_R$ = allelic richness; N = sample size).

| Population | Year | $H_e$ | $A_r$ | N |
|---|---|---|---|---|
| Miner Lake | 2020 | 0.81 | 9.26 | 41 |
| Miner Lake | 2016 | 0.80 | 9.36 | 58 |
| Miner Lake | 2006 | 0.80 | 9.24 | 37 |
| Mussigbrod Lake | 2020 | 0.75 | 7.50 | 32 |
| Mussigbrod Lake | 2012 | 0.75 | 7.69 | 50 |
| Mussigbrod Lake | 2006 | 0.75 | 7.88 | 48 |
| Pintler Lake | 2009 | 0.67 | 4.79 | 32 |

**Table 6.** Measures to achieve Mussigbrod, Miner and Pintler lakes grayling conservation strategies. Participating agency is Montana Fish, Wildlife & Parks (FWP).

| Waterbody | Conservation Measure | Lead Agency | Cost |
|---|---|---|---|
| Mussigbrod Lake | Monitoring every 6-8 yrs | FWP | $1,000 operations & 3 existing staff |

| | | | |
|---|---|---|---|
| Miner Lake | Monitoring every 6-8 yrs | FWP | $1,000 operations & 3 existing staff |
| Pintler Lake | Monitoring every 6-8 yrs | FWP | $1,000 operations & 3 existing staff |
| Twin Lakes | Monitoring, additional introductions | FWP | $10,000 operations & 5 existing staff |

***Ruby River***: Grayling were reintroduced to the Ruby River to establish a stable, naturally reproducing population (Byorth 1996). The upper Ruby River above Ruby Reservoir (Ruby River) was selected for grayling reintroduction because of size (≈ 41 unfragmented miles of habitat), low gradient (mean = 0.7%), abundant pool habitat, and low density of nonnatives (Kaya 1992; Liermann 2001). Arctic grayling currently exist in most of the mainstem habitat of the Ruby River above Ruby Reservoir (Figure 7). Grayling were initially introduced by stocking fry and fingerlings (1997-2005); however, the subsequent use of remote site incubators (2003-2008) had better results (Gander et al. 2019b). Reintroduction efforts concluded in 2008 and the population was deemed a self-sustaining UMR conservation population when natural reproduction was documented for 10 consecutive years and eDNA results showed a wide distribution (Gander et al. 2019b). Post-stocking measures of genetic diversity ($H_e$ and $A_r$) were relatively high and stable from 2010 to 2018 (Table 7). However, declines in $N_b$ suggest that periodic future monitoring is warranted, as the current estimates of $N_b$ indicate that genetic diversity may decline in future generations.

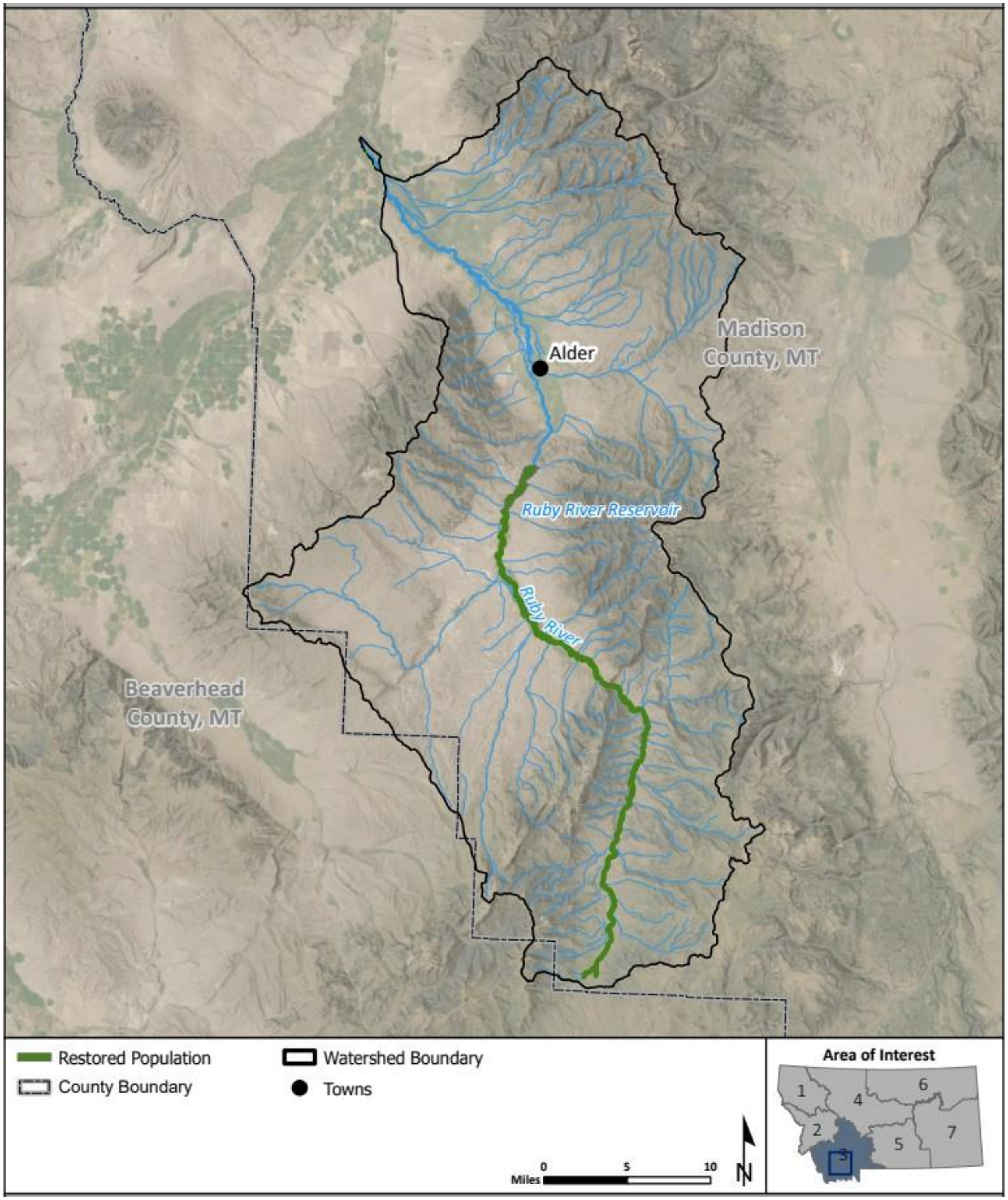


**Figure 7**. Distribution of the reintroduced Arctic grayling population in the upper Ruby River.

**Table 7.** Genetic monitoring results for grayling in the upper Ruby River, 2010 to 2018. Non-parenthesized sample sizes refer to mixed age samples used to estimate average expected

heterozygosity ($H_e$) and allelic richness ($A_r$) in a given year. Parenthesized samples refer to the number of samples from a given cohort used to estimate effective number of breeders ($N_b$).

| Year | $H_e$ | $A_r$ | $N_b$ | Sample Size |
|---|---|---|---|---|
| 2010 | 0.7930844 | 7.804212 | 23.5 (15.8, 36.9) | 25 (48) |
| 2011 | 0.8294469 | 9.054225 | 24.8 (12.6, 86.1) | 27 (19) |
| 2012 | 0.8554041 | 9.608342 | 42.1 (17.2, Inf.) | 27 (20) |
| 2013 | NA | NA | NA | NA |
| 2014 | NA | NA | 20.2 (11.6, 38.8) | NA (38) |
| 2015 | 0.8573938 | 9.438658 | 13.4 (7.0, 26.1) | 19 (36) |
| 2016 | 0.8588745 | 9.59538 | NA | 28 |
| 2017 | 0.8244626 | 8.000473 | 6.9 (3.9, 9.8) | 78 (76) |
| 2018 | 0.8502296 | 8.903817 | NA | 33 |

The conservation strategy for the Ruby River grayling population is to monitor genetic variation to maintain a stable or increasing genetic effective population size. If future declines in genetic variation are observed, conservation measures would include genetic infusion by introducing embryos via remote site incubators at similar locations and densities as during grayling introductions (Gander et al. 2019b). The Ruby River grayling population will be monitored next in 2023 by FWP and USFS staff (Table 8).

**Table 8.** Measures to achieve the Ruby River grayling conservation strategy. Participating agencies are Montana Fish, Wildlife & Parks (FWP) and the U.S. Forest Service (USFS).

| Waterbody | Conservation Measure | Lead Agency | Cost |
|---|---|---|---|
| Ruby River | Monitoring every 6-8 yrs | FWP, USFS | $5,000 operations & 6 existing staff |

***Madison River:*** Grayling were historically widely distributed and abundant in the Madison River but are now believed to be extirpated. The decline of grayling in the Madison River was more gradual than in neighboring drainages (e.g., Gallatin, Sun, Smith rivers) because land and water use was initially less severe in the Madison (Vincent 1962). The construction of Ennis and Hebgen reservoirs in 1901 and 1914, respectively, eliminated many spring creek complexes that served as the primary spawning areas for grayling in the Madison River. Specifically, Horsethief Springs, Grayling Creek, and South Fork Madison River contained abundant grayling populations prior to the construction of Hebgen Dam (Vincent 1962). Grayling were common throughout the Madison River between Ennis Dam and Hebgen Dam until the 1950s (Kaya 1990) but declined to a small remnant population near Ennis Reservoir by 1980 (Byorth and Shepard 1990). Competition with and predation by nonnative trout was considered the primary limiting factor for grayling in the Madison River, but this was exacerbated by the inundation of Hebgen Dam and ultimately both factors contributed to their extirpation (Vincent 1962). Arctic grayling in the Madison River are occasionally reported by anglers but not detected during standardized fisheries sampling. As a result, indigenous grayling have likely been extirpated from the drainage leaving only recently introduced fish at low abundances that cannot support a self-sustaining population. Arctic grayling in the Madison River are now considered to be reintroduced populations with reintroduction efforts ongoing (Figure 8).

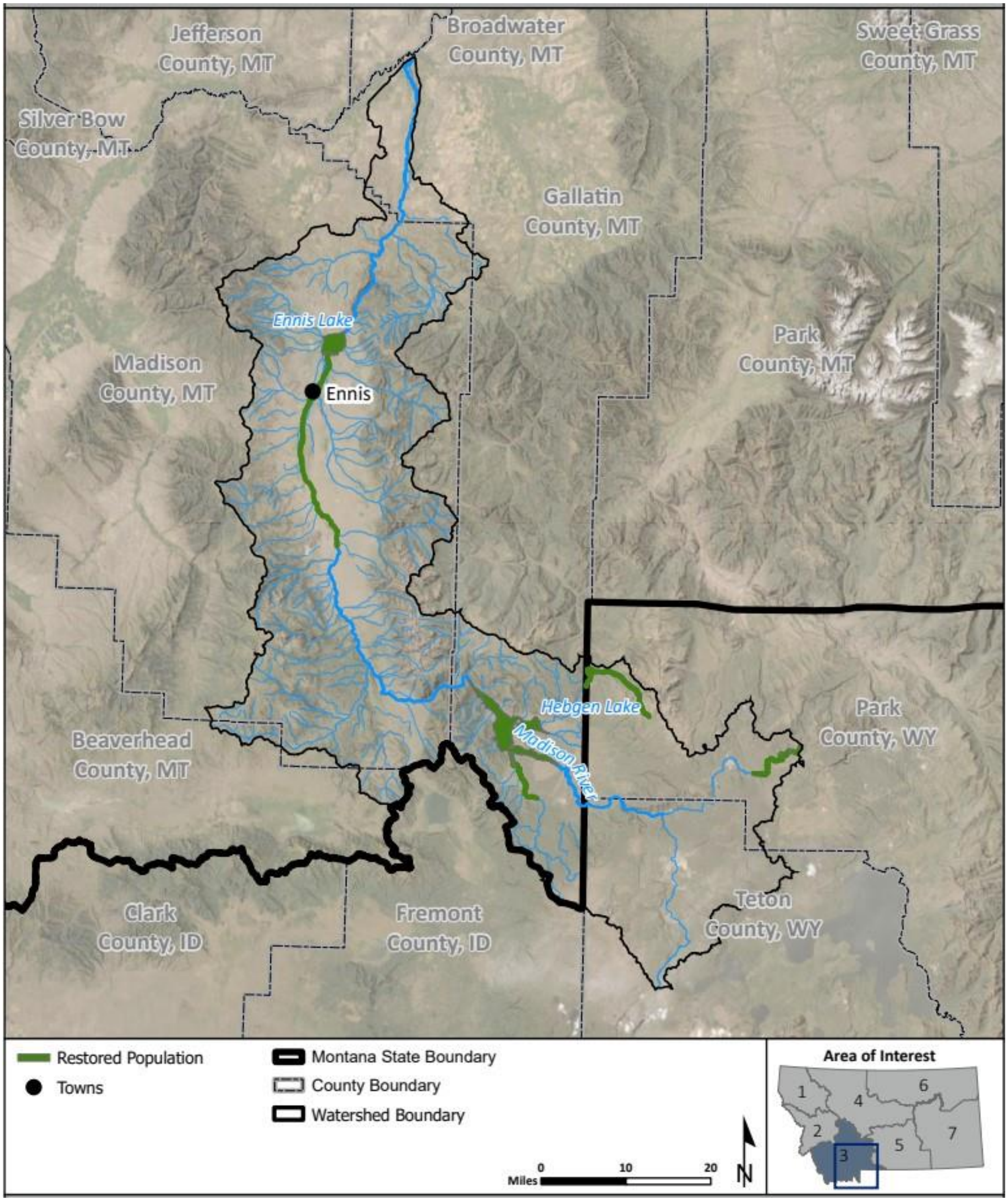


Figure 8. Current distribution of reintroduced grayling in the Madison River.

The conservation strategy for the Madison River is to establish at least two viable populations in Hebgen Reservoir and its tributaries. Previous unsuccessful reintroduction attempts in the Madison River drainage were concentrated in spring creeks near Ennis Reservoir. High densities of brown trout in the tributaries and the mainstem Madison River are believed to have precluded the establishment of viable populations. Therefore, future introductions in the Madison River drainage will be limited to Hebgen Reservoir and its tributaries. Introduction efforts will include at least 500,000 eggs/year for 3-5 consecutive years based on the large size of Hebgen Reservoir (5,080 hectares; Table 9). Introductions will also occur in the upper reaches of Grayling Creek and the Gibbon River where nonnative fish have been removed as well as the South Fork Madison River where densities of resident nonnative trout are low (Table 9). Grayling from populations with primarily Madison River genetic ancestry will be used for reintroductions when possible (Kovach et al. 2021). The Axolotl Lake population may also be used to meet demographic goals.

**Table 9.** Measures to achieve Madison River grayling conservation strategies. Participating agencies are Montana Fish, Wildlife & Parks (FWP), Yellowstone National Park (YNP), the U.S. Forest Service (USFS), and Northwestern Energy, who funds salary for FWP staff working on grayling conservation.

| Waterbody | Conservation Measure | Lead Agency | Cost |
|---|---|---|---|
| S. Fk. Madison | Introductions, monitoring | FWP, USFS | $5,000 operations & 6 existing staff |
| Gibbon River | Monitoring, additional introductions | YNP, FWP | $5,000 operations & 10 existing staff |
| Grayling Creek | Monitoring, additional introductions | YNP, FWP | $5,000 operations & 10 existing staff |

***Gallatin River:*** Indigenous grayling were extirpated from the Gallatin River over a century ago but introduced grayling presently exist in a reach of the Gallatin Canyon. Grayling were historically abundant and widespread throughout the mainstem Gallatin River, spring creeks, and lower reaches of other large tributaries (Kaya 1990); however, they were probably extirpated from the Gallatin River drainage by the early 1900s because of competition with and predation by nonnative species and habitat degradation mainly in the form of water withdrawals (Vincent 1962). Grayling currently exist in low abundances and are primarily limited to reaches in the Gallatin River between Big Sky and Wilson Bridge near Gallatin Gateway (Figure 1). Grayling inhabiting the Gallatin River probably dispersed from Green Hollow Pond, which supports a Big Hole genetic reserve population. Angler reports have been limited to adult fish and no grayling have been captured during long-term monitoring efforts in the Gallatin River or its tributaries, so it is unlikely that a self-sustaining population exists.

The conservation strategy for the Gallatin drainage is to establish grayling populations in Chiquita Lake and in one stream. In 2021 and 2022, 6,000 grayling fingerlings per year, which were the progeny of fish captured in Rogers Lake, were released into Chiquita Lake. Chiquita Lake is a small lake (1.4 hectares) in the headwaters of the North Fork of Spanish Creek where nonnative trout were removed as part of a Westslope Cutthroat Trout reintroduction. Additional stocking is planned for Chiquita Lake in 2023. Establishing a self-sustaining population in a different mountain lake within the Gallatin River drainage will be pursued should efforts in Chiquita Lake fail. Reintroductions in the upper Gallatin River or its tributaries will occur once reintroductions in the Madison River drainage are complete or hatchery capacity increases to ensure sufficient eggs and juvenile fish for larger introductions (> 500,000/year) are available for ongoing efforts. Based on historical occupancy and quality of existing habitats, the headwaters of the Gallatin River, Snowflake Springs near the Yellowstone National Park boundary, and the Taylor Fork are probable reintroduction locations. When possible, introductions in the Gallatin River drainage will occur with grayling of primarily Madison genetic ancestry (Kovach et al. 2021) but may be supplemented with fish from Green Hollow or Axolotl lakes to meet demographic goals.

**Table 10.** Measures to achieve Madison River grayling conservation strategies. Participating agencies are Montana Fish, Wildlife & Parks (FWP), Yellowstone National Park (YNP), the U.S. Forest Service (USFS), and Northwestern Energy, who funds salary for FWP staff working on grayling conservation.

| Waterbody | Conservation Measure | Lead Agency | Cost |
|---|---|---|---|
| Chiquita Lake | Introductions, monitoring | FWP, USFS | $5,000 operations & 6 existing staff |
| Gallatin streams | Introductions, monitoring | FWP, USFS, YNP | $5,000 operations & 6 existing staff |

***Mountain Lakes:*** Widespread, historical stocking in mountain lakes throughout Montana established 12 UMR grayling conservation populations (Figure 1; Tables 11 & 12). Introduced grayling populations with conservation value are self-sustaining and occur in lakes or reservoirs within the UMR (USFWS 2014). Viable grayling populations outside of the UMR have value as genetic reserves but are not explicitly considered as conservation populations because they occur outside of the geographic boundary of the Distinct Population Segment (DPS; USFWS 2020). Analysis of the genetic ancestry of all introduced populations, inside and outside of the DPS, identified potential donor sources for introductions in the Centennial Valley and Madison River drainages (Kovach et al 2021). Genetic monitoring also revealed a relatively stable trend in genetic variation for most established grayling populations (Table 12).

The conservation strategy for introduced UMR mountain lakes grayling populations is to monitor genetic variation to maintain a stable or increasing genetic effective population size. If future declines in genetic variation are observed, conservation measures would include genetic infusion from other populations with appropriate genetic ancestry, including those outside of the UMR, by introducing embryos via remote site incubators or stocking fry or fingerlings (Table 11).

**Table 11.** Characteristics and primary genetic ancestry of introduced grayling mountain lake populations (CV = Centennial Valley; M = Madison River; RB = rainbow trout; RBxCT = hybrid cutthroat; EB = brook trout; YCT = Yellowstone cutthroat trout; WCT = westslope cutthroat trout; WSU = white sucker).

| Lake Name | Drainage | Elevation (ft) | Lake Size (ac) | Total length (mm) | Other Species Present |
|---|---|---|---|---|---|
| Agnes Lake* | Big Hole | 7537 | 108 | 260-354 | None |
| Bobcat Lake*[CV] | Big Hole | 8405 | 6 | 190-305 | None |
| Odell Lake*[CV] | Big Hole | 8390 | 33 | 200-320 | RBxCT, EB |
| Schwinegar Lake*[CV] | Big Hole | 8350 | 4 | 101-305 | None |
| Emerald Lake* | Gallatin | 8980 | 14 | 254-301 | None |
| Grayling Lake*[M] | Gallatin | 8360 | 3 | 208-296 | None |
| Hyalite Reservoir* | Gallatin | 6704 | 158 | 285-431 | EB, YCT |
| Deer Lake*[M] | Gallatin | 9105 | 12 | 212-368 | None |
| Park Lake*[CV] | U. Missouri | 6320 | 32 | 152-305 | WCT |
| Lake LeVale*[M] | Sun | 7357 | 12 | 75-125 | None |
| Gibson Reservoir* | Sun | 4798 | 1288 | N/A | EB, RB, WCT, WSU |
| Grebe Lake* | Madison | 8023 | 156 | N/A | WCT |
| Cliff Lake | Clark Fork | 8593 | 16 | 183-284 | None |
| Rogers Lake[M] | Flathead | 3998 | 239 | <406 | WCT |
| Red Meadow Lake[M] | Flathead | 5605 | 16 | 178-279 | WCT |
| Meadow Lake[M] | Wyoming | 7900 | 50 | N/A | None |
| Elizabeth Lake[CV] | Belly | 4896 | 195 | N/A | RB |
| Cascade Lake | Yellowstone | 7995 | 30 | N/A | YCT |

* conservation population
[CV] primarily of Centennial Valley ancestry
[M] primarily of Madison River ancestry

**Table 12.** Heterozygosity ($H_e$) and allelic richness ($A_r$) of introduced grayling lake populations.

| Population | Year | $H_e$ | $A_r$ |
|---|---|---|---|
| Agnes Lake | 2020 | 0.83 | 9.46 |
| Agnes Lake | 2016 | 0.83 | 9.55 |
| Bobcat Lake | 2020 | 0.79 | 6.88 |
| Bobcat Lake | 2012 | 0.80 | 7.27 |
| Bobcat Lake | 2006 | 0.79 | 7.59 |
| Cliff Lake | 2020 | 0.79 | 7.76 |
| Deer Lake | 2020 | 0.71 | 5.98 |
| Deer Lake | 2017 | 0.70 | 6.09 |
| Elizabeth Lake | 2019 | 0.82 | 8.92 |
| Emerald Lake | 2020 | 0.82 | 8.18 |
| Emerald Lake | 2013 | 0.81 | 7.91 |
| Grayling Lake | 2020 | 0.74 | 5.56 |
| Grayling Lake | 2012 | 0.70 | 5.10 |
| Hyalite Reservoir | 2020 | 0.79 | 7.24 |
| Hyalite Reservoir | 2013 | 0.68 | 5.52 |
| Lake LeVale | 2020 | 0.82 | 7.70 |
| Odell Lake | 2020 | 0.80 | 9.18 |
| Odell Lake | 2006 | 0.81 | 9.09 |
| Park Lake | 2020 | 0.81 | 8.16 |
| Park Lake | 2016 | 0.83 | 8.19 |
| Red Meadow Lake | 2020 | 0.81 | 7.43 |
| Rogers Lake | 2020 | 0.78 | 7.63 |
| Schwinegar Lake | 2020 | 0.81 | 7.77 |

**Table 13.** Measures to achieve mountain lakes grayling conservation strategy. Participating agencies are Montana Fish, Wildlife & Parks (FWP) and the U.S. Forest Service (USFS).

| Waterbody | Conservation Measure | Lead Agency | Cost |
|---|---|---|---|
| Introduced Mountain Lakes | Monitoring every 6-8 yrs | FWP, USFS | $5,000 operations & 6 existing staff |

# UMR Grayling Genetic Reserve Brood Populations

The need to create a genetic reserve for Montana grayling was first identified in the 1980s when conservation actions for the Big Hole River population were initiated (Leary et al. 1991). Two brood reserves were subsequently created for Big Hole River grayling in the 1990s. Management goals for the Big Hole River genetic reserve populations are to 1) replicate the genetic diversity of the Big Hole River Arctic grayling population and 2) minimize genetic and phenotypic differentiation between the brood and wild Big Hole River populations. Attempts were made to create a genetic reserve for the Centennial Valley population prior to its crash in 2016. However, initial efforts were not successful and attempts to create a Centennial Valley genetic reserve are ongoing using non-indigenous populations of grayling with a primary ancestry from the Centennial Valley.

Brood populations may be self-sustaining or perpetuated through onsite spawning and replacement (stocking). Initial founding of self-sustaining or perpetuation of non-self-sustaining broods should occur using spawning practices that maximize parental contribution so as not to allow genetic diversity to decline from levels observed in wild populations. In these cases, two females should be spawned with 2 males using a 2x2 cross (Figure 9). The eggs of two females will be split into two equal groups with each split fertilized by one male. Eggs should be incubated separately so the number of parents successfully contributing to each year class is precisely known and contributions can be equalized (Table 7). Year classes should be marked with a unique tag so that different year classes may be crossed, which will convert year class differences in genetic diversity to within population genetic diversity. In both types of populations (stocked and self-sustaining), it will be occasionally necessary to infuse wild genetics to prevent genetic straying. For the Big Hole River brood, it is recommended that at least ten wild pairs of grayling are spawned and infused every ten years (Leary 1991). For the Centennial Valley brood, wild fish infusions are not presently possible due to low population levels but will be incorporated once abundances rebound. Infused wild genes should represent 10% or less of all genes in a given year class to prevent a genetic bottleneck (Leary 1991).

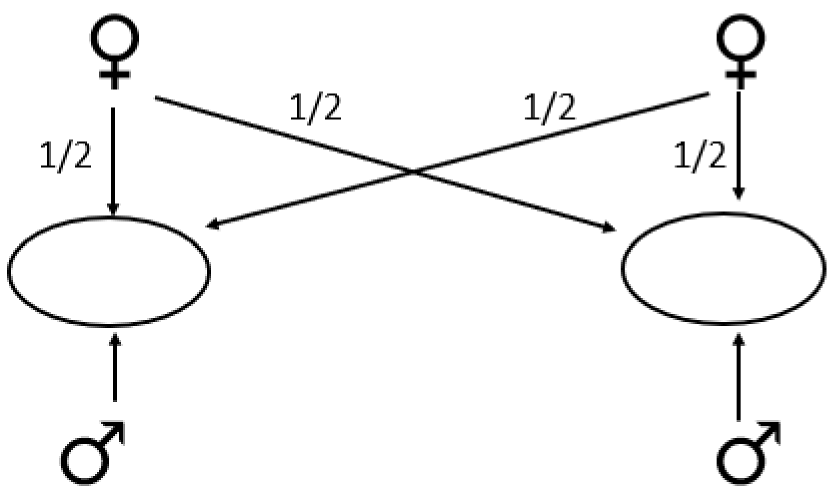


**Figure 9.** Schematic diagram for a typical 2x2 spawning cross.

***Axolotl Lake:*** A Big Hole River Arctic grayling genetic reserve was established in Upper Twin Lake (Axolotl Lake) in the Gravelly Mountains in 1991. No natural reproduction occurs in Axolotl Lake and the population is maintained by annually stocking age-1 fish obtained from onsite spawning of the Axolotl population. The purpose of the Axolotl Lake population was to establish an additional population with Big Hole River ancestry that could be used to augment natural grayling reproduction in the Big Hole or reestablish grayling in the Big Hole River in the event of extirpation. Direct introductions of captive fish are not recommended into the Big Hole River unless the population begins to rapidly lose genetic variation and is at immediate threat of extinction (Leary 1991). Axolotl Lake was the founding source of the reintroduced Ruby River population and is currently used for reintroduction efforts in the upper Madison River drainage.

The genetic diversity of the Axolotl Lake genetic reserve population diverged from the wild Big Hole River population following initial spawning efforts in Axolotl Lake (Leary 1991; Kovach et al. 2019). The allelic richness and expected heterozygosity of Axolotl Lake were found to be 8.5% and 1.0% lower, respectively, then the Big Hole River population in 2019. Genetic diversity was even lower when compared to the genetic variation of the Big Hole River in the early 1990s when the population was founded (Kovach et al. 2019). Although it was recommended that direct introductions to the Big Hole River do not occur except when necessary, remote site incubators have been used to introduce eggs to the upper mainstem Big Hole River and its tributaries (e.g., Rock Creek, Wise River, Governor Creek, McVey Creek). Successful introductions, such as McVey Creek may have contributed to a reduction in the genetic variation of the wild population (Table 15; Kovach et al. 2019).

Management goals for the Axolotl Lake genetic reserve population are to 1) replicate the genetic diversity of the Big Hole River Arctic grayling population and 2) minimize genetic and phenotypic differentiation between the brood and wild Big Hole River populations (Table 14).

Table 14. Conservation measures for the perpetuation of the Big Hole River Arctic grayling genetic reserve in Axolotl Lake.

| Waterbody | Conservation Measure | Lead Agency | Cost |
|---|---|---|---|
| Axolotl Lake | Incubate eggs separately | FWP | $5,000 operations & 6 existing staff |
| Axolotl Lake | Ensure equal contribution | FWP | $5,000 operations & 6 existing staff |
| Axolotl Lake | Spawn different year classes | FWP | $5,000 operations & 6 existing staff |
| Axolotl Lake | Equalize spawning contributions | FWP | $5,000 operations & 6 existing staff |
| Axolotl Lake | Remove all parents which contribute to annual brood replacement | FWP | $5,000 operations & 6 existing staff |
| Axolotl Lake | Infuse wild genes every ten years | FWP | $5,000 operations & 6 existing staff |

Progeny from the 2020 wild Big Hole River spawn were infused into the genetic reserve population beginning in 2022 when mature age-2 fish were first encountered. Refined management of the genetic reserve population will reduce genetic divergence with the wild Big Hole River population. Axolotl Lake grayling will be the source for introductions into recently restored Big Hole tributaries (e.g., French Creek) and may also be used to augment the Big Hole River population if $N_e$ declines below 50 for more than a generation (i.e., the population begins losing genetic variation, is potentially experiencing inbreeding depression, and is at risk of extinction).

***Green Hollow Reservoir:*** A second genetic reserve population was established in Green Hollow Pond in the Gallatin River drainage using fish from Axolotl Lake in 1998. Founder effects resulted in further genetic divergence from the wild Big Hole River population (Table 15; Kovach et al. 2019). A barrier on the inlet stream to Green Hollow Pond will be removed to allow natural spawning, which combined with continued genetic supplementation from Axolotl Lake, will increase the genetic effective population size and improve genetic variation. The goal of this population is to provide a source of UMR grayling for introductions outside of the Big Hole River drainage. Green Hollow Pond annually provides up to 400,000 eggs for reintroduction efforts in the Madison and Gallatin drainages.

**Table 15.** Expected heterozygosity ($H_e$) and allelic richness ($A_r$) of the Big Hole River and brood populations.

| Sample | $H_e$ | $A_r$ |
|---|---|---|
| Big Hole 1980s | 0.89 | 17.43 |
| Big Hole 1990s | 0.88 | 16.44 |
| Big Hole 2000s | 0.88 | 16.47 |
| Big Hole early 2010s | 0.89 | 16.41 |
| Big Hole late 2010s | 0.88 | 15.93 |
| GreenHollow Reservoir | 0.87 | 13.50 |
| Axolotl Lake | 0.87 | 14.58 |

***Handkerchief Lake:*** A genetic reserve for the Centennial Valley grayling population does not currently exist. Introductions of Arctic grayling into historically occupied waters of the Centennial Valley have been attempted using the progeny of wild fish in Red Rock Creek. Until recently, this population was considered resilient enough to withstand gamete removals for introductions throughout the valley. However, this is no longer possible given the recent decline in abundance of the grayling population in Upper Red Rock Lake and a genetic reserve for the Centennial Valley grayling population is needed. Management goals for the population are:

1. Replicate genetic variation of Centennial Valley grayling to protect against extirpation
2. Minimize genetic differentiation between genetic reserve and wild Centennial Valley populations

Handkerchief Lake, in northwest Montana, is outside the native range of grayling but contained a viable population of introduced grayling for over 50 years until it was treated with rotenone in 2013 to remove hybridized cutthroat trout (Grisak and Marotz 2002). As part of the public scoping process, it was agreed that grayling would be reintroduced in Handkerchief Lake if the population had high conservation value. Following the fish removal project, attempts to replicate the Centennial Valley population were made in Handkerchief Lake using progeny from wild Red Rock Creek fish. However, those efforts have only included stocking 12,000 grayling fry and fingerlings whereas the original Handkerchief Lake grayling fishery was established with 711,000 fish stocked on 14 occasions during the 1950s and 1960s (Grisak and Marotz 2002). Few grayling are believed to occupy Handkerchief Lake based on targeted sampling and angler reports. Because of the low number of contributing parents, surviving fish have reduced genetic variation, which is not representative of the current or historical Centennial Valley population.

To establish a population in Handkerchief Lake that most accurately represents the historic genetic variation of grayling in the Centennial Valley at abundances sufficient to serve as a brood source for conservation projects, FWP will introduce fish into the Handkerchief Lake population from multiple mountain lakes that appear to be primarily founded from the Centennial Valley grayling (i.e., Park, Bobcat, Schwinegar, Odell, Elizabeth lakes; Kovach et al. 2021). Once established, Handkerchief Lake will serve as the primary donor source for introductions into suitable habitat in the Centennial Valley and Red Rock River drainage. The following introduction strategies that maximize success of genetic and demographic management goals will be used (Table 16).

Table 7. Conservation measures for the creation and perpetuation of a Centennial Valley Arctic grayling genetic reserve in Handkerchief Lake.

| Waterbody | Conservation Measure | Lead Agency | Cost |
|---|---|---|---|
| Handkerchief Lake | Introduce remaining Centennial-origin fish in captivity | FWP | $5,000 operations & 6 existing staff |
| Handkerchief Lake | Introduce grayling from at least three mountain lakes with primarily Centennial Valley ancestry into Handkerchief Lake | FWP | $5,000 operations & 6 existing staff |
| Handkerchief Lake | Spawn at least 50 pairs from each of the three lakes using a 2x2 cross | FWP | $5,000 operations & 6 existing staff |
| Handkerchief Lake | Use a combination of stocking fry and remote site incubators for introductions | FWP | $5,000 operations & 6 existing staff |
| Handkerchief Lake | Collect genetic samples from all contributing parents for parentage-based tagging | FWP | $5,000 operations & 6 existing staff |
| Handkerchief Lake | Annually collect 100 genetic samples from grayling in Handkerchief Lake for at least two generations following augmentation | FWP | |
| Handkerchief Lake | Infuse wild genes when demographically possible | FWP | $5,000 operations & 6 existing staff |

## Literature Cited


AGW. 2017. Memorandum of understanding among Montana Fish, Wildlife & Parks, Montana Department of Natural Resources & Conservation, U. S. National Park Service – Yellowstone National Park, U. S. Bureau of Land Management, U. S. Fish and Wildlife Service, U. S. Forest Service Region 1, U. S. Natural Resources Conservation Service and supporting organizations concerning Montana Arctic grayling conservation and restoration. FWP, Helena.

Allendorf, F. W. 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology 5:181-190.

Allendorf, F. W., O. Berry, and N. Ryman. 2014. So long to genetic diversity, and thanks for all the fish. Molecular Ecology 23:23-25.

Allendorf, F. W., W. C. Funk, S. N. Aitken, M. Byrne, and G. Luikart. 2022. Conservation and the genomics of populations, third edition. Oxford University Press, Oxford, United Kingdom.

Beal, F. 1953. The history of Georgetown Lake. FWP, Helena.

Boltz, G. D. 2000. Fisheries investigations on the Red Rock Lakes National Wildlife Refuge, Montana. USFWS, Bozeman.

Boyd, J. 2014. Arctic grayling SSA expert elicitation workshop. USFWS, Helena, Montana.

Byorth, P. A. 1996. Upper Ruby River fluvial Arctic grayling reintroduction plan. FWP, Helena.

Byorth, P. A. 1997. Upper Ruby River fluvial Arctic grayling reintroduction. Environmental assessment and reintroduction plan. FWP, Helena.

Byorth, P. A., and B. B. Shepard. 1990. Ennis Reservoir/Madison River fisheries investigation. FWP, Ennis.

Byorth, P. A., and J. P. Magee. 1998. Competitive interactions between Arctic grayling and brook trout in the Big Hole River Drainage, Montana. Transactions of the American Fisheries Society 127:921-931.

Caballero, A., and A. García-Dorado. 2013. Allelic diversity and its implications for the rate of adaptation. Genetics 195:1373-1384.

Christie, M. R., M. J. Ford, and M. S. Blouin. 2014. On the reproductive success of early-generation hatchery fish in the wild. Evolutionary Applications 7:883-896.

Clancey, P. 1997. Madison River and Ennis Reservoir fisheries. FWP, Ennis.

Comte, L., L. Buisson, M. Daufresne, and G. Grenouillet. 2013. Climate-induced changes in the distribution of freshwater fish: observed and predicted trends. Freshwater Biology 58:625-639.

Davis, M. N. 2016. Winter survival and habitat as limiting factors for Arctic grayling at Red Rock Lakes National Wildlife Refuge. Master's thesis. Montana State University, Bozeman.

Deeds, J. F., and W. N. White. 1926. Water power and irrigation in the Jefferson River basin, Montana. U. S. Geologic Survey water-supply paper No. 580:41-116.

Evermann, B. W. 1893. A reconnaissance of the streams and lakes of western Montana and northwestern Wyoming. Bulletin of the U. S. Fish Commission for 1891. XI:3-60. Government Print Office, Washington.

Fausch, K. D. 1998. Interspecific competition and juvenile Atlantic salmon (*Salmo salar*): on testing effects and evaluating the evidence across scales. Canadian Journal of Fisheries and Aquatic Sciences 55:218-231.

Flynn, K. F., T. J. Johnson, W. S. Parker, and J. Lovell. 2019. Upper Red Rock Lake, MT: Preliminary engineering and feasibility analysis to improve winter habitat for Arctic grayling. CDM Smith, Inc. Helena, Montana.

Flynn, K. F. 2022. EFDC+ dissolved oxygen modeling of Upper Red Rock Lake. KF2 Consulting, Helena, Montana.

FWP. 1989. Application for reservations of water in the Missouri River basin above Fort Peck Dam, Volume 2, Reservation requests for waters above Canyon Ferry Dam. Unpublished Report, Helena.

FWP. 1907. Early records of grayling propagation in Montana. Bozeman.

FWP. 2014. Southwest Montana grayling reintroductions; Environmental Assessment. Ennis.

FWP. 2021. Candidate conservation agreement with assurances for fluvial Arctic grayling in the upper Big Hole River 2021 Annual Report. Dillon.

FWP. 2022. Montana fishing regulations 2022. Helena.

Gander, T., L. Bateman, T. Paterson, M. Duncan, R. Kovach, and M. Jaeger. 2019a. Centennial Valley Arctic grayling report 2010-2018. FWP, Bozeman.

Gander, T., L. Bateman, A. McCullough, R. Kovach, and M. Jaeger. 2019b. Ruby River Arctic Grayling restoration project completion report. FWP, Bozeman.

Gangloff, M. M. 1996. Winter habitat and distribution of Arctic grayling in Upper Red Rock Lake, Red Rock Lakes National Wildlife Refuge, Montana. Master's thesis. Montana State University, Bozeman.

Gillin, G. 2001. Literature review of fisheries information for the Centennial Valley, Montana. USFWS, Dillon, Montana.

Gillilan, S., and K. Boyd. 2009. Letter of findings: Narrows and Limestone creek surface flow investigation. Gillilan Associates, Inc and Applied Geomorphology, Bozeman, Montana.

Grisak, G., and B. Marotz. 2002. South Fork Flathead Watershed westslope cutthroat trout conservation program. Project No. 1991-01903, 143 electronic pages, (BPA Report DOE/BP-00005043-1)

Hansen, P. L., R. D. Pfister, K. Boggs, B. J. Cook, J. Joy, and D. K. Hinckley. 1995. Classification and management of Montana's riparian and wetland sites. No. 54. Montana Forest and Conservation Experiment Station, School of Forestry, The University of Montana, Missoula.

Harding, S. T. 1915. Irrigation development in Montana. Agricultural College Experimental Station, Bulletin #103, 310-312. Bozeman, Montana.

Henshall, J. A. 1906. A list of the fishes of Montana: with notes on the game fishes. Bulletin University of Montana No. 34, Biological Series No. 11, University of Montana, Missoula.

Henshall, J. A. 1907. Cultivation of the Montana grayling. Bureau of fisheries document, No. 628.

Kaeding, L. R., and G. D. Boltz. 1999. A study of Arctic grayling and their stream habitat in support of reserved water right applications, Red Rock Lakes National Wildlife Refuge, Montana. USFWS, Bozeman, Montana.

Katzman, L. M. 1998. Effects of predation on status of Arctic grayling at Red Rock Lakes National Wildlife Refuge, Montana. Master's thesis, Montana State University, Bozeman.

Kaya, C. M. 1990. Status report on fluvial Arctic grayling (*thymallus Arcticus*) In Montana. FWP, Helena.

Kaya, C. M. 1992. Restoration of fluvial Arctic grayling to Montana streams: assessment of reintroduction potential of streams in the native range, the Upper Missouri River Drainage above Great Falls. Montana State University, Bozeman.

Kovach, R. P., A. R. Whiteley, M. E. Jaeger, S. Painter, A. Lodmell, and R. F. Leary. 2019. Genetic monitoring informs conservation status and trend of Arctic grayling at the southern edge of their distribution. Canadian Journal of Fisheries and Aquatic Sciences 77(12):1934-1942.

Kovach, R., S. Painter, and A. Lodmell. 2019. Genetic letter on Axolotl Lake and Green Hollow Reservoir. University of Montana Conservation Genetics Laboratory, Missoula.

Kovach R., S. Painter, and A. Lodmell. 2021. Identification of Centennial origin populations based on genetic data. University of Montana Conservation Genetics Laboratory, Missoula.

Kovach R., S. Painter, and A. Lodmell. 2022. Big Hole and Centennial Valley Arctic grayling genetic status report no. 5347. University of Montana Conservation Genetics Laboratory, Missoula.

Leary, R. F. 1991. Establishment, maintenance, and use of a genetic reserve of Big Hole River Arctic grayling brood management plan. University of Montana Wild Trout and Salmon Genetics Lab Report 1991/5, Missoula.

Leary, R. F., S. Painter, A. Lodmell, and F. W. Allendorf. 2015. High genetic variation among and within Arctic grayling populations in the Upper Missouri River Drainage, Montana. University of Montana Conservation Genetics Laboratory Report 15/1, Missoula.

Levine, R. 2007. Arctic grayling (*Thymallus arcticus*) emergence and development in Odell Creek, Red Rock Lakes National Wildlife Refuge, Montana. Master's thesis. Montana State University, Bozeman.

Liermann, B. W. 2001. An evaluation of the reintroduction of fluvial Arctic grayling into the upper Ruby River. Master's thesis. Montana State University, Bozeman.

Lohr, S. C., P. A. Byorth, C. M. Kaya, and W. P. Dwyer. 1996. High-temperature tolerances of fluvial Arctic grayling and comparisons with summer river temperatures of the Big Hole River, Montana. Transactions of the American Fisheries Society 125:933-939.

Luikart, G., F. W. Allendorf, J. M. Cornuet, and W. B. Sherwin. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 89:238-247.

Lund, J. A. 1974. The reproduction of salmonids in the inlets of Elk Lake, Montana. Master's thesis, Montana State University, Bozeman.

Magee, J. M., and P. A. Byorth. 1994. Competitive interactions of fluvial Arctic grayling *Thymallus arcticus* and brook trout *Salvelinus fontinalis* in the upper Big Hole River, Montana. Fluvial Arctic Grayling Workshop.

Magee, J., E. Rens, and P. Lamothe. 2005. Fluvial Arctic grayling monitoring report 2004. FWP, Dillon.

McCullough, A. R. 2017. Relations among Arctic grayling, nonnative salmonids, and abiotic factors in the Big Hole River, Montana. Master's thesis. Montana State University. Bozeman.

Miller, R. R., J. D. Williams, and J. E. Williams. 1989. Extinctions of North American fishes during the past century. Fisheries 14(6):22-48.

Mogen, J. T. 1996. Status and biology of the spawning population of Red Rock Lakes Arctic grayling. Master's thesis, Montana State University, Bozeman.

Montana Code Annotated. 2000. United States Fish and Wildlife Service Red Rock Lakes-Montana Compact ratified. 85-20-801, Helena.

Myers, L. 1977. Interagency fisheries habitat inventory for the Centennial Valley. BLM, Dillon, Montana.

Nelson, P. H. 1954. Life history and management of the American Grayling (*Thymallus signifer tricolor*) in Montana. Journal of Wildlife Management 18:324-342.

Nico, L. G., and P. L. Fuller. 1999. Spatial and temporal patterns of nonindigenous fish introductions in the United States. Fisheries 24(1):16-27.

Olsen, J. R. 2014. Miner, Mussigbrod, Twin and Pintler lakes report 2006-2014. FWP Federal Aid Job Progress Report. Federal Aid Project Number: F-113 R7-13, Butte.

Olsen, J. R. 2020. Stream surveys in the Big Hole River Drainage 2017-2020. FWP Federal Aid Project Number: F-113-R, Butte.

Oswald, R. A., S. Hochhalter, and L. Rosenthal. 2008. Southwest Montana native fish research and conservation April 1, 2007–December 31, 2007. FWP Wildlife Grant Project Performance Report T-26-3. Helena.

Oswald D, S. Lula, S. Huchhalter, L. Rosenthall, A. Brooks, and T. Horton. 2007. Southwest Montana native species research and conservation. FWP Project Performance Report. T-26-3, Dillon.

Paterson, T. 2013. Estimation of the abundance and apparent survival of spawning Arctic grayling in Red Rock Creek; Red Rock Lakes National Wildlife Refuge. Master's thesis. Montana State University, Bozeman.

Peterson, D. P., and W. R. Arden. 2009. Ancestry, population structure, and conservation genetics of Arctic grayling (*Thymallus arcticus*) in the upper Missouri River, USA. Canadian Journal of Fisheries and Aquatic Science 66: 1758-1774.

Puchani, A.R. 2021. Success of Westslope cutthroat trout and arctic grayling conservation translocations in Yellowstone National Park, Montana and Wyoming, USA. Master's thesis. Montana State University, Bozeman, Montana 59717.

Randall, L. C. 1978. Red Rock Lakes National Wildlife Refuge: an aquatic history 1899–1977. USFWS, Kalispell, Montana.

Seavy, N.E., T. Gardali, G. H. Golet, F. T. Griggs, C. A. Howell, R. Kelsey, S. L. Small, J. H. Viers, and J.F. Weigand. 2009. Why climate change makes riparian restoration more important than ever: recommendations for practice and research. Ecological Restoration, 27(3), 330-338.

Shade, C., and S. Bonar. 2005. Distribution and abundance of nonnative fishes in streams of the western United States. North American Journal of Fisheries Management 25:1386-1394.

Shields, G.O. (editor). 1897. Fish and Fishing *in* Recreation, Those Elk Lake Trout. October 1897 Vol. VII, NO. 4. Pg. 394. https://play.google.com/books/reader?id=2-KfAAAAMAAJ&printsec=frontcover&output=reader&authuser=0&hl=en&pg=GBS.PA394

Sonderegger, J. L. 1981. Geology and geothermal resources of the eastern Centennial Valley. Montana Geological Society, Field and Conference Symposium Guidebook to Southwest Montana.

Streu, J. M. 1990. Select aspects of the life history and ecology of Arctic grayling (*Thymallus arcticus montanus)* [Milner] in the upper Big Hole River Drainage, Montana. Montana Natural Heritage Program, Helena.

Unthank, A. 1989. Historical overview of Red Rock Lakes National Wildlife Refuge grayling fisheries. USFWS, Lima, MT.

USDA. 2012. Riparian assessment: using the NRCS riparian assessment method. Environment Technical Note No. MT-2 (Rev. 2). Natural Resources Conservation Service, Bozeman, Montana.

USFWS. 1985. Red Rock Lakes National Wildlife Refuge fish management plan and project report 1984-1985. Creston Fisheries Center, Kalispell, Montana.

USFWS. 2006. Candidate Conservation Agreement with Assurances for fluvial Arctic grayling in the upper Big Hole River. Helena, Montana.

USFWS. 2009. Comprehensive conservation plan Red Rock Lakes National Wildlife Refuge. Lima, Montana.

USFWS. 2014. Endangered and threatened wildlife and plants; revised 12-month finding on a petition to list the upper Missouri River distinct population segment of Arctic grayling as an endangered or threatened species; proposed rule. Federal Register 79(161):49384-49422.

USFWS. 2018. Candidate conservation agreement with assurances for Arctic grayling in the Centennial Valley, Montana. Helena, Montana.

USFWS. 2020. Endangered and threatened wildlife and plants; finding on a petition to list the fluvial population of the Arctic grayling as endangered. Federal Register Docket No. FWS-R6-ES-2020-002.

USFWS. 2020. Revised 12-month finding on a petition to list the upper Missouri River Distinct Population Segment of Arctic grayling. Federal Register 85(142):54708–54753.

Vatland, S. J. 2015. Effects of stream temperature and climate change on fluvial Arctic grayling and nonnative salmonids in the upper Big Hole River, Montana. Doctoral dissertation. Montana State University, Bozema.

Vincent, R. E. 1962. Biogeographical and ecological factors contributing to the decline of the Arctic grayling, *Thymallus arcticus* Pallus, in Michigan and Montana. Doctoral dissertation. University of Michigan, Ann Arbor.

Waples, R. S., G. Luikart, J. R. Faulkner, and D. A. Tallmon. 2013. Simple life history traits explain key effective population size ratios across diverse taxa. Proceedings of the Royal Society B 280:20131339.

Waples, R. S., T. Antao, and G. Luikart. 2014. Effects of overlapping generations on linkage disequilibrium estimates of the effective population size. Genetics 197:769-780.

Warren, J. M., and M. Jaeger. 2017. Centennial Valley Arctic grayling adaptive management plan. USFWS and FWP, Lima, Montana.

Warren, J. M., M. Jaeger, K. Cutting, and K. Cook. 2019. Site-specific protocol for the Centennial Valley Arctic Grayling Adaptive Management Plan. USFWS and FWP, Lima, MT.

Warren, J. M., M. Jaeger, R. Kreiner, K. Cutting, L. Bateman, T. Gander, T. Paterson, J. Payne, and M. Duncan. 2022. Centennial Valley Arctic grayling adaptive management project annual report, 2021. USFWS and FWP, Lima, Montana.

Warren, J. M., M. Jaeger, T. Gander, K. Cutting, R. Kreiner, L. Bateman, and T. Paterson. 2022. Centennial Valley Arctic grayling adaptive management project spring update, 2022. USFWS and FWP, Lima, Montana.